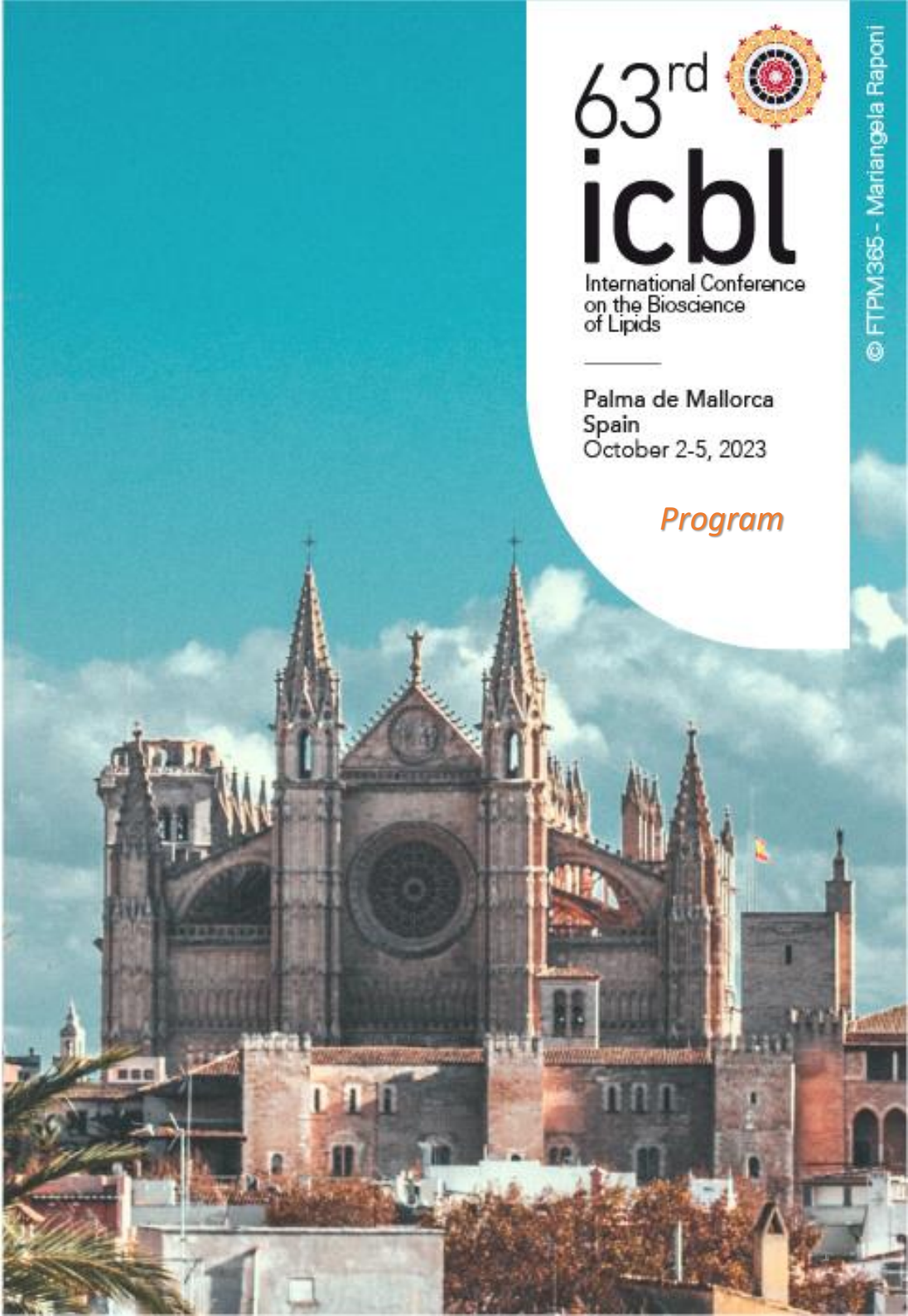
63rd icbl

International Conference on the Bioscience of Lipids

Palma de Mallorca
Spain
October 2-5, 2023

Program



Dear colleagues,

It is a great pleasure to welcome you to the 63rd edition of the International Conference on the Bioscience of Lipids (ICBL) that will be held in Palma de Mallorca (Spain) between the 2nd-5th of October 2023.

The theme of this year's conference will be is Advances in Lipid Research: from bench to bedside.

These are indeed special moments for lipid researchers. We are all experiencing, day by day, how rapidly the field is evolving. The solid advances in lipidome analysis, together with the irruption of spatially resolved techniques, have led us to astonishing lipid landscapes with high complexity in shape, composition and, consequently, function. But, how is this diversity regulated during homeostasis? How much can they tell us about pathologies? How might a reprogramming in lipid metabolism affect the development and progression of a disease or the response to treatments? We are facing all these new fascinating questions while many fundamental issues remain unanswered, such as understanding how lipid metabolism is specifically regulated to generate such diversity.

The program proposed herein aims to offer the ideal scenario to discuss hand in hand with basic, translational, and clinical lipid researchers the advances in the field but, more importantly, the new opportunities and the current limitations. We will encourage researcher-student interactions by organising "Meet the Expert" sessions at lunch. Early career researchers submitting the most original contributions will be selected for oral presentations and awards will be given to the best posters.

Last but not least, on Monday Oct 2nd we will start off with a symposium on Spatially Resolved Omics Techniques, while the last ICBL session will be a Joint Session, Lipids & Cancer, with the III International Workshop on Translational Cancer Research, to which ICBL attendees are invited to.

All the events will take place at the Auditòrium de Palma, located at the bay of the city, giving us the best excuse to enjoy long walks along the seafront while we share our impressions of the meeting.

We hope that you will join us in this international celebration of the Bioscience of Lipids in Palma.

Your local organizing committee,

Dr. Gwendolyn Barceló-Coblijn
Health Research Institute of the Balearic Islands, IdISBa, Palma - *Chair*

Dr. Patricia Aspichueta
University of the Basque Country, UPV/EHU, Bilbao

Dr. Jesús Balsinde
Institute of Molecular Biology and Genetics (IBGM), Valladolid

Dr. Gemma Fabriàs
Institute for Advanced Chemistry of Catalonia, IQAC-CSIC, Barcelona

Dr. Mariona Jové
Universitat de Lleida- IRBLLeida, Lleida

Dr. Reinald Pamplona
Universitat de Lleida-IRBLLeida
Lleida

Dr. Oliver B. Vögler
University of the Balearic Islands, IUNICS, IdISBa, Palma

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Monday, October 2

9:00-9:10	Wellcome
9:40-11:00	SPATIAL LIPIDOMICS
11:00-11:25	coffee break
11:25-13:45	SPATIAL PROTEOMICS
13:45-14:30	lunch break
14:30-15:30	SPATIAL TRANSCRIPTOMICS
15:30-16:00	Closing lecture
16:00-16:45	Round Table researchers & companies

Tuesday, October 3

9:00-11:10	Lipids in Metabolic Disorders
11:10-11:30	coffee break
11:30-13:55	Lipid in immunology
13:55-14:25	*meet the expert lunch* + poster viewing
14:25-15:20	Lipid enzymes as new drug targets
15:20-16:00	short talks
16:00-17:00	POSTER SESSION I
17:00-18:00	short talks
18:00-18:35	Lipid enzymes as new drug targets

Wednesday, October 4

8:30-10:35	Clinical Analysis & system biology
10:35-11:35	POSTER SESSION II
11:35-13:40	Modified Lipids in pathophysiology
13:40-14:10	*meet the expert lunch* + poster viewing
14:10-15:30	short talks
16:15-18:00	SOCIAL EVENT
19:00-22:00	GALA DINNER

Thursday, October 5

9:00-9:15	Welcome
9:15-9:50	LIPIDS AND CANCER
9:50-10:10	
10:10-10:45	
10:45-11:05	
11:05-11:25	coffee break
11:25-11:45	LIPIDS AND CANCER
11:45-12:20	
12:20-12:40	
12:40-13:00	
13:00-14:00	KEYNOTE TALK
14:00-14:30	ICBL 2024+Awards+farewell
14:30-15:30	*LUNCH ICBL + WkS* Poster Session for WkS

Monday, October 2

9:00-9:10 Welcome

Spatial Lipidomics

9:10-9:40 **Satellite Talk**. Lipid Imaging Mass Spectrometry: Challenges, Limitations and Achievements.
José Andrés Fernández González, University of the Basque Country, Leioa, Spain.

9:40-10:10 **Satellite Talk**. Integrated morphometric and molecular classification of central nervous system cancers using a unified platform with picosecond infrared laser mass spectrometry.
Arash Zarrine-Afsar, University Health Network, Toronto, Canada.

10:10-10:30 **Selected Abstract.** PPAR gamma role in the renal tubule-specific lipid metabolism in fibrosis context.
Lucía Martín-Saiz, Paris Cardiovascular Research Centre - INSERM, Paris, France.

10:30-11:00 **Satellite Talk**. The Power of MALDI Imaging in Disease-Based Research.
Björn Wendik, Bruker Daltonics, Freiburg, Germany.

11:00-11:25 Coffee break

Monday, October 2

Spatial Proteomics

11:25-11:55 **Satellite Talk**. MS-Imaging in basic and clinical research at the hospital: from the bench to the patient.
Eduardo Chicano Gálvez, Maimónides Biomedical Research Institute of Córdoba (IMIBIC), Córdoba, Spain.

11:55-12:15 **Selected Abstract.** Optimization of MALDI-MSI technologies on Zebrafish eleutheroembryos: A case study with endocrine disruptors.
Albert Menéndez-Pedriza, Institute of Environmental Assessment and Water Research (IDAEA-CSIC), Barcelona, Spain.

12:05-12:35 **Satellite Talk**. Lipidomic Changes in Aging Skin studied by MS Imaging.
Martina Marchetti-Deschmann, Institute of Chemical Technologies and Analytics (TU Wien), Vienna, Austria.

12:15-12:45 **Satellite Talk**. Molecular Intratumor Heterogeneity Assessed by Mass Spectrometry Imaging Has Prognostic Value in Primary Breast and Oral Cancers.
Piotr Widłak, Medical University of Gdańsk, Poland.

13:15-13:45 **Satellite Talk**. Insights into Enzyme Histochemistry by MALDI MSI.
Andreas Baumeister, Shimadzu Europa GmbH, Düsseldorf, Germany.

13:45-14:30 Lunch break

Monday, October 2

Spatial Transcriptomics

14:30-15:00 **Satellite Talk**. Childhood obesity: Cartography of adipose tissue in space and time. Spatial transcriptomics map of human pediatric adipose tissue. Understanding hypertrophic adiposity in children through spatial transcriptomics.
Josep C. Jiménez-Chillarón, Sant Joan de Déu Reseach Institute, Bonsailab – 10x Genomics, Barcelona, Spain.

15:00-15:30 **Satellite Talk**. Multi-omic Spatial Profiling at Single-Cell Resolution to Unravel Complex Biology.
Aida Freire Valls, Diagnostica Longwood, Barcelona, Spain

Victoria Menéndez García, MD Anderson Cancer Center, Madrid Spain

15:30-16:00 **Satellite Talk**. Introduction of the JST-ERATO Lipidome Atlas Project in Japan.
Makoto Arita, Keio University, Faculty of Pharmacy, Tokyo, Japan.

16:00-16:45 Round Table researchers & companies - Conclusions

Monday, October 2

17:15 - 17:30 ICBL 2023 Opening Ceremony.

17:30- 18:30 Van Deenen Lecture

Edward A. Dennis, University of California at San Diego, La Jolla, California, USA.

18:30 Welcome cocktail

Tuesday, October 3

Lipids in Metabolic Disorders

9:00-9:35 **Plenary Talk.** Role of mitochondrial fusion proteins in the transfer of phospholipids from the endoplasmic reticulum to the mitochondria.
Antonio Zorzano, Institute for Research in Biomedicine (IRB), Barcelona, Spain.

9:35-9:55 **Selected Abstract.** Combined effect of ketogenic diet and GDNF injection on Schwann Cells in mouse model of Krabbe's disease.
Gaetan Ravaut, Université du Québec à Montréal (UQAM), Montréal, Canada.

9:55-10:30 **Plenary Talk.** Lipid droplets drive lipid mediator production and modulate ferroptosis.
Toni Petan, Jožef Stefan Institute, Ljubljana, Slovenia.

10:30-10:50 **Selected Abstract**. Tracking ether lipid synthesis and breakdown in human adipocyte differentiation.
Sabrina Sailer, Institute of human genetics, Medical University of Innsbruck.

10:50-11:10 **Selected Abstract**. Dietary derived short chain fatty acids impair brain health: A shift in our understanding of short chain fatty acids health promotive benefits.
Raymond Thomas, University of Western Ontario, Ontario, Canada.

11:10-11:30 Coffee break

Lipid in Immunology

11:30-12:05 **Plenary Talk.** TBA
Carlos Fernández-Hernando, Yale School of Medicine, Connecticut, USA.

Tuesday, October 3

12:05-12:25 **Selected Abstract.** Knockout of cardiolipin synthase disrupts cardiac development in neonatal mice.
Michael Schlame, New York University Grossman School of Medicine, New York, USA.

12:25-13:00 **Plenary Talk.** STAT-1-Controlled Lipin-2 Is a Master Regulator of the Antiviral and Anti-Inflammatory Responses to Interferon.
María Ángeles Balboa García, Institute of Molecular Biology and Genetics-CSIC, Valladolid, Spain.

13:00-13:20 **Selected Abstract.** Mitochondrial lipidomes are tissue specific. The phospholipid to cholesterol ratio regulates UCP1 activity.
Sarah Brunner, Technical University of Munich, School of Life Sciences, Freising, Germany.

13:20-13:55 **Plenary Talk.** Novel insights into the phospholipase A2 family.
Makoto Murakami, University of Tokyo, Japan.

13:55-14:25 Meet the Expert lunch + Poster viewing

Lipid enzymes as new drug targets

14:25-15:00 **Plenary Talk.** Design, synthesis and pharmacological evaluation of soluble epoxide hydrolase inhibitors for pain: focus on visceral pain and chemotherapy-induced neuropathic pain .
Santiago Vázquez, University of Barcelona, Spain.

15:00-15:20 **Selected Abstract.** Evaluate the Therapeutic Functions of Enzyme-X in Atherosclerotic Cardiovascular Diseases.
Law Shi Hui, Kaohsiung Medical University, Kaohsiung, Taiwan.

Tuesday, October 3

15:20-15:40 **Short Talk.** Agilent LCMS technologies and workflows to afford a confident lipid profiling.
Jaume Morales i Sediles, Agilent, Barcelona, Spain.

15:40-16:00 **Short Talk.** European Research Council (ERC) grants: all you need to know before applying.
Ino Agrafioti, European Research Council (ERC), Brussels, Belgium.

16:00-17:00 Poster Session I

17:00-17:20 **Selected Abstract**. Advanced (super-resolution) microscopy for dissecting lipid membrane organization and dynamics.
Christian Eggeling, Leibniz Institute of Photonic Technologies, Jena, Germany.

17:20-17:40 **Selected Abstract.** Diversity of membrane phospholipids; biochemical mechanism and disease relevance.
Takao Shimizu, National Center for Global Health and Medicine, Tokyo, Japan.

17:40-18:00 **Selected Abstract.** The roles of polyunsaturated fatty acids in vivo revealed by analyzing polyunsaturated fatty acid-deficient mice.
Takehiko Yokomizo, Juntendo University, Tokyo, Japan.

18:00-18:35 **Plenary Talk.** Endocannabinoid and paracannabinoid control of pain processing.
Daniele Piomelli, University of California, Irvine, USA.

18:35-19:30 Steering Committee

Wednesday, October 4

Clinical Analysis & System Biology

8:30-9:05 **Plenary Talk.** Motor Neuron Disease or Sensory Neuropathy? L-Serine as a modulating factor.
Throsten Horneman, University Hospital Zürich, Switzerland.

9:05-9:25 **Selected Abstract.** Q-RAI data-independent acquisition for lipidomic quantitative profiling.
Jing Kai Chang, National University of Singapore, Republic of Singapore.

9:25-10:00 **Plenary Talk.** Quantifying Lipid Species in Large-Scale Human Studies: Approaches and Challenges.
Gerhard Liebisch, University Hospital Regensburg, Germany

10:30-10:35 **Plenary Talk.** Lipidomics and multiomics for molecular discovery in disease
Cristina Legido-Quigley, Kings College London, United Kingdom.

10:35-11:35 Poster Session II

Modified Lipids in pathophysiology

11:35-12:10 **Plenary Talk.** Oxidative phospholipidomics: the cross talk between the chemistry and the biological role in inflammation and chronic diseases
Rosario Domingues, University of Aveiro, Portugal.

12:10-12:45 **Plenary Talk.** Lipoxidation of cysteine residues: selectivity and diversity of structural and functional implications
Dolores Pérez-Sala, Center for Biological Research Margarita Salas, CSIC, Madrid, Spain.

Wednesday, October 4

12:45-13:05 **Selected Abstract**. The emerging role of the mitochondrial fatty-acid synthase (mtFAS) in the regulation of energy metabolism.
Sara Tucci, Medical Center - University of Freiburg, Germany.

13:05-13:40 **Plenary Talk.** The biological effects of protein lipoxidation and its analysis by mass spectrometry.
Corinne M Spickett, Aston University, Birmingham, United Kingdom.

13:40-14:10 Meet the Expert lunch + Poster viewing

14:10-14:30 **Selected Abstract**. Electronegative low-density-lipoprotein induces insulin resistance and hypertriglyceridemia: a new insight into the pathogenesis of gestational diabetes mellitus.
Shao-Chi Hung, Kaohsiung Medical University, Kaohsiung, Taiwan.

14:30-14:50 **Selected Abstract**. Life and death in adipose tissue: a matter of epigenetics?
Lara Coppi, Università degli Studi di Milano, Milan, Italy.

14:50-15:10 **Selected Abstract**. Are 3D cultures able to model in vivo tumor behavior? A spatial lipidomics and multiomics investigation.
Gábor Balogh, Institute of Biochemistry Szeged, Hungary.

15:10-15:30 **Selected Abstract**. Phosphatidic acid phosphatase contains a novel RP domain that regulates its phosphorylation and function in lipid synthesis.
George Carman, Rutgers University, New Jersey, USA.

16:00-18:00 Social Event

19:00-22:30 Gala Dinner

Thursday, October 5

9:00-9:15 Welcome

Lipids and Cancer

9:15-9:50 **Plenary Talk.** Circulating Metabolic Biomarkers: From Liver Inflammation to Cancer.
Jesús Mª Bañales, Biodonostia Health Reseach Institute, Donostia, Spain.

9:50-10:10 **Selected Abstract**. Combining lipidomics, metabolomics and cytokinomics to characterize the immunometabolic landscape associated with organ failure and mortality in acutely decompensated cirrhosis.
Cristina López-Vicario, August Pi i Sunyer Biomedical Research Institute, Barcelona, Spain.

10:10-10:45 **Plenary Talk.** The potential of lipid nanoemulsions for the development of advanced cancer therapies.
María de la Fuente, Health Research Institute of Santiago de Compostela, Santiago, Spain.

10:45-11:05 **Selected Abstract**. A redox stress-modulated phospholipase A2 remodels lipids to regulate ferroptosis in cancer
Stephen Ruiz, Memorial Sloan Kettering Cancer Center, New York, USA.

11:05-11:25 Coffee break

11:25-11:45 **Short talk**. Accelerating Biomarker Discovery with Next Generation Proteomics
Bernat Coll-Martínez, Diagnostica Longwood, Barcelona, Spain.

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11:45-12:20 **Plenary Talk.** The Lipotype Hypothesis.
Giovanni d'Angelo, Swiss Federal Institute of Technology, Lausanne, Switzerland.

Thursday, October 5

12:20-12:40 **Selected Abstract**. Unlocking the potential of MALDI-IMS for an in-depth characterization of the tumor microenvironment: lipids as a Trojan horse.
Karim Pérez, Health Research Institute of the Balearic Islands, Palma, Spain.

12:40-13:00 **Selected Abstract**. Modulation of tumor immunity by autotaxin-producing cells in the tumor microenvironment.
Gabor Tigyi, University of Tennessee Health Science Center, Tennessee, USA.

13:00-14:00 Closing Keynote Lecture
Environmental constraints on cell proliferation
Matt Vander Heiden, Koch Institute at MIT, Cambridge, Massachusetts, USA.

14:00-14:30 ICBL 2023Awards Ceremony and Presentation of ICBL 2024

14:30-15:30 Lunch ICBL & Workshop

About the Speaker

Dr. José A. Fernández González

Dept. of Physical Chemistry. Faculty of Science and Technology, University of the Basque Country (UPV/EHU), Leioa, Spain

I started working at the University of the Basque Country (UPV/EHU) as a Ramon y Cajal researcher (2001, first promotion) and put together my own research group. I currently have two research lines: one centered in the study of the aggregation of molecules of biological relevance using mass-resolved laser spectroscopy (MRLS) and a second one in lipids imaging mass spectrometry (LIMS). The starting hypothesis of the latter is that lipids are excellent biomarkers for early detection, diagnosis and prognosis of diseases, due to their connection with the cell metabolism: any alteration of the cell homeostasis results in a fast and deep alteration of the lipid fingerprint. LIMS enables mapping the distribution of such fingerprint directly in tissues, establishing a direct correlation between lipid alteration and the tissue/cell affected by the disease. Our activity in this research line includes development of new methodologies, software and new equipment, such a matrix deposition device that enables achieving spatial resolutions better of 5 micron/pixel, always trying to make LIMS advance towards its application in clinics. Some of our achievements in the field of LIMS include the publication of the first images of lipid distribution in human brain, the first map of the lipidome of the human nephron or the first maps of lipid distributions in human colon at high spatial resolution and their alteration in the context of a neoplasia. We are currently carrying out studies of lipid alteration in the context of several pathologies, such as colon cancer, Crohn's disease, ulcerative colitis, melanoma, and clear cells renal cell carcinoma, among others. This is eminently a translational research line and relies on the collaboration with several companies, hospitals and other research institutions.

Altogether, our research has so far resulted in +160 publications, 13 PhD thesis, 2 licensed software and two patents, one of which was the basis for a start-up company: IMG Pharma Biotech S.L.

About the Speaker

Dr. Arash Zarrine-Afsar

University Health Network. Faculty of Medicine, University of Toronto. Point Surgical Inc. Canada and Switzerland

Dr. Zarrine-Afsar is a physical biochemist with interest in the development of various spectroscopic and spectrometric methods to understand the dynamics of biological systems, in particular transition to the disease state. He is currently Principal Investigator at University Health Network and leads a group of researchers focused on development of rapid diagnostic methods using mass spectrometry. He holds a position as Assistant Professor in the Dept. of Surgery and Graduate Dept. of Medical Biophysics at University of Toronto's Faculty of Medicine. The research in his laboratory over the past 5 years has further matured Picosecond Infrared Laser Mass Spectrometry (PIRL-MS) as a rapid pathology determination tool where 10-second laser sampling of tissue molecular content has allowed differentiation of cancer types and subtypes faster than currently possible in the standard of care. Dr. Zarrine-Afsar additionally serves as the Chief Technology Officer of Point Surgical Inc.

Dr. Björn Wendik, Bruker Daltonics, Freiburg, Germany

Björn is a developmental biologist who was trained in Würzburg/Bavaria and in Freiburg/Baden-Württemberg. Already during his studies he used many different imaging techniques and image analysis tools. For his PhD thesis he worked predominantly with Zebrafish and studied the development of insulin producing islet cells and also studied axon guidance mechanisms of motoneurons using life cell imaging and a lot of confocal microscopy. After his postdoctoral training, he worked as a lecturer at the University of Freiburg where he was also responsible for the imaging core facility. His career in life science industry started as a confocal specialist for high speed life cell imaging, using spinning disc confocal microscopes. He moved then to a position as a Field Application scientist into the spatial biology field, where he worked with highplexing technologies in the immunooncology field and in pathology.

About the Speaker

Dr. Eduardo Chicano-Gálvez

Head of the IMIBIC Mass Spectrometry and Molecular Imaging Unit (IMSMI)
Maimónides Biomedical Research Institute of Córdoba (IMIBIC; Spain)

Eduardo Chicano-Gálvez is the Head of the IMIBIC Mass Spectrometry and Molecular Imaging Unit (IMSMI) at the Maimónides Biomedical Research Institute of Córdoba (IMIBIC; Spain). In his core facility he provides highly specialised support in the latest mass spectrometry techniques available to researchers and users who require it by 4 main analytical lines: metabolomics, lipidomics, proteomics (all LC-MS/MS based) and MS-Imaging (multiomics).

More specifically, he is responsible for optimizing and analyzing the MS-Imaging experiments, encompassing levels ranging from lipidomics and metabolomics to proteomics. Additionally, he handles the analysis of quantitative proteomics data acquired through LC–MS methods like DIA, DDA, and PRM.

Eduardo holds a Ph.D. from the University of Córdoba (Spain) and has also earned a Master's degree in Bioinformatics from the International University of Andalusia (UNIA). He is the recipient of a competitive contract under the “Programme for Linking Technicians to Common Research Support Structures” funded by the Andalusian Health Service since 2012.

He actively collaborates with researchers globally, with a primary focus on the biomedical field while also extending his involvement to environmental, agricultural, and other basic/clinical research projects. Eduardo's ongoing efforts are centered on the discovery of novel prognosis and diagnosis biomarkers, encompassing both fluid and tissue-based analyses. Furthermore, he is engaged in the development of innovative approaches like spatialomics to combine different MS-Imaging omics data, LC–MS data and optical microscopy data through the utilization of laser capture microdissection, AI, neural networks, classical machine learning methodologies, and systems biology principles.

About the Speaker

Dr. Martina Marchetti-Deschmann

Institute of Chemical Technologies and Analytics – TU-Wien, Vienna, Austria

She obtained her PhD in Chemistry at the University of Vienna and has been working in the field of Instrumental Analytical Chemistry for over 20 years. Her research is focused on mass spectrometry and hyphenated techniques for biomolecule identification, quantification, detailed characterization and spatial localization. Her research areas include laser-based Analytical Chemistry, in particular laser-assisted mass spectrometry.

International research stays in Amsterdam at AMOLF (NL), in Leiden at the Medical University (NL), the Academy of Sciences in Brno (CZ), and at the Vanderbilt University (US) allowed her to become an expert in Mass Spectrometry Imaging (MSI) over the past years. Her group was the first lab that combined molecular and elemental imaging in multi-instrument approaches (MS & Infrared, (Immuno)histology, Fluorescence, XRF or AFM, but also MALDI & LA-ICP MSI). Today, Martina is an internationally recognized expert for instrument advances and innovative methodologies to put multi-measurements of a sample into practice to gather comprehensive information down to the cellular level.

Martina seeks to advance mass spectrometric research, education and professionalization. Besides other activities she is board member of the International Mass Spectrometry Foundation (IMSF) and founding member of the MS Imaging Society (MSIS), being also president of the latter since 2021. She published over 139 peer-reviewed articles, 7 book chapters and holds three patents. Martina received the eLearning Award at TU Wien in 2010, was awarded the Beynon Prize from the Journal "Rapid Communications in Mass Spectrometry" in 2007 and the Fritz Feigl Prize in 2013 awarded by the Austrian Society of Analytical Chemistry (ASAC).

Prof. Piotr Widłak

Medical University of Gdańsk, Poland

Prof. Piotr Widlak received an M.Sc. in Molecular Biology (1988), Ph.D. in Biomedicine (1993), and habilitation in Biochemistry (2001), and also holds a titular (state) professorship in Medical Sciences (2007). He performed post-doctoral research at the Karolinska Institute, Stockholm/Huddinge (1994-1995), and UT Southwestern Medical Center, Dallas (1996-1997). In 1997 he joined the faculty of the Maria Sklodowska-Curie National Research Institute of Oncology, Gliwice Branch, one of the largest clinical and medical research institutes in Poland. He was the Deputy Director for Research (2006-2015), and the Chairman of the Center for Translational Research and Molecular Biology of Cancer (2010-2021). In 2022 he joined staff of the Medical University of Gdańsk, a top medical school in Poland, as a Director of the Clinical Research Support Centre.

Dr. Widlak has authored and co-authored over 200 peer-reviewed papers, with a number of citations exceeding 4,000. His major focus of research have been molecular mechanisms involved in cellular responses to stress as well as applications of proteomics and metabolomics in molecular oncology.

Dr. Andreas Baumeister, Izasa-Scientific -
Shimadzu Europa, Düsseldorf, Germany

SHIMADZU
Excellence in Science

2008-2014: BSc and MSc studies at Münster University in Chemistry, Physic and Business Chemistry. Master Thesis at Klaus Dreisewerd's lab: Laser wavelength and fluence dependency of MALDI analysis of non-covalent complexes.

2014-2017: PhD studies at Münster University, Research at Klaus Dreisewerd's lab: Laser wavelength an fluence dependency of AP-MALDI and development of a SALDI technique to analyze insect pheromones and other complex lipid mixtures.

Since 2018: Product specialist at European head quarter of Shimadzu: Application development and customer support for MALDI-TOF and MALDI imaging.

Dr. Josep C. Jiménez-Chillarón, Sant Joan de Déu Research Institute, Bonsailab – 10x Genomics, Barcelona, Spain

Over the last 15 years I have worked in the broad Field of Developmental Origins of Health and Disease.

In particular, my research has focused on understanding the molecular mechanisms that link neonatal nutrition with later risk of obesity and diabetes, by combining animal models and Clinical Cohorts. Currently, we are exploring the potential role of Epigenetic mechanisms in mediating childhood obesity and obesity-related metabolic diseases in humans and transgenerational inheritance of diabetes risk in murine models.

More recently, we are exploring the signals that mediate hypertrophy and its long-lasting effects by combining genomics (spatial transcriptomics) and fluxomics (isotpe tracing) in vivo.

I have published my results in top-rank journals in the fields of Metabolism and Diabetes, including Cell Metabolism, Diabetes, Diabetologia, Molecular Metabolism or Endocrinology.

I have written 3 book chapters and I am co-inventor in 2 patents derived from our research.

Dr. Aida Freire Valls, Longwood Diagnostica – Nanostring, Barcelona, Spain.

Aida Freire Valls has a PhD in life sciences from Heidelberg University, where she specialized on flow cytometry and gene expression technologies that she applied to tumor microenvironment and immunology using mouse models studies. She is currently a Technical Sales Specialist at NanoString working with distributors in Europe, Middle East and Africa.

About the Speaker

Dr. Makoto Arita

Human Biology-Microbiome-Quantum Research Center (WPI-Bio2Q) at Keio University, Tokio, Japan

Makoto Arita received his Ph.D. from the Graduate School of Pharmaceutical Sciences, University of Tokyo in 1997. Currently he is a Professor of Pharmaceutical Sciences, and a Core Director of Human Biology-Microbiome-Quantum Research Center (WPI-Bio2Q) at Keio University, and a Team Leader of RIKEN Center for Integrative Medical Sciences. Dr. Arita has experience leading multidisciplinary research teams as a principal investigator for “Biology of LipoQuality” a program project supported by JSPS Grant-in-aid for Scientific Research on Innovative Areas (FY2015-2020). He successfully organized the 60th International Conference on the Bioscience of Lipids (ICBL2019, Tokyo) as a chair, and serves as an Executive Editor for the Progress in Lipid Research. Now he is leading JST-ERATO Lipidome Atlas Project (FY2021-2026) to pioneer the spatiotemporal biology of lipid diversity through a creation of the Lipidome Atlas, and to discover unknown molecules associated with important biological processes.

Dr. Edward A. Dennis

University of California at San Diego, La Jolla, CA, USA

Edward A. Dennis received his B.A. from Yale University in 1963 and his Ph.D. from Harvard University in 1968. Following the completion of his postdoctoral fellowship at Harvard Medical School, he was appointed (1970) assistant professor at the University of California, San Diego (UCSD). He became Professor in 1981 and then Distinguished Professor in 2004 in the Dept. of Chemistry and Biochemistry and Dept. of Pharmacology at the School of Medicine of UCSD.

He has since served as Chair of the Dept. of Chemistry and Biochemistry, Editor-in-Chief of the Journal of Lipid Research (2003–2018) and Director of the LIPID MAPS NIH Glue Grant Consortium (2003–2014).

He was the recipient of the American Society of Biochemistry and Molecular Biology's Avanti Award in Lipid Enzymology in 2000, the European Federation for Lipid Science and Technology's European Lipid Science Award in 2008, the American Chemical Society, San Diego Section, Distinguished Scientist Award in 2016, and the Bert Vallee Award in Biomedical Science from the American Society of Biochemistry and Molecular Biology (2020). Prof. Dennis' career research focus has been on the structure, function, mechanism, and inhibition of the enzyme phospholipase A2 as well as on signal transduction, inflammation, lipid metabolism, eicosanoid action, and lipidomics.

About the Speaker

Dr. Antonio Zorzano

University of Barcelona. Biomedical Research Institute Barcelona, CIBERDEM, Spain

Antonio Zorzano is a Full Professor of Biochemistry and Molecular Biology at the University of Barcelona, Director of Complex metabolic diseases and mitochondria laboratory at the IRB Barcelona, and Programme Head at CIBERDEM. Professor Zorzano received his PhD in Biology at the University of Barcelona, and did postdoctoral studies with Emilio Herrera (Hospital Ramon y Cajal, Madrid), Neil Ruderman (Boston University Medical Center), and Paul Pilch (Boston University Medical School). He has worked as Visiting Professor at Boston University Medical School. He has supervised 40 Ph.D. theses, and has coordinated international consortia funded by different European agencies. He is co-inventor of 22 patents, and has published over 360 scientific articles (more than 45,000 citations), with key discoveries published in leading journals, and an h-index of 96 (Google Scholar). He has been founder of biotechnological companies in Spain and in UK.

Professor Zorzano's research focuses on the regulation of metabolism and its interplay with insulin resistance, obesity, type 2 diabetes, and liver diseases. His current interest links metabolism with mitochondrial dynamics, autophagy, and mitochondrial stress. A global goal of his group is to identify and validate molecular targets that permit the prevention or treatment of metabolic diseases by using cell-based systems, genetically modified mice, and translational approaches.

Dr. Toni Petan

Jožef Stefan Institute in Ljubljana, Liubliana, Slovenia

Toni Petan received his PhD in Biochemistry and Molecular Biology from the University of Ljubljana, Slovenia, where he worked on phospholipases A2 and membrane enzymology. Following his postdoctoral studies in cancer cell and molecular biology at Harvard Medical School, he returned to Slovenia and formed a research group at the Jožef Stefan Institute in Ljubljana, combining his passion for lipids with cancer research. Currently, he serves as an associate professor at Jožef Stefan International Postgraduate School and leads an enthusiastic group of young scientists. His work primarily focuses on a fascinating and long-neglected organelle, the lipid droplet, particularly exploring its roles in fatty acid trafficking and signalling in response to cellular stress. Some of the research topics his group is currently investigating include: 1) the roles of lipid droplets in the production of inflammatory and mitogenic lipid mediators, 2) the interplay between lipid droplets and autophagy, and 3) the function of lipid droplets as managers of membrane homeostasis and modulators of ferroptosis.

About the Speaker

Dr. María Ángeles Balboa

Institute of Molecular Biology and Genetics - CSIC, Valladolid, Spain

María A. Balboa obtained her B.S. in Biology, M.S. in Biochemistry and Ph.D. in Immunology from the Autónoma University of Madrid, Spain in 1984, 1986 and 1992 respectively. To receive training in lipid signaling she moved to the U.S.A. where she worked as a Posdoctoral Fellow at the Dept. of Pharmacology in the University of California at San Diego under the supervision of Prof. Paul Insel. In 1995, she moved to the Dept. of Chemistry and Biochemistry of the same university to work under the supervision of Prof. Edward Dennis, a worldwide expert in phospholipases and lipids, and remained there for 5 years. She returned to Spain in 2000 and obtained a "Ramon y Cajal" research position at the Instituto de Biología y Genética Molecular (IBGM), Valladolid. In 2004 she obtained a permanent position as a tenured scientist of the Spanish National Research Council (CSIC), and was promoted to "Investigador Científico" in 2009. Her group has focused on how innate immune cells respond to stimuli by changing their lipid composition and how lipids influence immune responses. Within this area the group has revealed that lipid-modifying enzymes that participate in the de novo lipid synthesis are important for innate immune cell activation and, as a consequence, they influence inflammation-based diseases.

One of her groups' main achievements in this field is that lipin-2 is a break for the activation of the inflammasome NLRP3 and IL-1β production. The work is very relevant because human mutations in the gene that encodes for LPIN2 produces an autoinflammatory disease known as the Majeed Syndrome. Based on their results this syndrome has been classified as a NLRP3 inflammosomopathy and new possible treatments would be available for patients.

Her group has obtained continuous funding from regional, national and private bodies (Junta de Castilla y Leon, Spanish government, among others). She is currently a member of the "Sociedad Española de Bioquímica y Biología Molecular", and a member of the CIBERDEM (CIBER for the study of Diabetes and Metabolic diseases, Valladolid node). She collaborates in evaluation activities for the "Agencia Estatal de Investigación", Biomedicine Section, as "Gestora" (since April 2022). As part of her academic activities she also participates in the Master of Biomedical Investigation, University of Valladolid since 2012.

About the Speaker

Dr. Makoto Murakami

University of Tokyo Graduate School of Medicine,
Tokyo, Japan

I am a biochemist with a long-standing interest in phospholipase A2s (PLA2s) and lipid biology. My interest and commitment to the analysis of PLA2 and lipid mediators has its roots about 35 years ago, when I was a graduate student at the University of Tokyo, with the discovery of elevated levels of sPLA2-IIA in inflammation. In 1994-1995, I worked as a postdoctoral fellow in the F. Austen's lab at Harvard University in Boston and characterized the whole view of eicosanoid biosynthesis in mast cells. In 1995-2004, a period when a number of PLA2 enzymes were identified, I examined their basic properties in cultured cells as an associate professor at Showa University, Tokyo. After 2005, I shifted my main research from in vitro to in vivo analyses of PLA2s using knockout/transgenic mice as a principal investigator at the Tokyo Metropolitan Institute of Medical Science. In 2017, I moved to the present position as a professor at the University of Tokyo Graduate School of Medicine. Now, I am one of the leading scientists in the PLA2 and lipid research field, and my present research interests involve the roles of various PLA2s in allergy, immunity, cardiovascular diseases, metabolic syndrome, cancer, reproduction, and skin homeostasis, with a catchphrase of "the research of QOL (Quality of Lipids) for QOL (Quality of Life)".

I have given 53 invited lectures in various international conferences and organized the 6th International Conference on Phospholipase A2 and Lipid Mediators in 2015 (chair) and the 60th International Conference on the Bioscience of Lipids in 2019 (co-chair). I have been awarded the Young Investigator Award for the Pharmaceutical Society of Japan in 1999, Young Investigator Award for the Japanese Society of Inflammation and Regeneration in 2000, Investigator Award for the Tokyo Metropolitan Institute of Medical Science in 2008, Investigator Award for the Terumo Science Foundation in 2014, Investigator Award for the Bureau of Social Welfare and Public Health, Tokyo Metropolitan Government in 2015, Eicosanoid Research Foundation's Outstanding Achievement Award in 2022, and MEXT Award for Science and Technology in 2023. I am a committee member of the Japanese Biochemistry Society, Japanese Lipid Biochemistry Society, and Japanese Society of Inflammation and Regeneration.

About the Speaker

Dr. Santiago Vázquez

Faculty of Pharmacy and Food Science, University of Barcelona, Institute of Biomedicine of the UB, Barcelona, Spain

I am a Full Professor in Organic and Medicinal Chemistry at the Faculty of Pharmacy and Food Science, University of Barcelona (UB) and a member of the Institute of Biomedicine of the UB (IBUB). I have over 25 years' experience working on organic and medicinal chemistry, with focus on the synthesis of new compounds with biological activity. After my PhD in the UB (1996, PhD Extraordinary Prize of the UB), I spent two years at the University College London as a Marie Curie Research Fellow (supervisor: William B. Motherwell). Back to Barcelona in 2000 as Assistant Professor, I obtained a "Ramón y Cajal contract" in 2001. Later, I was promoted to Associate Professor in 2005 and to Full Professor in 2020. During 2016 I was a "Salvador de Madariaga" grant holder at The Institute of Cancer Research (UK).

After working several years in pure organic chemistry -novel synthetic methodology, chemistry of polycyclic hydrocarbons-, more recently my research has focused on the synthesis of novel channel blockers -human NMDA and P2X7, M2 channel of the influenza A virus- and novel enzyme inhibitors, including 11β-HSD1 inhibitors and soluble epoxide hydrolase (sEH) inhibitors. I have established successful collaborations with several biological groups in Europe (e. g., Prof. Lieve Naesens, KU Leuven; Prof. Scott P. Webster, University of Edinburgh) and the USA (e. g., Prof. William F. DeGrado, UCSF; Prof. Lawrence H. Pinto, Northwestern University; Prof. Bruce D. Hammock, UCD; Prof. Jon W. Johnson, University of Pittsburgh). I have published more than 100 research articles, half of them in highly reputed journals (Nature Communications, Angew Chem Int Ed, J Am Chem Soc, J Med Chem, Antiviral Res, Eur J Med Chem).

So far, I have supervised 18 PhD Thesis (+ 2 ongoing), and several MSc Research projects. I have participated as co-I in 16 research projects and as PI in 8 research projects from public funding agencies. I have been the PI in 10 research contracts with chemical and pharmaceutical companies (e.g., Amino Chemicals, Boehringer Ingelheim, Ern, Menadiona, Salvat). I am co-inventor in 12 patents related with new chemical entities with biological activity and in one patent for a new process for the manufacturing of opioids. I have successfully applied to several valorisation programs for transferring knowledge from the University to the private sector. Indeed, 4 patents have been transferred to the private sector and one is already in exploitation.

About the Speaker

Dr. Jaume C. Morales, Agilent, Barcelona, Spain

Agilent

Since 1991, 20 years in technical service of Hewlett Packard and Agilent Technologies. The functions within the support group were initially to start and organize the whole subject of courses to customers as well as to teach them. Once launched, my main dedication was to provide technical support to customers in the entire range of products of the LPA (Liquid Phase Analysis) GPA (Gas Phase Analysis) and Mass Spectrometry line, which includes instrumentation mentioned above. Providing support from installation, deployment, verification and repair to training in specific and customer-specific applications. As for Capillary Electrophoresis and UV-VIS I was part of the NTP (New Technology Products) group, which means a small number of engineers who give international support. During the year 95 I was part of a European project (internal division) that consisted of the unification of the material used to teach the software courses of the Analytics applications. Personally, I dedicated myself to developing the course material of one of these applications for all of Europe. Since 2000 I have specialized more thoroughly in LC/MSD and CE/MSD techniques in both Quadrupole, Trap and TOF/QTOF & IMS. As well as in the associated techniques of NanoLC. At the same time I specialized in the field of Biotechnology and more specifically in Proteomics and Metabolomics, giving support at national and European level to customers in installation, tuning and repair of our solutions in this area of applications. - Dedication of 50% of my time as an advisor to field engineers at European level supporting them as an interface between them and the Support product manager. on LC/MSD Trap and LC/MSD TOF/QTOF products. European support for Metabolomics and Proteomics applications based on high resolution LCMS.

Since 2011 MS Product Specialist for Spain and Portugal as Business Manager of LCMS with responsibility for sales results, marketing and training to the sales team. Specialization on workflows and applications development for Life Science (Pharma, Clinical research, Toxicology, Metabolomics and Lipidomics) as well as for Applied Markets (Food, Environment, Forensics, Chemical & Energy). Customer support and consultancy for best solution for their needs concerning Agilent Portfolio.

About the Speaker

Daniele Piomelli, PhD, MD (hon)

Daniele Piomelli studied pharmacology and neuroscience with James H. Schwartz and Eric Kandel at Columbia University (1983-1988), and with Paul Greengard at the Rockefeller University (1988-1990). In 2000, two of his mentors (Kandel and Greengard) were awarded the Nobel Prize for their contributions to physiology and medicine. After working at the INSERM in Paris (France) and at the Neurosciences Institute in San Diego, with Nobel Laureate Gerald Edelman, Daniele joined the University of California, Irvine, where he is now Louise Turner Arnold Chair in Neurosciences and Distinguished Professor of Anatomy and Neurobiology, Pharmacology and Biological Chemistry. Daniele is an author of >420 peer-reviewed articles in journals such as Nature, Science, Nature Medicine, PNAS and Nature Neuroscience, three full-length books, and >35 patents. He founded the department of drug discovery and development (D3) at the Italian Institute of Technology in Genoa (Italy), which he directed from 2007 to 2016, and three biopharmaceutical start-ups based on discoveries made in his lab. Since 2018, Daniele serves as Editor-in-Chief of Cannabis and Cannabinoid Research, the only peer-reviewed journal entirely dedicated to the study of cannabis, its derivatives, and their endogenous counterparts in the human body. He directs the NIDA Center of Excellence ICAL (Impact of Cannabinoids Across the Lifespan) and UCI's Center for the Study of Cannabis.

About the Speaker

Dr. Ino Agrafioti

European Research Council Executive Agency (ERCEA) of the European Commission, Brussels, Belgium.

Dr. Ino Agrafioti is a biologist (BA Biological Sciences, University of Oxford; MSc in Bioinformatics, Imperial College London; PhD Theoretical Genomics, Imperial College London; MSc Public Policy and Management, Athens University of Economics and Business) who after a long journey through interdisciplinarity decided that she prefers science policy instead. Since 2015, she is the Coordinator of the Panel LS1 Molecules of Life: Biological Mechanisms, Structures and Functions, which funds projects in the disciplines of Molecular Biology, Biochemistry, Structural Biology, Molecular Biophysics, Chemical Biology and Synthetic Biology, at the European Research Council Executive Agency (ERCEA) of the European Commission.

About the Speaker

Dr. Thorsten Horneman

Institute for Clinical Chemistry
University Hospital Zürich, Switzerland

I am a professor of Lipidology and head of the lipidomics research facility at the Institute for Clinical Chemistry, University Zurich. My and my labs primary interest is the world of sphingolipids and in particular human diseases associated with pathological changes in SL metabolism. Here we pioneered by identifying several new lipid metabolizing enzyme and metabolic pathways. We also discovered a class of atypical and neurotoxic 1-deoxySphinglipids (1-deoxySL) as pathogenic metabolites. 1-DeoxySL cause the inherited peripheral neuropathy HSAN1 and are emerging factors to be involved in other diseases such as the diabetes, the diabetic neuropathy and cancer.

My lab has long-standing experiences in LC-HRMS-based lipidomics (targeted and untargeted) and metabolic studies. We developed several distinctive analytical methods and metabolic labelling techniques to investigate the structure, function and metabolism of sphingolipids with a particular focus on rare and atypical sphingolipid metabolites. In this context, we also established several lipid metabolites and lipid based metabolic signatures as diagnostic and prospective biomarkers for cardio-metabolic diseases including NAFLD, CVD, MetS and T2DM.

Dr. Gerhard Liebisch

University Hospital of Regensburg, Germany

Gerhard Liebisch obtained his PhD at the University of Regensburg. He is a research associate at the Institute of Clinical Chemistry and Laboratory Medicine at the University Hospital of Regensburg and responsible for the instrumental analytics lab (https://lipidomics-regensburg.de/).

His research interests focus on the development of mass spectrometric methods for quantification of lipid species. For more than 20 years, these methods have been applied in large-scale clinical studies and basic research including the use of stable isotope labelled lipid(s)/-precursor to trace the transport and metabolism of lipids. He is a co-author of more than 250 papers in peer reviewed international journals, editorial board member of The Journal of Lipid Research.

He is a co-founder of the Lipidomics Standards Initiative and a board member in the International Lipidomics Society.

Professional Experience:
2020 – Present Head of Research (Inst. for Clinical Chemistry, University Zürich)
2015 – Present Professor for Clinical Chemistry and Biochemistry (University Zürich)
2002 – 2015 Senior Scientist. Group leader Research “Sphingolipid Metabolism and Lipidomics”. Institute for Clinical Chemistry, University Hospital Zürich
2006 – Present Clinical Chemist (FAMH). Administrative, diagnostic and consulting tasks in the diagnostic service facility of the Institute for Clinical Chemistry, University Hospital Zürich. Coordination of Laboratory ICT projects
2001 – 2002 Research Scientist (Postdoc). Institute for Cell Biology, Group Eppenberger/Walliman. Topic: Cellular energy metabolism
2000 – 2001 Research Scientist (Postdoc). University of Potsdam, Germany. Topic: Creatine Kinase and Muscle Energy Metabolism

Dr. Cristina Legido-Quigley

Steno Diabetes Center Copenhagen and an Associate Professor at King’s College London, United Kingdom

Dr. Cristina Legido-Quigley is the Head of Systems Medicine at Steno Diabetes Center Copenhagen and an Associate Professor at King’s College London.

Her main area of interest is neurometabolism and how the brain copes with disease, as well as finding clinical tests for healthy aging, Alzheimer's, cognition, diabetes and metabolic diseases. Her discoveries span fatty molecules that are important for cognition, small molecules that in liver alert to tissue damage, together with modulating molecular pathways for improving the treatment of diabetes. She is also researching algorithms for better personalised diagnoses in the clinic.
She has been a group leader at King's College London since 2006. In 2018 she moved to Steno a hospital and research center in Denmark to be the Head of Systems Medicine. She shares her findings in scientific publications in the biotechnology and medical field.

Dr. Rosario Domingues

University of Aveiro, Portugal

Rosário Domingues graduated in Pharmaceutical Sciences, University of Coimbra (1990), received her Ph.D. degree in Chemistry (1998), and Habilitation in Biochemistry (2014) at University of Aveiro (UA). Since 2016, she held the contract of Associated Professor with habilitations in the Mass Spectrometry Centre, Dept. of Chemistry, University of Aveiro (UA). She is the head of the Lipidomic laboratory at UA,) and is the Director of the Doctoral Program in Biochemistry at UA. She has over 25 years of research experience in the field of mass spectrometry, and is a well-established researcher in the field of as Lipidomics, namely in Lipidomics in Heath and Disease, Oxidative Lipidomic, Marine lipidomics, in Glycomics and in the study of the modifications in biomolecules ten book chapters, 372 articles published in international journals with referee. She coordinated and participated in several research projects funded by national and European programs (27 in total). In the present, she is the coordinator of the European project Cost Action CA19105 Pan-European Network in Lipidomics and EpiLipidomics.

About the Speaker

Dr. Dolores Pérez-Sala

Margarita Salas Center for Biomedical Research - CSIC, Mardid, Spain

Dr. Dolores Pérez-Sala obtained her MD from Universidad de Extremadura and her PhD from Universidad Complutense de Madrid, Spain. During her PhD she carried out stays at University of Pennsylvania (USA) and Sussex University (UK). She was a postdoctoral fellow at Harvard University Medical School (Boston, USA), where she worked on the isoprenylation and methylation of retinal G proteins. On her return to Spain, she joined the Centro de Investigaciones Biológicas, (CIB-CSIC), where she worked as a postdoctoral fellow in several research lines including the role of isoprenylation and methylation in cancer and vascular biology and the mechanisms of regulation of gene expression by inflammatory mediators, such as nitric oxide and cyclopentenone prostaglandins. From these lines she developed an interest for the modification of proteins by reactive species, and particularly for the modification of cysteine residues as a regulatory mechanism. At present, she leads the Group of Posttranslational Modification of Proteins (http://cib.csic.es/research/structural-and-chemical biology/posttranslational-modification-proteins). This group has identified the first targets for modification by cyclopentenone prostaglandins and explored the interplay between these modifications and enzymatic modifications of cysteine residues in proteins of the Ras superfamily, among others. This line has led to the study of lipoxidation of several protein targets and more recently of cytoskeletal proteins of the intermediate filament family, unveiling the role of these structures as redox sensors. She is the author of over 130 publications.

Dr. Corinne Spickett

Aston University, Birmingham, United Kingdom

Corinne Spickett is currently a Professor at Aston University, following a move from the University of Strathclyde in January 2011. Her first degree was in biochemistry at Oxford University and she has a D.Phil. (Oxon) on the application of NMR to study yeast bioenergetics in vivo. After further postdoctoral work using NMR to investigate stress responses in plants and glutathione metabolism in pre-eclamptic toxaemia, she became a Glaxo-Jack Research Lecturer in the Dept. of Immunology at the University of Strathclyde. Since then, she has been working on the analysis of phospholipid oxidation by electrospray mass spectrometry and the biological effects of oxidized lipids, especially as relating to atherosclerosis and inflammation, and has published extensively in this field. She has also applied her expertise in analysis of phospholipids to lipidomic studies of LDL in chronic kidney disease and yeast membrane changes. More recently, she expanded her research to include lipidomics as well as ox-lipidomics, analysis of protein oxidation and formation of lipoxidation products during inflammation, and has been developing label-free, semi-targeted approaches to their identification in biological samples.

Prof Spickett was Treasurer of the Society for Free Radical Research Europe from 2007-16, and then on SFRR-E Council. She is a member of the Steering Committee of the International HNE-Club. She was a Workgroup Leader in the COST Actions B35 on Lipid Peroxidation Associated Disorders and CM1001 on Chemistry of non-enzymatic protein modification, and is now responsible for communication and dissemination in CA19105 EpiLipidNET. She was the Coordinator of the H-2020 Innovative Training Network “MASTRPLAN” on MASS spectrometry TRaining network for Protein Lipid adduct Analysis, and is currently a group Leader in the Innovative Training Network project MemTRain. At Aston University she is Director of Research for the Biosciences Research Group.

Dr. Jesús Mª Bañales

Biodonostia Health Research Institute, Donostia, Spain
University Hospital in San Sebastián (Spain). University of Navarra (Pamplona). Mayo Clinic (Rochester, USA) and the Universidad Área Andina (Bogotá, Colombia)

Dr. Jesús Bañales is an Ikerbasque Research Professor and Head of the Liver Diseases Group at the Biodonostia Health Research Institute - Donostia University Hospital in San Sebastián (Spain). He is also Full Professor of Biochemistry at the University of Navarra (Pamplona), as well as Assistant Professor of Medicine/Sciences at the Mayo Clinic (Rochester, USA) and at the Universidad Área Andina (Bogotá, Colombia).

His research group on Liver Diseases consists of 25 multidisciplinary researchers focused on studying the molecular mechanisms involved in liver pathophysiology and the search for new diagnostic and therapeutic strategies.

Prof. Bañales has authored more than 200 scientific publications in high-impact journals and has led over 50 national and international competitive research projects. He is a member of the Editorial Board of scientific journals such as Hepatology, Journal of Hepatology (Associate Editor), and Nature Reviews Gastroenterology & Hepatology. He has been recognized with prestigious international research awards, including the EASL Emerging Leader Award 2018, UEG Rising Star Award 2018, and "SER Navarra Researcher Award" 2020.

Dr. Giovanni D'Angelo

Swiss Federal Institute of Technology,
Lausanne, Switzerland

Giovanni D'Angelo obtained his PhD in Cell Biology from the Consorzio "Mario Negri" SUD, Santa Maria Imbaro, Italy. After post-doctoral training at the Telethon Institute for Genetics and Medicine, Italy he moved to the Institute of Protein Biochemistry, at the National Research Council of Italy as a principal investigator. Since 2018, Giovanni is an Assistant Professor and Kristian Gerhard Jebsen Chair on Metabolism at EPFL. His main interest is understanding the meaning of compositional variability in cell membranes by studying the mechanisms by which the lipid composition is determined.

Dr. Matt Vander Heiden

Director of the Koch Institute at MIT, Cambridge, MA
Member of MIT Center for Precision Cancer Medicine, Ludwig Center at MIT
Member, Broad Institute of Harvard and MIT
USA

Matthew Vander Heiden is the director of the Koch Institute at MIT, the Lester Wolfe (1919) Professor of Molecular Biology, and a member of the Broad Institute. He is a practicing oncologist and instructor in medicine at Dana-Farber Cancer Institute/Harvard Medical School. He earned his doctoral and medical degrees from the University of Chicago, where he worked in the laboratory of Craig Thompson. Vander Heiden then completed a residency in internal medicine at Boston's Brigham & Women's Hospital and a hematology-oncology fellowship at Dana-Farber Cancer Institute/Massachusetts General Hospital. He was a postdoctoral fellow in the laboratory of Lewis Cantley at Harvard Medical School, where he was supported by a Mel Karmazin Fellowship from the Damon Runyon Cancer Research Foundation. In 2010, Vander Heiden joined the MIT faculty. His work has been recognized by many awards including the Burroughs Wellcome Fund Career Award for Medical Sciences, the AACR Gertrude B. Elion Award, the HHMI Faculty Scholar Award, and an NCI Outstanding Investigator Award. Vander Heiden serves on the scientific advisory board of Yale Cancer Center, Agios Pharmaceuticals, Aeglea Biotherapeutics, iTeos Therapeutics, Evelo Therapeutics, CyteGen, and Auron Therapeutics, of which he is also an academic founder. He is part of the investment advisory board for DROIA Venture Fund.

Lipid Imaging Mass Spectrometry: Challenges, Limitations and Achievements

José A. Fernández, University of the Basque Country, Leioa, Spain

Lipid imaging mass spectrometry (LIMS) is experiencing a fast increase in popularity, due to its ability to map lipid distribution in tissues without previous labelling. Lipids constitute an important portion of the metabolome and its deep knowledge is required to establish a connection between genotype and phenotype. A cell's lipidome is strictly regulated and changes with its metabolic stage or with the appearance of a pathology. Therefore, LIMS constitutes a kind of new histology that enables visualizing a tissue section from its molecular composition. Its characteristics make the technique suitable to make an impact in the diagnostic units of the hospitals. However, so far, the jump into clinics has not taken place.

In this seminar, I will give a personal vision of the state-of-the-art of the technique, what it has accomplished, what the main challenges are in order for LIMS to become a really useful technique in medicine and which are its limitations.

Integrated morphometric and molecular classification of central nervous system cancers using a unified platform with picosecond infrared laser mass spectrometry

Arash Zarrine-Afsar, University of Toronto, Toronto, Canada

Introduction. Capitalizing on the coupling between lipid metabolism and cancer formation, untargeted mass spectrometry profiling of tissue lipidome with ambient ionization methods has constituted an attractive strategy for rapid determination of cancer types through comparing MS1 profile of a query specimen to that of a validated library of known cancer metabolic lipid fingerprints. One such method is Picosecond InfraRed Laser Mass Spectrometry (PIRL-MS) that has been successful in discriminating molecular subgroups of medulloblastoma cancers in 10 seconds with minimal tissue consumption (< 1 mm3) and no thermal damage outside the sampling zone.
Methods. Local tissue banks containing ~3,000 frozen CNS specimens over 20 different classes of adult and pediatric cancers were subjected PIRL-MS and building a comprehensive molecular signature library. 10-second MS1 PIRL-MS spectra were collected on a Waters Xevo G2 XS quadrupole Time-of-Flight mass spectrometer. Multivariate modeling of the MS1spectra using both supervised dimensionality reduction and unsupervised clustering methods were conducted to validate the model performance through cross validation, and subsequently with blind sample predictions towards the determination of the sensitivity and specificity values for cancer type classification, and to inform mass-to-charge (m/z) values most important for such differentiation. The molecular identities of these m/z values were determined through targeted high-resolution tandem mass spectrometry and chromatography on a Synapt qTOF (Waters).
Results. We have investigated 150 pediatric brain cancers (3 morphometrically distinct types and 7 molecularly different classes) and close to 700 adult brain cancers (16 morphometrically distinct classes and 4 molecularly distinct types). The sensitivity and specificity of the integrated morphometric and molecular diagnosis for pediatric brain cancers with 10-second PIRL-MS was > 94%. This classification could use as little as only 18 tissue lipids whose identities were determined with targeted chromatography and tandem mass spectrometry. The classification of the adult brain cancers with PIRL-MS is currently ongoing but preliminary data suggests > 84% sensitivity and specificity across 16 morphometric classes and select molecular types. Novel Aspect. A single PIRL-MS platform may fulfil the function of sequencing and immunostaining instruments for CNS cancer classification using downstream correlated phenomic (metabolomic) changes associated with both morphometrically and molecularly distinct cancer types. This analysis takes a few seconds of total collection and analysis time and does not need specimen preparation. Conflict of Interest Disclosure: Consultant and founder, inventor of licensed patent to Point Surgical Inc. with financial interest.

Expanding the horizons of in-depth 4D-Lipidomics™ in MSI by SpatialOMx®

Björn Wendik, Bruker Daltonics, Frieburg, Germany

Lipids are a diverse group of compounds with many crucial and varied biological functions and are known to change in response to stress and disease. Therefore, lipids are an interesting class of compounds for biomedical research. The complexity of lipid samples makes their analysis directly from tissue sections extremely challenging.

MALDI Mass Spectrometry Imaging (MALDI-MSI) has emerged as a powerful tool for the in-situ examination of lipids. Here we present the advantages of the timsTOF Flex and ScimaX technologies for MALDI-MSI measurements of lipids.

The integration of a native ESI/MALDI dual ion source for seamless switching between ionization modalities within seconds enabling powerful SpatialOMx® applications. SpatialOMx is a powerful tool that enables researchers to gain a more comprehensive understanding of the biomolecules expressed in e.g., cancer tissues and their relationship to tissue pathology. By integrating MALDI Imaging with classical lipid LC-MS/MS PASEF workflows, it is possible to identify biomolecules based on 4D-omics data and spatially locate specific biomolecules within a tissue section, providing a deeper understanding of the tissue microenvironment and its role in e.g., cancer progression.

Bruker technologies as timsTOf Flex, with the additional annotation criteria from CCS values, MALDI 2 to boost the sensitivity of lipids and small molecules, and the extreme resolution power of the MRMS technology, offers excellent tools to develop the spatialOMX concept, and reveal the relevant biological information in the image, while the acquisition process is standardized.

MS-Imaging in basic and clinical research at the hospital: from the bench to the patient

Eduardo Chicano-Galvez, Maimonides Biomedical Research Institute of Cordoba, Spain

In recent years, MS-Imaging has undergone a remarkable transformation, transitioning from a mere "analytical curiosity" for tissue molecular mapping to a substantial and tangible reality in today's scientific landscape. This evolution has solidified its position as a well-established field, showcasing its ability as an immensely valuable tool across both clinical research and basic scientific inquiry.

This dynamic technique has evolved beyond its initial applications to encompass a comprehensive range of capabilities. Not only does it empower researchers to explore the molecular intricacies of biological samples, but it also could serve as a bridge between laboratory discoveries and direct patient impact. This transformative shift highlights how MS-Imaging has matured into a potent force that could solve the divide between laboratory research and practical clinical applications.

Moreover, due to its versatility, MS-Imaging is no longer limited to single-omic analysis. It has expanded its capabilities to encompass the integration of various omics approaches. By combining different sample levels such as genomics, proteomics, metabolomics, lipidomics, and even optical microscopy, this approach provides an outstanding opportunity to unravel the intricate molecular underpinnings of health and disease.

During this presentation, we will delve into specific examples that highlight the versatility of MS-Imaging. On one hand, we will see its application in basic research, where it has enabled scientists to unravel intricate proteomic landscapes, providing insights into the intricate workings of biological models. On the other hand, we will focus on clinical research attempts that aim to fully harness the potential of MS-Imaging in clinical settings. These efforts are motivated by the aspiration to transform scientific discoveries into tangible solutions for diagnostics and patient care.

Lipidomic Changes in Aging Skin studied by MS Imaging

Martina Marchetti-Deschmann, Institute of Chemical Technologies and Analytics – TU-Wien, Vienna, Austria

Our skin is constantly exposed to solar radiation, high oxygen levels, and environmental pollutants. These are accelerant stress factors for premature skin aging, tissue inflammation, and photocarcinogenesis. Such oxidative stress activates cutaneous lipoxygenases and nonenzymatic lipid peroxidation. Oxidized lipids (oxLip) can act as danger-associated molecular patterns (DAMPs) and as members of the senescence-associated secretory phenotype (SASP), the signaling cocktail of senescent cells. Our study aims to target bioactive oxidized phospholipids of the SASP and expand the list of possible candidates. We present a systematic investigation of lipids (Lips) and their chemically-driven oxidation products (oxLipS) generated in a controlled environment by reactive oxygen species (ROS) after UV exposure. Results are correlated to results from skin-equivalents investigated by mass spectrometric Imaging (MSI).

We investigate and characterize oxLipS generated via chemical pathways by high-resolution mass spectrometry (HRMS). Oxidized products of PAPC (oxPAPC) result from reactions with peroxides, from hydroxylation, consecutive fatty acid chain cleavage or loss of arachidonic moiety, to name a few. DNPC and DPPC instead, cannot generate oxLipS, thus serving as internal standard for semi-quantitative information. Electrospray (ESI) HRMS provided crucial information on oxLipS generated by ROS formed in solution after UV irradiation. Accurate, MS data allowed for the identification of newly formed species. As pre-requisit for MSI, Lipids were also investigated by matrix-assisted laser desorption/ionization (MALDI) HRMS to identify ionization biases.

Findings were translated to skin-equivalent samples by firstly doping a collagen matrix with LipS and oxLipS of known composition and concentration. Qualitative, semi-quantitative, and spatial information about oxLipS in the skin equivalents was generated by MALDI MSI. Furthermore, fibroblasts were embedded in the collagen matrix and also exposed to UV light to assess the effect of cells on lipid oxidation. Results from the skin models were also compared to oxlip distributions in skin biopsies from young and old donors.

We acknowledge financial support of the Federal Ministry Republic of Austria for Education, Science and Research and TU Wien (HRSM 2016), and the Christian Doppler Research Association for funding SKINMAGINE. This work was partially supported by COMULIS (CA17121) and EpiLipiNet (CA 19105).

Molecular Intratumor Heterogeneity Assessed by Mass Spectrometry Imaging Has Prognostic Value in Primary Breast and Oral Cancers

Piotr Widłak, University of Gdańsk, Gdańsk, Poland

Intra-tumor heterogeneity (ITH) results from the coexistence of genetically distinct cancer cell (sub)populations, their phenotypic plasticity, and the presence of heterotypic components of the tumor microenvironment. Here we addressed the potential association between phenotypic ITH revealed by mass spectrometry imaging (MSI) and the prognosis of breast cancer and oral cancer. Tissue specimens resected from 59 patients with locally advanced HER2-positive invasive ductal carcinoma (BC) and 77 patients with locally advanced oral squamous cell carcinoma (OC) were included in the study. A 5-year (BC) and 3-year (OC) follow-up was available for all patients which enabled their separation into two groups: with no evidence of disease (NED) and with progressive disease (PD). After on-tissue trypsin digestion tissue specimens were analyzed by MALDI-MSI and peptide maps of all cancer regions were segmented using an unsupervised approach to reveal their intrinsic heterogeneity. We found that in both cancer types intra-tumor similarity of spectra was higher in the PD groups and the diversity of clusters identified during image segmentation was higher in the NED groups, which indicated a higher level of ITH in patients with more favorable outcomes. Signature of molecular components detected by MSI (the hypothetical identity of imaged tryptic peptides) that correlated with long-term outcomes could be associated with proteins involved in immune functions. Furthermore, a positive correlation between the level of tumor-infiltrating lymphocytes (or lymphocytic host response) and heterogeneity revealed by MSI was observed in both tumor types. We proposed that a higher level of ITH revealed by MSI in cancers with a better prognosis could reflect the presence of heterotypic components of the tumor microenvironment such as infiltrating immune cells enhancing the response to the treatment.

Insights into Enzyme Histochemistry by MALDI MSI

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Aim:
Enzymatic reactions are commonly detected by reacting a substrate and enzyme, using the reaction products in a subsequent reaction to produce a color response, then measuring the absorbance. Existing methods of detecting enzymatic reactions require both the primary reaction between the substrate and enzyme and the secondary reaction for a color response.
This study investigated mass spectrometry imaging as technique to detect the products of enzymatic reactions directly in mouse brain sections and whole Drosophila sections.

Methods:
Acetylcholine was applied to the surface of each tissue sample and the degradation product choline was detected. Choline produced by this reaction was differentiated from endogenous choline by using deuterium-labeled acetylcholine-d9 as the substrate. The substrate was sprayed followed by two-step vapor deposition method for CHCA matrix application. AP-MALDI-MSI measurements were performed using an iMScope imaging mass microscope (Shimadzu) at a spatial resolution of 5 µm or 10 µm, respectively. Data were analyzed using IMAGEREVEAL MS software.

Results and discussion:
Deuterium-labeled acetylcholine-d9 and choline-d9 were detected and used for calculation of cholinesterase activity. The images of mouse brain sections revealed high AChE activity in the corpus striatum, hippocampus and hypothalamus, and low AChE activity in the corpus callosum and cerebellar cortex. In case of a Drosophila section, ChE activity was spread unevenly, with high ChE activity in the cerebrum and thoracoabdominal region.

Conclusion:
A new MSI-based enzyme histochemistry method investigate enzyme activity in different areas of tissues without using color-developing reactions.

Childhood obesity: Cartography of adipose tissue in space and time. Spatial transcriptomics map of human pediàtric adipose tissue. Understanding hypertrophic adiposity in children through spatial transcriptomics

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Obesity is characterized by the accumulation of fat mass and is often associated with adipose tissue dysfunction. It is especially worrying in children because obesity-associated co-morbidities, such as type 2 diabetes, or cardiovascular disease, appear earlier in life and show stronger severity, which together reduce life expectancy. In obese children the expansion of the white adipose tissue (WAT) is mostly accomplished through hypertrophy (increase in adipocyte size). In turn, hypertrophic adiposity is strongly associated with adipose tissue inflammation, insulin resistance, and diabetes.

Our aim is to assess the (potential) signals associated to hypertrophy during the development of childhood obesity.

Specifically, we collected subcutaneous WAT biopsies from children with obesity and their matched lean controls (N=8; 4 Control + 4 OB; ages 4-12). Children with obesity exhibited hyperglycemia, moderate hyperinsulinemia, and high circulating TAG and cholesterol content. Likewise, the WAT from obese children was already hypertrophic. We embedded the samples onto the 10X Genomics slide system, generated the cDNA, and prepared the libraries for sequencing. Ongoing-preliminary results will be discussed, including the methodological specific modifications required for working with white adipose tissue samples.

Multi-omic Spatial Profiling at Single-Cell Resolution to Unravel Complex Biology

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Victoria Menéndez García, MD Anderson Cancer Center, Madrid Spain

In this seminar you will be introduced to how our platforms allow researchers to combine whole tissue imaging, gene expression, and protein data. Achieve multicellular analysis with GeoMx® Digital Spatial Profiler, or zoom in to view single cells with the CosMx™ Spatial Molecular Imager (SMI). Join us to see into the private lives of these cells in situ, at both cellular and subcellular levels supported by AtoMx™ Spatial Informatics Platform (SIP). Gain spatial biology insights anytime, anywhere using AtoMx™, a cloud-basedsolution that provides advanced data analytics and global collaboration capabilities.

Introduction of the JST-ERATO Lipidome Atlas Project in Japan

Makoto Arita, Keio University, Tokyo, Japan

Abnormal lipid metabolism is often a background factor of diseases, which may lead to the discovery of new seeds for drug discovery and medical applications such as early diagnosis and treatment. Recent advances in mass spectrometry have provided a major impact on lipid biology, suggesting that the lipid molecules analyzed in the past are only the tip of the iceberg. We established a LC-TOF/MS-based untargeted lipidomics platform equipped with MS-DIAL4 software that enables us to identify the structural diversity of >8,000 lipids in human and mouse tissues, cells, and commensal bacteria. Also, the Lipidome Atlas Project is ongoing by utilizing the timsTOF fleX MALDI-2-based imaging mass spectrometry to visualize the local environment created by specific lipids on the dynamics and functions of multicellular systems. We aim to elucidate the mechanisms that create, regulate, and recognize lipid diversity and its localization in vivo, as well as to elucidate diseases caused by the disruption of such mechanisms.

PPAR gamma role in the renal tubule-specific lipid metabolism in fibrosis context

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Introduction: Unresolved inflammation of the tubule-interstitium with ensuing tubulo-interstitial fibrosis plays a key role in the progression of chronic kidney disease. However, the links between metabolic pathways of kidney tubular epithelial cells (TEC) and the sustained inflammatory milieu that promotes fibrosis have not been studied in detail. Previous data showed a powerful cytoprotective effect of peroxisome proliferator-activated receptor-g (PPARg) agonism not only in glomerular epithelial cells in experimental RPGN but also on tubule-interstitial fibrosis. PPARs compose possibly the best-recognized sensor system for fatty acids and we hypothesize that altered PPAR-coordinated-TEC metabolism participates to sustain the inflammatory milieu in renal physiology and diseases.
Materials and methods: Here we present a specific tubule-dependent lipidomic analysis by MALDI imaging mass spectrometry of kidney sections belonging to four groups: control mice, TEC selective PPARg-deficiency KO mice, fibrotic control mice and fibrotic KO mice. Fibrosis was induced by retroorbital injection of nephrotoxic serum during three consecutive days. 21 days after the injection, mice were euthanized and snapped frozen kidney sections of 15 µm thickness were prepared in a cryostat. MALDI-IMS analyses were performed at 10 µm of spatial resolution using a MALDI-LTQ-Orbitrap XL. Data were acquired with mass resolution of 60000 at m/z = 400, in scanning range of 550-1000 for negative-ion mode. Lipid assignment was based upon comparison between the experimental m/z and the species in the software's LIPID MAPS database. Prisim8 and Orange-Biolab programs were used to conduct statistical analysis. After MALDI-IMS, IF staining of the different tubules was performed over the same tissue section in order to correlate each structure with its lipid fingerprint. Additionally, different enzymes involved in the lipid metabolism were assessed by PCR in renal tissue of the four groups.
Results and discussion: Altogether, 16 lipid families were identified by MALDI-IMS accounting for 190 different lipid species. Differences in renal structures were highlighted using ANOVA analysis and some classification models whose classification accuracy were around 90%. The most striking observation was the significant decrease in PEs relative abundance in fibrotic model compared to control kidney highlighted in proximal tubules. Moreover, in PPARg KO mice we observed an upregulation in the expression.

Optimization of MALDI-MSI technologies on Zebrafish eleutheroembryos: A case study with endocrine disruptors

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The Zebrafish (Danio rerio) has become a powerful model organism in a wide range of scientific fields, including ecotoxicology for presenting various advantages concerning other common model organisms. One of the most important is that toxicological data can be extrapolated not only to aquatic species but also to other vertebrates, including humans. Moreover, zebrafish embryos are considered an excellent alternative animal model with fewer ethical restrictions, ensuring the fulfilment of the 3R's principle (Replacement, Reduction, and Refinement) in animal research.

Bulk omic technologies have contributed to environmental toxicology to deepen an organism's response to pollutants at the molecular level. However, reporting the molecular information of individual cell types in addition to their spatial organization is unachievable. To overcome these issues, breakthrough technologies have emerged to encompass single-cell and spatially resolved omics, including mass spectrometry imaging (MSI). Particularly relevant is the use of Matrix-Assisted Laser Desorption/Ionization (MALDI-MSI) owing to it providing a favourable balance between sample preparation, chemical sensitivity, and spatial resolution. Despite its outstanding features, MALDI approaches have some limitations in lipidomics studies. For instance, the conditions for the optimal ionization of certain lipid classes (i.e., sterols) or the spatial resolution compared to other MSI techniques. For that reason, state–of–of–the–art techniques such as laser-post ionization coupled with the MALDI (MALDI-2) tool emerged as a potential new game-changer in the MSI field.

In this study, we developed and optimized several spatial lipidomics protocol to analyse zebrafish embryo sections using both MALDI and MALDI-2-MSI with a lateral spatial resolution of up to 5 µm. Our results revealed the presence of different lipid clusters corresponding to different sections of the zebrafish embryo. Results demonstrate the usefulness of spatial omics studies in this biological model, particularly underlining possible lipid biomarkers for relevant tissues such as eye or brain. Therefore, MALDI-2 approach has been used to characterize the lipidomic disruption on the eyes induced by a particular endocrine disruptor (Tributyltin (TBT)), an environmental pollutant which has been recently hypothesized to produce an important ocular damage.

Role of mitochondrial fusion proteins in the transfer of phospholipids from the endoplasmic reticulum to the mitochondria

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The ER is the primary site for the synthesis of phospholipids, which are essential components of cellular membranes, including mitochondrial membranes. However, mitochondria have limited capacity for de novo synthesis of phospholipids and heavily rely on the ER for their supply. ER-mitochondria contact sites, known as mitochondria-associated membranes (MERCs), serve as platforms for lipid transfer. MAMs contain specific proteins, such as the ER-resident ORP5/8, VPS13A, VPS13D, VAT1, which facilitate lipid exchange between the ER and mitochondria.

Mitochondrial fusion proteins, such as Mitofusin 1 and 2 (MFN1 and MFN2) and Optic atrophy 1 (OPA1), mediate the physical fusion of mitochondria, allowing the mixing of their contents, including lipids. Our laboratory has reported that MFN2 has the capacity to bind some phospholipids such as phosphatidylserine (PS), and can also isolate it from membranes and to form rigid domains enriched in PS. As a result, MFN2-deficient livers showed a reduced capacity to transfer PS from ER to mitochondria and, consequently, mitochondrial conversion of PS into phosphatidylethanolamine (PE) was also reduced. In the talk, we will analyze the implications of a deficient mitochondrial PS transfer in liver pathology. Furthermore, we will discuss the potential functional interaction between MFN2 and PS carriers, and also the potential role of MFN2 variant ERMIT2 into PS transfer. This study will shed light into the function of MFN2 in MERCs that could explain the associated metabolic pathologies such as insulin resistance, type 2 diabetes and liver diseases.

Lipid droplets drive lipid mediator production and modulate ferroptosis

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Lipid droplets (LDs) have a central role in fatty acid metabolism and signaling, but their contribution to the distribution of various types of fatty acids within the cell is not well understood. Here we show that LDs manage the trafficking of PUFAs to control lipid mediator production and ferroptosis sensitivity. On the one hand, we find that the incorporation of PUFAs into triglycerides stored within LDs and their release via adipose triglyceride lipase (ATGL) are required for the conversion of PUFA into cyclooxygenase- and lipoxygenase-derived lipid mediators. ATGL regulates these biosynthetic pathways independently of the group IVA cytosolic phospholipase A2 (cPLA2α), but it also promotes the incorporation of LD-derived PUFAs into membrane phospholipids, which are substrates for cPLA2α. On the other hand, when cellular redox defences are compromised, LD turnover becomes a major determinant of cell survival. We find that inhibition of glutathione peroxidase 4 (GPX4) triggers diacylglycerol acyltransferase (DGAT)-mediated LD biogenesis, which in turn reduces lipid peroxidation and prevents ferroptotic cell death. However, the concurrent LD breakdown via lipolysis and lipophagy promotes lipid peroxidation over time, suggesting that a fine balance between LD biogenesis and breakdown is required for the protection of cells against ferroptosis. Finally, we demonstrate that DGAT inhibition is sufficient to increase cancer cell sensitivity to ferroptosis as well as suppress lipid mediator production and cancer cell proliferation in vitro and in vivo. Thus, LDs control PUFA trafficking to fine-tune lipid oxygenation pathways responsible for both lipid mediator signalling and lethal lipid peroxidation during ferroptosis. Targeting LD turnover may thus be a valid strategy in the fight against cancer.

STAT-1-Controlled Lipin-2 Is a Master Regulator of the Antiviral and Anti-Inflammatory Responses to Interferon

María Ángeles Balboa, Institute of Molecular Biology and Genetics - CSIC, Valladolid, Spain

Interferons (IFN) are crucial antiviral and immunomodulatory cytokines that exert their function through the regulation of a myriad of genes, many of which are not yet characterized. Lipin-2 is a member of a family of phosphatidic acid phosphatase enzymes which are central to lipid metabolism, as they provide the diacylglycerol that is used within the de novo pathway for phospholipid and triacylglycerol biosynthesis. The gene encoding for lipin-2, LPIN2, is mutated in patients that suffer from an autoinflammatory disease known as Majeed syndrome. These patients experience recurrent flares of fever and inflammation in their joints and skin, and their macrophages exhibit an exacerbated production of IL-1β due to an increased classical activation of the NLRP3 inflammasome. Here we reveal that lipin-2 is regulated by IFN in a STAT-1-dependent manner. Lipin-2 inhibits viral replication both in vitro and in vivo. Moreover, lipin-2 also acts as a regulator of inflammation in a viral context by reducing the signaling through TLR3 and the generation of ROS and mtDNA release that ultimately activate the inflammasome NLRP3. Inhibitors of mtDNA exit from mitochondria restrict IL-1β production in lipin-2-deficient animals in a model of viral infection. Finally, analyses of databases from COVID-19 patients show that LPIN2 expression levels negatively correlate with the severity of the disease. They also negatively correlate with the expression of the inflammatory genes IL6, VEFGA and CCL3, related with the cytokine storm, the endothelial dysfunction, and the increased recruitment of innate immune cells that characterize patients with severe COVID-19. Interestingly, the expression levels of canonical interferon-regulated genes such as MX1, OAS1 and OAS2 positively correlated with the expression of LPIN2. Overall, these results uncover novel regulatory mechanisms of the IFN response driven by lipin-2, and open new perspectives for the future management of patients with LPIN2 mutations.

Novel insights into the phospholipase A2 family

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In essence, “phospholipase A2” (PLA2) means a group of enzymes that release fatty acids and lysophospholipids by hydrolyzing the sn-2 position of glycerophospholipids. To date, more than 50 enzymes possessing PLA2 or related lipid-metabolizing activities have been identified in mammals, and these are subdivided into several families in terms of their structures, catalytic mechanisms, tissue/cellular localizations, and evolutionary relationships. Many of the PLA2 enzymes recognize the differences in the fatty acyl and/or head group moieties of their substrate phospholipids, and several enzymes catalyze even non-PLA2 reactions such as phospholipase A1, lysophospholipase, neutral lipid lipase, and transacylase reactions. From a functional viewpoint, the PLA2 superfamily has mainly been implicated in signal transduction, driving the production of a wide variety of bioactive lipid mediators. However, a growing body of evidence indicates that PLA2s also contribute to phospholipid remodeling or recycling for membrane homeostasis, fatty acid □-oxidation for energy production, and barrier lipid formation on the body surface. Accordingly, PLA2 enzymes are considered one of the key regulators of a broad range of lipid metabolism, and perturbation of specific PLA2-driven lipid pathways often disrupts tissue and cellular homeostasis and may be associated with a variety of diseases. The functions of individual PLA2s rely on their enzymatic properties, their tissue/cellular distributions, lipid composition in target membranes, and spatiotemporal availability of downstream enzymes and cofactor(s) in various pathophysiological settings. During the last two decades, the functions of various PLA2s have been clarified by studies based on knockout/transgenic mice and/or human diseases caused by mutations of these enzymes, along with comprehensive lipidomics. In this talk, I will make an overview of our recent findings on the novel biological roles of PLA2s and their underlying lipid pathways, focusing mainly on (1) unique actions of sPLA2s on extracellular vesicles and gut microbiota, (2) functional segregation of cPLA2s by mobilizing distinct lipid mediators, and (3) regulation of phospholipid catabolism and turnover by iPLA2s.

Design, synthesis and pharmacological evaluation of soluble epoxide hydrolase inhibitors for pain: focus on visceral pain and chemotherapy-induced neuropathic pain

Santiago Vázquez, Universitat de Barcelona, Spain

Soluble epoxide hydrolase (sEH), an enzyme belonging to the cytochrome P450 pathway of the arachidonic acid cascade, has been suggested as a pharmacological target for the treatment of pain-related disorders. Indeed, a representative sEH inhibitor (sEHI), EC5026, is currently in clinical trials for the management of neuropathic pain (NP) [1].

Recently, we have designed, synthesized and pharmacologically evaluated novel series of potent benzohomoadamantane-based sEHI and a selected compound presented excellent efficacy in a murine model of cerulein-induced acute pancreatitis [2]. Herein, we will present further medicinal chemistry around these sEHI in order to improve the DMPK properties of previous hits. After an extensive in vitro screening cascade, molecular modelling, and in vivo pharmacokinetics studies, three candidates were assessed in vivo in murine models of allodynia and pain.

First, two compounds presented anti-allodynic effect, in a dose-dependent manner, in a murine model of capsaicin-induced allodynia. Next, the most potent compound showed robust analgesic efficacy in the cyclophosphamide-induced murine model of cystitis, a well-established model of visceral pain [3]. Finally, considering that chemotherapy-induced NP (CINP), a severe side effect of several anticancer agents, is a largely unmet medical need, our third sEHI was evaluated in a murine model of paclitaxel-induced NP. Subcutaneous administration of this candidate completely reversed, in a dose dependent manner, the paclitaxel-induced sensory hypersensitivity. Also, administration of the sEHI 30 min before paclitaxel treatment fully prevented the development of the neuropathic allodynia.

Collectively, these results suggest interstitial cystitis/pain bladder syndrome and CINP as new indications for sEHI.

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Endocannabinoid and paracannabinoid control of pain processing

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The intoxicating constituent of cannabis, 9-tetrahydrocannabinol, produces its pharmacological effects by activating cannabinoid receptors in the brain and peripheral tissues. The primary endogenous ligands for these receptors are two lipid-derived transmitters, the endocannabinoids anandamide and 2-arachidonoyl-sn-glycerol (2-AG). Anandamide is produced by both neural and non-neural cells, and is deactivated via a two-step process consisting of transmembrane transport followed by intracellular hydrolysis. Anandamide hydrolysis is catalyzed by the intracellular serine amidase, fatty-acid amide hydrolase (FAAH). Along with the endocannabinoids, the body produces a family of ancillary substances that are similar to the endocannabinoids in chemical structure, biogenesis, and deactivation, but differ from them in function. These substances, called paracannabinoids (Greek pará 'side by side'), activate various receptors to produce effects that can be either synergistic or antagonistic to those of the endocannabinoids. One such substance, palmitoylethanolamide (PEA), is an agonist for the nuclear receptor PPAR-alpha and acts synergistically with anandamide to regulate both pain initiation and pain chronification. PEA is deactivated by the intracellular cysteine amidase, N-acylethanolamine acid amidase (NAAA). In my talk, I will provide an outline of drug classes that selectively interfere with the deactivation of anandamide and PEA, focusing on their pharmacological properties and therapeutic potential. The analgesic properties of these agents suggest that they might complement or possibly replace opioid drugs.

Motor Neuron Disease or Sensory Neuropathy? L-Serine as a modulating factor

Thorsten Hornemann, University of Zurich, Switzerland

The enzyme Serine-palmitoyltransferase (SPT) catalyzes the first and rate-limiting step in the de-novo synthesis of sphingolipids. Certain mutations in SPT cause the Hereditary Sensory Neuropathy Type 1 (HSAN1). HSAN1 is a peripheral neuropathy characterized by progressive pain and sensory loss associated with impaired wound healing capacity. Metabolically, the SPT-HSAN1 mutations induce a permanent change in the substrate specificity of SPT, thereby shifting its affinity from the canonical substrate L-Serine to L-Alanine. This substrate change results in the formation of an atypical class of 1-deoxySphingolipids (1-deoxySL) which are toxic to peripheral sensory neurons in vitro and vivo.

In contrast, amyotrophic lateral sclerosis (ALS) is a degenerative disease of the lower and upper motor neurons. Clinical hallmarks include a rapidly progressing muscle atrophy, speech and swallowing difficulties, fasciculation and spasticity finally leading to premature death due to respiratory insufficiency.

Recently, we identified a group of heterozygous SPT variants in eight unrelated families with early onset ALS. All identified SPT-ALS variants cluster in a specific domain of the SPT enzyme that is important for its interaction with the regulatory subunit ORMDL3. Consequently, the SPT-ALS variants showed an impaired homeostatic control of the enzyme resulting in an excessive overproduction of ceramides and other SL species.

Restricting L-Serine limits this overproduction but instead resulted in an increased use of L-Alanine by the hyperactive SPT mutant and consequently an increased 1-deoyxySL formation. This indicates that limiting L-serine availability could result in a phenotypic shift from a motor to a sensory phenotype. This hypothesis was confirmed in an isolated SPT-ALS family in which individual family members showed an either sensory or a motor phenotype in response to endogenous L-Serine availability.

In summary, our data indicate that mutations located in specific domains of SPT can results in either a sensory neuropathy or a motor neuron disease. Limiting L-serine availability in the SPT-ALS condition leads to a metabolic shift and a phenotypic conversion from a motor to sensory phenotype.

Quantifying Lipid Species in Large-Scale Human Studies: Approaches and Challenges

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Accurate quantification of lipid species in large-scale human studies is essential for understanding their role in health and disease. Here we will discuss high-throughput methods for robust and accurate quantification of lipid species that include both direct infusion and LC-coupled mass spectrometry. Analysis must be coupled with automated data processing including quality control assessments. In addition to appropriate analytical methods, sample stability and efficient lipid extraction are critical for successful clinical lipidomics.

Lipidomics and multiomics for molecular discovery in disease

Cristina Legido-Quigley, Kings College London, United Kingdom

During my presentation, I will center on lipidomics and clinical omics, specifically their application in exploring the brain-liver axis. Over the years, our research has led to the development of methodologies, including mass spectrometry omics analyses, big data analyses, multiomics and causality testing like clinical interventions and statistics like Mendelian Randomization.

I will begin by providing an overview of lipidomics technology. The presentation will highlight three examples of our recent discoveries in disease lipid pathways. Firstly, we will explore findings concerning sphingolipid pathways in the liver, recent results holding promise for liver fibrosis treatment.

Secondly, I will delve into our research on lipid pathways in Alzheimer's disease, shedding light on primary fatty acid amides, very interesting sedative molecules that the brain makes, and their possible role in innate immunity in neurodegeneration.
Lastly, I will discuss our discoveries related to lipid pathways in cardiometabolic disease, which have implications for managing cardiovascular and metabolic disorders in both children and adults and we are currently testing in the clinic.

I will also touch upon the complexity of precision medicine and the challenges we encounter while integrating lipidomic data or biomarkers into clinical practice. Despite these challenges, I will emphasize the opportunities that lie ahead, particularly in preventive medicine and better treatments.

Oxidative phospholipidomics: the cross talk between the chemistry and the biological role in inflammation and chronic diseases

Rosário Domingues, University of Aveiro, Portugal

Phospholipids (PL) are widely present in living systems. They demonstrate a remarkable structural diversity that is expressed by the different phospholipid classes, each characterized by distinct polar head groups and esterification with saturated and/or unsaturated fatty acids. These characteristics are crucial in defining the unique lipidome of cells, tissues, and organelles, which is essential for their proper functioning. Noticeable the esterification with polyunsaturated fatty acids (PUFA) makes these biomolecules particularly susceptible to oxidation, either by enzymatic mediated oxidation, by radical induce oxidation and /or by photooxidation. The oxidation of the phospholipids can lead to the formation of different types of oxygenated and truncated phospholipid oxidation products (oxPL), formed by oxidation of the esterified PUFA which can disrupt the typical phospholipidome and contribute to cellular dysfunction and disease. Oxidized phosphatidylcholines have been detected in several chronic diseases, including neurodegenerative and cardiovascular diseases, associated with pro inflammatory effects, and suggested as potential biomarkers. Oxidized cardiolipin is a well known player in cell apoptosis while oxidized phosphatidylethanolamine is a key mediator in ferroptosis cell death. Interestingly, the polar head groups of certain phospholipid classes, the phosphatidylserine and phosphatidylethanolamine are also prone to suffer modification by oxidation and also by reaction with glucose under hyperglycemic condition, as occur in diabetes, obesity and metabolic syndrome. Thus, glycated phosphatidylethanolamine can be used as biomarker of uncontrolled diabetes.

Different type of modified lipids (epilipids) seems to display a specific modulatory capacity of the immune response, exhibiting either pro inflammatory, as reported of oxidized phosphatidylethanolamine and their glycated/glycoxidized formed, or with a role in diabetes comorbidities and in other chronic diseases, while oxidized phosphatidylserine exhibit anti inflammatory effects, evidencing a potential role in resolution of inflammation. However these oxidize dPL are usually present in low abundance, and usually overlooked and robust lipidomics approaches are need to detect these epiphospholipids in biological samples and to unveil their potential as biomarker of chronic and non communicable diseases.

Lipoxidation of cysteine residues: selectivity and diversity of structural and functional implications

Dolores Pérez-Sala, Margarita Salas Center for Biomedical Research - CSIC, Madrid, Spain

Cysteine residues are key elements in redox signaling and are targets for a plethora of modifications, including various forms of oxidation, lipoxidation and lipidation, which can convey great structural and functional diversity. Cysteine modifications can impact multiple protein features, including activity, interactions, degradation and subcellular localization. Cysteine residues are often preferred targets for the addition of electrophilic lipids or lipoxidation. Although this is a non-enzymatic modification, it is not random, and there are factors from the protein targets or the cellular context that influence its selectivity. Moreover, given the great structural diversity of electrophilic lipids, the functional consequences of lipoxidation can be highly varied. Indeed, certain proteins can be activated or inhibited depending on the modifying lipid moiety, or their subcellular localization or assembly can be differentially altered. In addition, there can be interplay between lipoxidation and other posttranslational modifications, resulting in fine tuning of protein properties and signaling pathways. We will provide several examples of proteins differentially regulated by various electrophilic lipids, paying special attention to certain cytoskeletal proteins of the intermediate filament family, which behave as sensors of redox and electrophilic stress, and participate in the integration of cytoskeletal responses.

The biological effects of protein lipoxidation and its analysis by mass spectrometry

Corinne M. Spickett, Aston University, Birmingham, UK

Lipid peroxidation leads to formation of a variety of reactive products, including short-chain esterfied and non-esterified aldehydes as well as alpha, beta-unsaturated alkenals. These can covalently modify proteins either by Schiffs base formation or Michael addition, a process known as lipoxidation, leading to altered function. Protein lipoxidation alters protein activity and protein interactions, thus affecting metabolic and signaling pathways, and many cellular processes. Lipoxidation can be considered as a post-translational modification of proteins. Its analysis can be challenging because of the wide variety of modifications possible and their heterogenous nature. Antibodies against some lipoxidations have been developed and used to good effect, but the best approach to determine the site and type of modification is liquid chromatography-tandem mass spectrometry (LC-MS/MS), which can sequence peptides and identify post-translational modifications. In this lecture, the mechanisms of lipoxidation will be described briefly and the principles of its analysis by LC-MS/MS will be explained. The biological effects of lipoxidation will be illustrated using examples such as the metabolic enzyme pyruvate kinase, the redox-sensitive dual specificity phosphatase PTEN in the AKT signaling pathway, and cytoskeletal proteins. The importance of lipoxidation in cell physiology and medicine will be discussed alongside the challenges of understanding its effects.

Circulating Metabolic Biomarkers: From Liver Inflammation to Cancer

Jesús Bañales, Biodonostia Health Research Insitute, Donostia, Spain

Biomarkers have significant potential in personalized medicine. They can be used for diagnostic purposes, prognosis estimation, therapeutic decisions, treatment response assessment, and evaluation of intermediate endpoints, among other applications. The emergence of liquid biopsy and the advent of the multi-omic era have revolutionized the diagnosis and management of diseases by revealing biomarkers present in biological fluids.

During the development and progression of diseases, metabolic changes occur in target tissues and/or cells. These changes can lead to modifications in the metabolomic profiles of biofluids (such as lipids, bile acids, and amino acids) which can be effectively detected using high-resolution techniques collectively known as "metabolomics."

The objective of this presentation is to explore various strategies of metabolomic-based liquid biopsy aimed at discovering non-invasive biomarkers for inflammatory diseases and cancer. Special emphasis will be placed on non-alcoholic fatty liver disease (NAFLD), where the value of serum metabolomics analysis in accurately estimating disease stage and assessing cardiovascular and genetic risks will be thoroughly evaluated. Additionally, novel evidence supporting the early detection of primary liver cancers using serum metabolomics will be presented and comprehensively discussed.

The Lipotype Hypothesis

Giovanni D'Angelo, Swiss Federal Institute of Technology, Lausanne, Switzerland

Single-cell genomics techniques have allowed for the deep profiling of individual cells in multicellular contexts. These new technologies have enabled the building of cell atlases where hundreds of different cell types are categorized according to their transcriptional and epigenetic states. These analyses have led to the depiction of detailed cell transcriptional landscapes that could be interpreted in terms of cell identity. Nonetheless, transcription represents only one axis in the establishment of cell phenotypes and functions and post-transcriptional events crucially concur to cell identity in ways that cannot be simply derived from transcriptional profiles. Thus, the chemical composition of individual cells and the activity of metabolic pathways are likely as good descriptors of cell identity as transcriptional profiles are. Moreover, accumulating findings assign to lipid metabolism an instructive role towards the establishment of cell identity, yet our understanding of the integration of transcriptional and lipid metabolic programs in cell fate determination remains superficial. Here I will report on our attempts to investigate lipidomes at single cell levels and at high spatial resolution by MALDI imaging mass spectrometry.

Environmental constraints on cell proliferation

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Complex regulatory mechanisms enable cell metabolism to match physiological state. How specific cancers use metabolism to support proliferation is determined both by cell intrinsic factors (such as tissue of origin and driver mutations) as well as the nutrients available to cells within the tumor. Accumulating evidence suggests that nutrient availability in tissues is heavily influenced by tissue site and non-cancer cells in the tissue, such that tissue location is a major determinant of nutrient availability leading to each tissue having a unique nutrient environment. This has implications for understanding how cancer cells use nutrients to support proliferation and survival. Thus, we find that to metastasize, cancer cells have to adapt to the nutrient conditions found within a particular organ, and that nutrient conditions can also impact gene expression in cells. Interestingly, in nutrient constrained conditions, cancer cells lack the flexibility to engage new metabolic pathways, addicting them to the salvage of some nutrients to save metabolic resources to synthesize other biomass components. This is particularly evident with respect to lipid metabolism in environments such as the brain where only very specialized lipids are found. This topic will be discussed, including how it influences sensitivity and resistance to cancer therapy.

Combined effect of ketogenic diet and GDNF injection on Schwann Cells in mouse model of Krabbe's disease

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Background: Krabbe disease mainly affects children and is characterized by the loss of function of the galactosylceramidase enzyme (GALC). This dysfunctional lysosomal protein cannot recycle myelin inducing accumulation of cytotoxic psychosine. In Twitcher mice (Twi), a model of Krabbe disease, this accumulation occurs in Schwann cells inducing cell death and neuroinflammation. It was recently observed that ketogenic diet (KDiet) increases the lifespan of children and rescues some of their physiological functions.
Methods: Twi mice have been exposed to KDiet or control diet 20 days post-weaning (P20) with or without Glial cell line-Derived Neurotrophic Factor (GDNF) injection, known to induce Schwann cells differentiation. Proteomic analyses were performed at P42 in sciatic nerve and brain tissue. Neuroinflammation and the presence of functional Schwann cells were evaluated in sciatic nerves at P25, P35 and P42.
Results: Compared to control diet, Twi mice increased lifespan when fed with KDiet. The brain proteomic analysis revealed that KDiet-fed Twi mice show an attenuation of the neuroinflammatory pathways. This was confirmed by our analyses showing that Twi mice present an inflammatory profile (evaluation of IL-6 and TNF-) which is improved when mice were fed with KDiet. In sciatic nerve, the proteomic analysis revealed that in Twi mice, ApoD recruitment is strongly increased compared to WT mice. ApoD is an important player in neuroinflammation resorption. In sciatic nerves of Twi mice, we were almost unable to detect functional Schwann cells (as seen by the expression of SOX10). SOX10 expression is partially rescued when mice were fed with KDiet (25% of recovery at P35 and 50% of recovery at P42). Interestingly, this effect is potentialized by GDNF injection.
Conclusions: Our study shows that KDiet, increases Twi mice lifespan. This is associated with partial restauration of neuronal integrity and neuroinflammation. These effects are potentialized by GDNF injection.

Tracking ether lipid synthesis and breakdown in human adipocyte differentiation

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Adipose tissue not only serves as an energy reservoir but also secretes hormones responsible for stimulating lipid metabolism. During obesity, adipose tissue is not able to serve as a buffering system for storing excess amounts of lipids and releases higher levels of free fatty acids. Evidence is present that also the amount and composition of ether lipids is changed during the course of acquired obesity. Alkylglycerol monooxygenase (AGMO) is a lipolytic enzyme that catalyses the breakdown of alkylglycerols and lyso-alklyglycero-phospholipids whereas plasmanyl ethanolamine desaturase (PEDS1) is an anabolic enzyme responsible for plasmalogen synthesis. We know from our experiments that AGMO and PEDS1 are active in several murine tissues and cell lines but the physiological role in human adipose tissue and adipocyte differentiation is still enigmatic.

To examine the relevance of AGMO and PEDS1 and ether lipid metabolism in human adipocyte biology we employed tissues from patients who underwent abdominoplasty surgery. Gene expression analyses of enzymes involved in ether lipid metabolism like PEDS1 together with classical adipose specific genes and AGMO enzymatic activity was analysed in isolated in vivo differentiated adipocytes and correlated to basic blood parameters of abdominoplasty patients. Furthermore, the relevance of AGMO and PEDS1 during adipocyte differentiation was looked at by RNAi mediated knockdown. Additionally, we investigated dynamics in ether lipid metabolism by pulse-chase experiments during adipogenesis using a fluorescently labeled ether lipid and the lipid composition of in vivo differentiated adipocytes.

Our findings indicate a possible connection of certain ether lipid enzymes to basic blood lipid parameters and an equilibrium of ether lipid degradation and plasmalogen synthesis.

Dietary derived short chain fatty acids impair brain health: A shift in our understanding of short chain fatty acids health promotive benefits

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Introduction: There is emerging recognition in the scientific community that the gut microbiota communicates with the brain to modulate health and functional outcomes. However, it is unclear how this communication occurs. Short chain fatty acids (SCFA) derived from gut microbial fermentation of dietary carbohydrates have been proposed as a route or mechanism via which this communication occurs. Conversely, gut microbes derived short chain fatty acid's role in improving metabolic health is well accepted and recognized in the scientific community. In this presentation, we seek to show that SCFA health promotive properties does not appear to accrue in improving brain health or function.

Materials and Methods: Acute dose of short chain fatty acids (50-1000µM at 60:20:20 ratio acetate/propionate/butyrate) were administered to both SHSY5Y cells and adult Long Evans rats representing concentrations reported in human systemic circulation and lipidome, neuronal cell cycle, morphology,spatial brain lipid metabolism and behavioral phenotypes assessed.Results and Discussion: We observed systemic exposure of the brain to acute doses of SCFAs at concentrations reported in human blood circulation alters the neurolipidome, mitochondria morphology, induced apoptosis, reduced ATP production and respiration in neurons. Similarly, when adult rats were exposed to acute dose of SCFAs; we observed that exposure spatially disrupted lipid metabolism in defined brain anatomical regions associated with deficits in cognition and memory. A sex specific effect was also observed with exposure with females showing more adverse outcomes in most evaluations conducted.

Conclusion: These findings present evidence for SCFA even at low dose (50µM) inducing adverse effects on brain lipid composition, function, behavioral phenotype impacting brain health outcome. Collectively, these results present new discoveries that gut microbes derived short chain fatty acids does not appear to confer health promotive benefits to brain following acute systemic exposure.

Knockout of cardiolipin synthase disrupts cardiac development in neonatal mice.

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Introduction: Cardiolipin is the specific phospholipid of mitochondria. Constitutive deletion of cardiolipin biosynthesis is embryonic lethal but the effect of cardiomyocyte-specific deletion of cardiolipin biosynthesis hasnot been established.

Materials & methods: We created a novel mouse model by deleting cardiolipin synthase (Crls1) in cardiomyocytes under the myosin-heavy chain promoter. Mouse hearts were analyzed by echocardiography, histology, electron microscopy, and mass spectrometry on both proteomics and lipidomics platforms.

Results & discussion: Crls1 knockout (KO) mice were born with reduced levels of cardiolipin and failed to increase the myocardial concentration of cardiolipin during postnatal maturation of the heart. In addition, Crls1KO mice were unable to accumulate respiratory proteins to the same level as controls and developed early-onset heart failure, from which they died at the age of 2 weeks. We present comprehensive proteomics and lipidomics data in a mouse model of rapidly progressing heart failure induced by cardiolipin deficiency.

Conclusions: Our data suggest that cardiolipin is specifically required for the postnatal maturation of the heart because it is essential to support the massive increase in the density of respiratory enzymes in mitochondrial cristae, which occurs during the neonatal period. Thus, lack of cardiolipin disrupts neonatal cardiac development.

Mitochondrial lipidomes are tissue specific - The phospholipid to cholesterol ratio regulates UCP1 activity

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Introduction: Lipid species composition is conserved at the sub-cellular level, but it is unclear whether organelle lipidomes differ between organs. Mitochondria are the provider of cellular energy from aerobic respiration in all tissues. Those present in brown adipose tissue (BAT) contain uncoupling protein (UCP)1 enabling them to convert chemical energy stored as triglycerides into heat; a process called non-shivering thermogenesis. Therefore, we asked whether UCP1 in BAT requires a specific lipidomic environment in mitochondria that differs from those of other tissues including white adipose tissue (WAT), muscle and liver.
Methods: Mitochondria were isolated from mouse tissues using differential centrifugation. Their purity was confirmed after a full proteome analysis through comparison to ER and mitochondria associated membrane (MAM) fractions isolated by density gradient centrifugation. Lipidomes were analyzed using quantitative mass spectrometry. To test the functional relevance of specific mitochondrial lipid compositions, respiration including UCP1 activity was investigated using microplate-based respirometry; in (A) primary brown adipocytes, where the mitochondrial cholesterol importer STARD3 was manipulated using RNAi and lentiviral overexpression; and in (B) isolated mitochondria, that were incubated with donor vesicles containing different amounts of glycerophospholipids (GPL) and free cholesterol (FC)
Results: We identified that BAT mitochondria contained almost no cholesterol. The GPL to FC ratio (GPL/FC) was up to 50-fold higher in brown than white adipose tissue, liver or muscle mitochondria and could be related with adipose tissue browning. Overexpression of STARD3 in brown adipocytes increasing the mitochondrial FC content inhibited UCP1-dependend respiration, whereas a knockdown lowering FC levels promoted it. Coinciding with this observation, loading of isolated mitochondria with FC prevented UCP1 function, while treatment with GPL boosted it.
Conclusion: We show, that the lipidomic organization of mitochondria (i.e. it's GPL/FC) is a critical parameter for BAT function and propose that targeting it might be a promising strategy to promote UCP1 activity.

Evaluate the Therapeutic Functions of Enzyme-X in Atherosclerotic Cardiovascular Diseases

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Atherosclerotic cardiovascular disease (ASCVD) remains the leading cause of death globally. Although intensively lowering low-density lipoprotein-cholesterol (LDL-C) reduces the occurrence of ASCVD; however, that cannot preclude the disease onset but instead increase the risk of all-cause mortality. Finding a novel therapeutic approach that targets atherogenic lipoproteins is of utmost importance. Previously, we separated LDL-C into five subfractions (L1~L5) with increasing electronegativity. L5-LDL elevated in ASCVD shows atherogenic properties owing to high contents of ceramide and lysophosphatidylcholine (LPC); in contrast, L1-LDL shows no harmful effects. Both ceramide and LPC can be hydrolyzed into harmless metabolites by bioengineered-Enzyme-X (Enzyme-X) with dual enzymatic activities: ceramidase and lysophospholipase A1. In this study, we aimed to evaluate its therapeutic functions in vivo.pCMV6-Enzyme-X was transfected into HEK 293T cells for Enzyme-X overproduction. Nickel column-equipped liquid chromatography was to purify Enzyme-X; ultra-performance liquid chromatography-mass spectrometry was used to monitor catalytical functions. Eight-week-old apoeem1Narl (apoE−/−) mice fed with high-fat diet (HFD) for additional eight weeks to show early onset of atherosclerosis (HFD group). To evaluate the therapeutic functions, HFD-fed apoE−/− mice received Enzyme-X thrice a week for eight weeks (HFD+Enzyme-X group). Besides, LPC was injected into normal-chow diet (NCD)-fed apoE−/− mice to promote atherosclerosis (LPC group); co-treated with Enzyme-X was to examine therapeutic effects (LPC+Enzyme-X group). ApoE−/− mice fed with NCD served as control.Ceramide and LPC levels were elevated (2~7 times) in HFD and LPC groups. Besides, they showed an early onset of atherosclerosis, including enhanced collagen deposition (increased 54% in HFD; 50% in LPC), increased elastic fiber fragmentation, and lipids accumulation in the aorta (increased 52% in HFD; 45% in LPC). Notably, injecting with Enzyme-X showed a significant reduction (2~6 times) of plasma ceramide and LPC. The disease groups that received Enzyme-X also showed reduced collagen deposition (reduced 46% in HFD+Enzyme-X; 54% in LPC+Enzyme-X), elastic fiber fragmentation, and lipid accumulation (reduced 59% in HFD+Enzyme-X; 38% in LPC+Enzyme-X). Finally, Enzyme-X prevents the formation of atherosclerotic plaques in aorta.In conclusion, dual enzymatic functions of Enzyme-X can prevent disease progression.
Conclusion: We show, that the lipidomic organization of mitochondria (i.e. it's GPL/FC) is a critical parameter for BAT function and propose that targeting it might be a promising strategy to promote UCP1 activity.

Life and death in adipose tissue: a matter of epigenetics?

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Background: Krabbe disease mainly affects children and is characterized by the loss of function of the galactosylceramidase enzyme (GALC). This dysfunctional lysosomal protein cannot recycle myelin inducing accumulation of cytotoxic psychosine. In Twitcher mice (Twi), a model of Krabbe disease, this accumulation occurs in Schwann cells inducing cell death and neuroinflammation. It was recently observed that ketogenic diet (KDiet) increases the lifespan of children and rescues some of their physiological functions.

Methods: Twi mice have been exposed to KDiet or control diet 20 days post-weaning (P20) with or without Glial cell line-Derived Neurotrophic Factor (GDNF) injection, known to induce Schwann cells differentiation. Proteomic analyses were performed at P42 in sciatic nerve and brain tissue. Neuroinflammation and the presence of functional Schwann cells were evaluated in sciatic nerves at P25, P35 and P42.

Results: Compared to control diet, Twi mice increased lifespan when fed with KDiet. The brain proteomic analysis revealed that KDiet-fed Twi mice show an attenuation of the neuroinflammatory pathways. This was confirmed by our analyses showing that Twi mice present an inflammatory profile (evaluation of IL-6 and TNF-) which is improved when mice were fed with KDiet. In sciatic nerve, the proteomic analysis revealed that in Twi mice, ApoD recruitment is strongly increased compared to WT mice. ApoD is an important player in neuroinflammation resorption. In sciatic nerves of Twi mice, we were almost unable to detect functional Schwann cells (as seen by the expression of SOX10). SOX10 expression is partially rescued when mice were fed with KDiet (25% of recovery at P35 and 50% of recovery at P42). Interestingly, this effect is potentialized by GDNF injection.

Conclusions: Our study shows that KDiet, increases Twi mice lifespan. This is associated with partial restauration of neuronal integrity and neuroinflammation. These effects are potentialized by GDNF injection.

Diversity of membrane phospholipids; biochemical mechanism and disease relevance

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Introduction: Glycerophospholipids constitute the main component of biological membrane using their amphipatic properties. Most usually, they contain saturated or mono-unsaturated fatty acids at sn-1 position, while polyunsaturated fatty acids are positioned at sn-2 of glycerol background. Such diversity and asymmetry combined together with polar head groupd, yield over 1,000 different species. Although Kennedy pathway (de novo pathway) and Lands` cycle (remodeling pathway) have been proposed over 60 years ago for membrane biogenesis, no molecular mechanism of diversity/asymmetry formation and its biological consequence remained ambiguous.Experimental proceduresBy in silico analyses and PCR procedures, we have identified 10 new genes in human and mice, which incorporate different types of fatty acyl-CoAs to lysophospholipids. Enzyme activities with diffferent acyl-CoAs and lysophospholipids were determined using cell lysates expressing individual enzymes. Transcrpitional and post-translational modifications were determined. The phospholipid products are analyzed and quantitated by LC-MS. Individual enzymes are deleted either globally or tissue-specifically using Crispr-Cas9 systems for phenotype analyses.

Results and Discussion: Arachidonic acid is mainly incorporated to sn-2 position of PC, PS etc by the action of LPCAT3 (novel nomenclature LPLAT12, see Ref. JBC 298, 101470). The deletion of LPCAT causes neonatal lethality due to malnutrition and failure of cylomicron production. Liver specific KO mice are protected from ferroptosis. These phenotypes were not observed by knockout of eicosanoid pathway, suggesting unique role of arachidonate-containing membrane lipids. Docosahexaenoic acid is incorporated in de novo pathway to lysophosphatidic acid to produce DHA-phosphatidic acid by the enzyme AGPAT3 (LPLAT3). Genetic ablation of AGPAT3 causes blindness, male infertility and neuronal disorders. Elucidation of molecular mechanism and transport of DHA from liver to individual tissues crossing blood barriers is ongoing projects.

Conclusion: More than 1,000 different species of glycerophospholipids are produced by 10-15 lysophospholipid acyltransferases in both MBOAT and AGPAT family. Biological significance and impact on diseases of membrane diversity have been explored using global and tissue specific KO mice.Ref. Shimizu, T. (2009) Ann. Rev. Pharmacol. Toxicol. 49, 123-150; Harayama, T. and Shimizu, T. (2020) J. Lipid Res. 61, 1150-1160.

Phosphatidic acid phosphatase contains a novel RP domain that regulates its phosphorylation and function in lipid synthesis

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Introduction: Phosphatidic acid (PA) phosphatase (PAP) is an evolutionarily conserved Mg2+-dependent enzyme that plays a key role in lipid homeostasis by controlling the cellular levels of its substrate, PA, and its product, diacylglycerol. These lipids are essential intermediates for the synthesis of triacylglycerol and membrane phospholipids. They also function in phospholipid synthesis gene expression, lipid droplet formation, and vesicular trafficking. The importance of PAP to lipid homeostasis and cell physiology is exemplified in yeast, mouse, and human by a host of cellular defects and lipid-based diseases associated with loss or overexpression of enzyme function. PAP function is largely controlled by its cellular location, which is mediated by phosphorylation and dephosphorylation. Multiple phosphorylations sequester PAP in the cytosol and protect it from proteasomal degradation. The endoplasmic reticulum-associated PAP phosphatase complex recruits and dephosphorylates PAP allowing the enzyme to associate with and dephosphorylate its membrane-bound substrate PA. PAP contains domains/regions that include the N-LIP and haloacid dehalogenase (HAD)-like catalytic domains, N-terminal amphipathic helix for membrane binding, C-terminal acidic tail for PAP phosphatase interaction, and a conserved tryptophan within the WRDPLVDID domain required for enzyme function.
Methods: Through bioinformatics, molecular genetics, and biochemical approaches, Results and discussion: we identified a novel RP (regulation of phosphorylation) domain that regulates the phosphorylation state of PAP. We showed that the RP mutation results in a 57% reduction in the endogenous phosphorylation of the enzyme, an increase in membrane association and PAP activity, but reduced cellular abundance.
Conclusion: This work not only identifies a novel regulatory domain within PAP but emphasizes the importance of the phosphorylation-based regulation of PAP abundance, location, and function in lipid synthesis. That disruption in the regulation of PAP function through alterations in phosphorylation leads to broader disruptions in lipid synthesis and cellular growth raises the suggestion that the RP domain may represent a possible therapeutic target for inhibiting growth of pathogenic fungi for which the domain is well conserved.

The roles of polyunsaturated fatty acids in vivo revealed by analyzing polyunsaturated fatty acid-deficient mice

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Introduction:Backgrounds Although polyunsaturated fatty acids (PUFA) are known as important sources of energy and bioactive lipids, the precise roles of each fatty acid are not known. To clarify the roles of each fatty acid, we try to establish PUFA-deficient mice.
Methods: PUFA-deficient mice were established by feeding fatty acid desaturase (FADS) 1/2-deficient mice with PUFA-deficient chow for two months. Lipidomic analyses were performed to confirm PUFA-deficiency.
Results: Lipidomic analyses revealed that PUFAs were deficient, and saturated and monounsaturated fatty acids were intact in most tissues of PUFA-deficient mice. These PUFA-deficient mice exhibited multiple phenotypes including male infertility, fatty liver, osteoporosis, anemia and bipolar disorder-like behavior. Supplementation of specific PUFA molecules rescued the phenotypes. For example, male infertility of PUFA-deficient male mice was due to the decrease in testosterone production, which was rescued by feeding omega-6 fatty acids. The bipolar-like behavior of PUFA-deficient mice was rescued by feeding the mice with docosahexaenoic acid.
Conclusions: Our PUFA-deficient mice will be useful to clarify the role of each PUFA molecule in various mouse disease models.

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Electronegative low-density-lipoprotein induces insulin resistance and hypertriglyceridemia: a new insight into the pathogenesis of gestational diabetes mellitus

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GDM is a common pregnancy disorder linked to adverse maternal outcomes. GDM also increases the risk of metabolic syndrome and cardiovascular disease. The current mechanistic understanding of GDM is insulin resistance (IR) and hypertriglyceridemia. Nevertheless, the etiology of IR and hypertriglyceridemia remain unclear. Low-density lipoprotein (LDL) can be divided into five subfractions (L1–L5) based on electronegativity. L5-LDL is the most electronegative subfraction and is significantly elevated in patients with metabolic syndrome and diabetes. Furthermore, our preliminary data show that plasma L5-LDL was elevated since the first trimester and significantly increased in the second and third trimesters of GDM mothers. This study aims to investigate whether L5-LDL is a trigger and how L5-LDL induces insulin resistance and triglyceride (TG) accumulation in the liver.Animal and cell models were constructed to determine how L5 promotes insulin resistance and TG accumulation in the liver. Purified human L5-LDL, L1-LDL, or saline were administered to eight-week-old C57BL/6 or LOX-1-/- mice through the tail vein twice a week for six weeks. In the end, mice were sacrificed, and the liver samples were obtained. We analyzed the activity of the insulin receptor substrate-1 (IRS-1)/phosphatidylinositol 3-kinase (PI3K)/serine/threonine-protein kinase B (Akt) signaling. Triglycerides, peroxisome proliferator-activated receptor alpha (PPARα), carnitine palmitoyltransferase I (CPT1), and glycerol-3-phosphate acyltransferase (GPAT) were assessed using oil red staining and western blotting. The human liver cancer cells (HepG2) were treated with L5-LDL or controls to evaluate the mechanisms of insulin resistance in GDM.The results demonstrated that phosphorylation of IRS-1 and Akt were significantly reduced in the livers of L5-injected mice, whereas triglyceride was increasingly accumulated. These effects were attenuated in LOX-1-/- mice. In hepatocytes, L5-LDL impaired the IRS-1/PI3K/Akt signaling pathway associated with IR. On the other hand, L5-LDL downregulated PPARα, CPT1, and upregulated GPAT activity, finally leading to TG deposition. More investigations regarding the hormone dysregulation of GDM mothers will be executed in the future.L5-LDL cause insulin resistance and TG accumulation in the liver, which leads to hypertriglyceridemia in the peripheral blood. Our study provides new insights into the pathogenesis of GDM and future treatments.

Q-RAI data-independent acquisition for lipidomic quantitative profiling

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Introduction: Untargeted lipidomics has been increasingly adopted for hypothesis generation in a biological context or discovery of disease biomarkers. Most of the current liquid chromatography mass spectrometry (LC-MS) based untargeted methodologies utilize a data dependent acquisition (DDA) approach in pooled samples for identification and MS-only acquisition for semi-quantification in individual samples. In this study, we present for the first time an untargeted lipidomic workflow that involves data independent acquisition (DIA), using the newly implemented Quadrupole Resolved All-Ions (Q-RAI) acquisition function on the Agilent 6546 quadrupole time of flight (Q-TOF) mass spectrometer. This would be more advantageous over DDA workflows, where MS2-based quantification is not applicable and would hence enable an easier development of targeted lipidomic assays for specific compounds of interest.
Materials and Methods: Lipid extracts from commercial human plasma were obtained via Butanol-Methanol extraction and ran using an in-house DDA method on an Agilent 6546 Q-TOF. This is followed by data processing and analysis on MetaboKit, a software enabling DDA-based spectral library construction and extraction of MS1 and MS2 peak areas, for reproducible identification and quantification of lipids in DIA analysis. A biological application of this workflow was then tested on Ceramide synthase 2 (CerS2) null nice.
Results and Discussion: Lipid coverage of the Q-RAI DIA workflow was comparable with other tools while quantification at MS1 and MS2 levels was comparable to multiple reaction monitoring (MRM) targeted analysis. Analysis of serum from CerS2 null mice using the Q-RAI DIA workflow identified 90 lipid species significantly different between CerS2 null and wild type mice. The most significant changes were registered on sphingolipids carrying C22 and C24 fatty acyl chains having significantly lower levels and long chain C16- and C18-containing species having significantly higher levels in CerS2 null mice, which are well-characterized changes associated with this phenotype.
Conclusion: Our results show the Q-RAI DIA as a reliable option to perform simultaneous identification and reproducible quantification of lipids in biological studies. This solution may potentially be extended to larger scale applications and analysis of polar metabolites in the near future.

Advanced (super-resolution) microscopy for dissecting lipid membrane organization and dynamics

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Molecular interactions are key in cellular signaling. In the membrane they are especially influenced by the organization and mobility of lipids. We present different advanced fluorescence microscopic tools that are able to determine such organization mobility and potentially extract interaction dynamics. Specifically, the direct and non-invasive observation of the interactions in the living cell is often impeded by principle limitations of conventional far-field optical microscopes, for example with respect to limited spatio-temporal resolution. We depict how novel details of molecular membrane dynamics can be obtained by using advanced microscopy approaches such as the combination of super-resolution MINFLUX microscopy. We highlight how these tools can reveal novel aspects of membrane bioactivity such as of the existence and function of potential lipid rafts.

Are 3D cultures able to model in vivo tumor behavior? A spatial lipidomics and multiomics investigation

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Introduction: Metabolic reprogramming, including dysregulated lipid metabolism, is a central feature of cancer cells. Most normal cells build up their membranes from dietary lipids. In contrast, cancer cells reactivate the de novo lipogenesis. The rewiring of lipid metabolism has been linked to the activation of oncogenic signaling pathways and crosstalk with the tumor microenvironment. To understand the aggressiveness and metastatic potential of tumors, the exploration of tumor heterogeneity is of great interest.

Methods: 3D mouse and human breast cancer cell cultures with different aggressiveness and 4T1 subcutaneous mouse allografts were used as in vitro and in vivo models, respectively. Subsequent, thin cryosections were prepared from the frozen samples, and areas corresponding to ca. 100 cells were dissected by laser-microdissection (LMD). From the spheroid sections, the middle circles were dissected to represent the radial organization. From the tumor sections, different regions were dissected based on their morphology to represent tumor heterogeneity. The LMD samples obtained from adjacent, parallel cryosections were treated according to the omics methods to be applied, like quantitative spatial lipidomics (Varga-Zsíros et al. 2023), proteomics and transcriptomics.

Results: We identified a radial gradient in the lipidomic profile of 4T1 spheroids, which correlated well with nutrient availability, i.e., monoene lipid species accumulated in the center, whereas polyene species displayed higher concentrations at the edges. Toward the spheroid center, we also detected increasing levels of ether lipids, sphingolipids, and lysosomal marker lipids. By using 4T1 mouse allografts, we showed that subcutaneous tumors displayed notable lipidomic alterations from intact toward partially necrotic regions. Similarly to 3D cultures, one of the most pronounced changes was the accumulation of ether lipids, which might correlate with tumor aggressiveness. Integrative proteomics and transcriptomics data supported these lipidomic changes and revealed further comprehensive cancer-associated disturbances.

Conclusion: We demonstrate that spatially resolved multiomics analysis of 3D cultures are able to mimic in vivo conditions. We expect that such a systemic level investigation of tumor heterogeneity will improve our understanding of cancer initiation and progression and will help to identify new therapeutic targets.

The emerging role of the mitochondrial fatty-acid synthase (mtFAS) in the regulation of energy metabolism

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Background: Malonyl-CoA synthetase (ACSF3) catalyzes the first step of the mitochondrial fatty acid biosynthesis (mtFAS). The end-product of this pathway is lipoic acid, an essential cofactor for several mitochondrial enzymes involving in energy production. Mutations in ACSF3 cause CMAMMA a rare inborn error of metabolism. The clinical phenotype is very heterogeneous, with some patients presenting with neurologic manifestations. In some children, presenting symptoms such as coma, ketoacidosis and hypoglycemia are suggestive of an intermediary metabolic disorder. The overall pathophysiological mechanisms are not understood.
Materials and Methods: In order to study the role of mtFAS in the regulation of energy metabolism we performed a comprehensive metabolic phenotyping with Seahorse technology in fibroblasts from healthy controls and ACSF3-deficient patients. SILAC-based proteomics and lipidomic analysis were performed to investigate the effects of hypofunctional mtFAS on proteome and lipid homeostasis of complex lipids.
Results: Our data clearly confirmed an impaired mitochondrial flexibility characterized by reduced mitochondrial respiration and glycolytic flux due to a lower lipoylation degree. These findings were accompanied by the adaptational upregulation of β-oxidation and by the reduction of anaplerotic amino acids as compensatory mechanism to address the required energy need. Finally, lipidomic analysis demonstrated that the content of the bioactive lipids sphingomyelins and cardiolipins was strongly increased.
Discussion: Our data clearly demonstrate the role of mtFAS in metabolic regulation. Moreover, we show that mtFAS acts as mediator in the lipid-mediated signaling processes in the regulation of energy homeostasis and metabolic flexibility.

Combining lipidomics, metabolomics and cytokinomics to characterize the immunometabolic landscape associated with organ failure and mortality in acutely decompensated cirrhosis

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Introduction: Patients with decompensated cirrhosis present chronic systemic inflammation causally linked to the development of multi-organ failure, a condition with high short-term mortality known as acute-on-chronic liver failure (ACLF). In this study we analyzed the systemic secretome through a combination of lipidomics, metabolomics and cytokinomics to identify biomarkers associated with ACLF development and mortality in patients with AD cirrhosis.

Material & Methods: Targeted lipidomics of immunomodulatory lipid mediators and untargeted metabolomics were performed by liquid chromatography-mass spectrometry on plasma and serum samples collected at hospital admission in 766 decompensated cirrhotic patients. Plasma cytokines/chemokines/growth factors were evaluated using multiplex bead-based immunoassay technology. A multifactorial regression network was built to identify significant associations between high-dimensional multi-omics data and outcome of AD cirrhosis.

Results & Discussion: Plasma lipidomics identified a lipid mediator signature characterized by increased prostaglandin (PG) E degradation products and reduced sphingosine-1-phosphate (S1P) levels that was significantly associated with ACLF development in decompensated patients. A signature associated with 90-day mortality composed of PGE degradation products, eicosapentaenoic acid (EPA), and 17-hydroxy-DHA (17-HDHA) was also identified in these patients. Likewise, serum metabolomics uncovered a signature associated with ACLF development and mortality that included metabolites of the tryptophan pathway (quinolinic acid), glucuronidation (glucuronic acid), and norepinephrine (hydroxy-3-methoxyphenylglycol sulphate). Moreover, cytokinomics revealed that interleukin-6 was the only cytokine significantly associated with ACLF and/or mortality. All these biomarkers significantly correlated with neutrophil blood counts except S1P, which correlated with platelets. Finally, the multi-omics integration identified a network of interactions between the omega-3 17-HDHA and the dicarboxylic acids, which frequently accumulate in the circulation in conditions of mitochondrial dysfunction.

Conclusion: The network-based multi-omics analysis allowed us to describe the circulating immunometabolic landscape of patients with decompensated cirrhosis and to identify new candidate biomarkers associated with clinical outcome and short-term survival.

A redox stress-modulated phospholipase A2 remodels lipids to regulate ferroptosis in cancer

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Introduction: Ferroptosis is a non-apoptotic, iron-dependent, and phospholipid peroxide (PLOOH) driven cell death mechanism. Cellular responses to monitor excess PLOOH include the reductive activity of glutathione peroxidase 4 (GPX4) and enzymes such as FSP1 to produce metabolites that have radical-trapping antioxidant activity. However, it remains unclear how certain mutations in cancer and dysfunctional lipid metabolism, specifically in lung adenocarcinoma (LUAD), contribute to promote ferroptosis resistance.

Method & Results: Through mechanistic investigation using genetic models, confocal microscopy, lipidomics, and in vivo studies, we discovered a novel function for a redox stress modulated phospholipase A2 (PLA2) enzyme in regulating ferroptosis through its unique specificity in recognizing and remodeling PLOOH. Under increased oxidative stress, PLA2 localizes to the endomembrane system, which includes the endoplasmic reticulum (ER), Golgi network, and lysosomes, which are essential propagation sites of PLOOH in ferroptosis. We also found that PLA2 activity and expression is controlled by NRF2 and its principal negative regulator KEAP1, which is a gene commonly mutated in LUAD. Lipidomic analyses further showed a distinct shift in cellular lipid composition when PLA2 function is perturbed and when ferroptosis is triggered. Furthermore, we identified a clinical-stage PLA2 inhibitor and its ability to synergize with GPX4 inhibitors to increase the susceptibility of cancer cells to undergo ferroptosis. Finally, we show anti-tumor efficacy in orthotopic and subcutaneous models of LUAD, where treatment with a PLA2 inhibitor alone can inhibit tumor growth and prolong survival substantially.

Conclusion: Therefore, our work presents a novel function for a conserved lipid metabolism enzyme, PLA2, and its potential to be a target to induce cell death through ferroptosis in a subset of lung cancers.

Unlocking the potential of MALDI-IMS for an in-depth characterization of the tumor microenvironment: lipids as a Trojan horse

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Introduction: Given that the tumor microenvironment (TME) plays a crucial role in tumor promotion and metastasis, a deeper comprehension of the intricate network orchestrating TME heterogeneity is critical to developing new tools for patient stratification and identifying biomarkers for immunotherapy. To address this challenging complexity in the specific context of colon cancer (CC), we aim to characterize the lipidome of the tumor immune microenvironment (TIME), which intimately influences tumor fate and its response to therapy.

Methods: 10 CC patients and 7 healthy controls (HC) were enrolled in the Gastroenterology Dept. of the University Hospital Son Espases. Cells were isolated from peripheral blood by FACS. Sorted cells (~10^5 cells) were applied on poly-L-lysine-coated glass slides and analyzed by Matrix-Assisted Laser Desorption Ionization imaging mass spectrometry (MALDI-IMS). In parallel, 5 CC patients who underwent colon surgical resection were enrolled in the General Surgery Unit. Tumor-infiltrating lymphocytes (CD4+, CD8+ T Cells, and B Cells) and tumor-associated macrophages (M1 and M2-like) were isolated after tumor enzymatic disaggregation and measured following the same method. Last, the lipidome of tumor sections was analyzed by MALDI-IMS.

Results and Discussion: The method developed allowed us to establish the lipidome using a relatively low number of cells. Main membrane lipid species were identified in circulating and tumor-infiltrating immune cell populations. The analysis showed a distinctive pattern of lipid species profile for every cell type, confirming the lipid fingerprint's specificity. Furthermore, the comparison between HC and CC patients revealed a significant impact of clinical conditions on arachidonic acid-containing phosphatidylethanolamine (PE) and PE-plasmalogen species, and their prominence was even higher in tumor-infiltrating cells. Finally, we could compare between all isolated immune cell subsets and different immune infiltrates detected on tumor tissue images, identified by their lipidome using MALDI-IMS.

Conclusions: Each immune cell type shows a unique lipid fingerprint, which was altered in CC patients. The sensitivity of the methodology developed provides a unique tool to analyze the lipidome of minor subsets of immune cells. Once characterized, these profiles can be recognized and localized within the tumor tissue using imaging techniques (MALDI-IMS), enabling an in-depth approach to TME characterization.

Modulation of tumor immunity by autotaxin-producing cells in the tumor microenvironment.

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Although metastasis is the principal cause of cancer-related deaths, the molecular aspects of the role of stromal cells in the establishment of the metastatic niche remain poorly understood. One of the most prevalent sites for cancer metastasis is the lungs. Several studies have shown that many cell types in the lung stroma could potentially provide a rich source of autotaxin (ATX), an enzyme with lysophospholipase D activity which generates lysophosphatidic acid (LPA), a bioactive lipid mediator, that promotes cancer progression. The ATX-LPA axis has been documented to support cancer cell proliferation, migration, survival, invasion, angiogenesis, metastasis and therapeutic resistance in both humans and mice. In the present study, We sought to identify individual autotaxin-producing stromal cells are responsible for the progression of melanoma to lung metastases.

We investigated the contribution of ATX derived from (1) alveolar type II epithelial (ATII) pneumocytes; (2) fibroblasts and (3) CD11b+ myeloid cells, in the pathophysiology of metastasis. To achieve this, we used the B16-F10 syngeneic melanoma murine model, which readily metastasizes to the lungs when injected intravenously. We used the Cre/lox system to generate three conditional knockout (KO) mice, in which ATX is specifically deleted in (1) ATII cells (i.e. Sftpc-KO), (2) fibroblasts (i.e. Type 1 collagen-KO), and (3) CD11b+ myeloid cells (i.e. LysM-KO).

We found that targeted KO of ATX in ATII cells, and CD11b+ myeloid cells, but not fibroblasts, significantly reduced the metastatic burden of melanoma by 30% ($p=0.0283$) and 50% ($p=0.0002$), respectively, compared to their WT counterparts. Moreover, we found upregulated levels of IFNγ (unpaired t-test, $p<0.0001$) and TNFα ($p=0.0003$), which could favor the increase in infiltrating CD8+T cells observed in the tumor regions of Sftpc-KO mice. LysM-KO mice presented profound a 50% decrease of lung neutrophils ($p=0.0004$) together with upregulated level of IL-27 ($p=0.0494$), compared to LysM-WT mice.

Our data identify for the first time the contribution of host ATII and CD11b+ cells of innate immunity as stromal sources of ATX in the progression of melanoma to lung metastasis. Importantly, our data from the CD11b+ myeloid KO mice suggest that some ATX-producing cells can modulate the tumor microenvironment by impairing the anti-tumor immunity.

Liver organoids as 3D model for assessing bioactive lipids metabolic effect: A novel application

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Introduction: The bioactive lipids, fatty acid esters of hydroxyl fatty acids (FAHFAs), were described as positive regulators of glucose metabolism, exhibiting anti-diabetic activity. In metabolic disorders like cancer and diabetes mellitus, the evaluation of glucose uptake is crucial. FAHFAs have been tested in animal models for their activity and interaction across organs, including the liver. 3D cell models allow predictability of FAHFAs efficacy and bioactivity in humans, replacing and reducing the use of animals. Organoids are 3D cell systems used to simulate organ properties in vitro. These assays coupled with modern methods empower new and accurate systems in metabolic disease studies. Since the liver is a key metabolic organ tightly controlled by insulin, this work introduces a novel strategy for hepatic analysis by determining bioactive lipids influence on glucose activity.
Material and Methods: Using Pluripotent Stem Cells (iPSC), we developed a liver 3D model to test the FAHFAs activity by applying the non-radioactive 2DG (2-deoxy-glucose) assay. We used confocal microscopy to determine the morphological state of mature liver organoids. Organoids were exposed to FAHFAs and 2DG to assess the glucose uptake activity, followed by direct liquid-liquid extraction using an in-house protocol. We used HILIC-based UHPLC-MS/MS to quantify the 2DG uptake accurately and for further non-targeted metabolomic analysis. MS-DIAL and MetaboAnalyst were used for identity, quantity, and statistics.
Results and discussion: The results showed a significant increase ($p<0.05$) in the glucose uptake across the bioactive lipids-stimulated groups, resembling a lipokine-like activity of the FAHFAs. The bioactive lipids stimulated the organoids at the same level, likely to have same trigger mechanisms. Compared to drug studies, liver organoids proved to be a rapid, reproducible, and useful model under the conditions needed for reducing animal use. The UHPLC, ESI and MS/MS system confirmed to be an exact set-up in metabolic evaluations. Further metabolomics analysis will be conducted to understand metabolic changes in liver organoids and different cell-origin organoids will be tested equally.
Conclusion: The novel approach of using organoids to evaluate bioactive lipids was able to assess the FAHFAs activity accurately and can be used for evaluation across other organs.

Lipid signatures of Alzheimer´s disease-associated retinal inflammation: the imbalance of the endocannabinoid system

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Introduction: Alzheimer's disease (AD) develops extra-cerebral manifestations in the retina, which is then considered a "window to the brain". Here, we explored for the first time the possible alterations of the endocannabinoid system (ECS) and the onset of gliosis in the retina of AD mice, since ECS dysregulation has recently been shown in AD brains.
Materials and Methods: The retinas explanted from 12 month-old Tg2576 (TG) mice over-expressing the amyloid precursor protein (APP) were used. The main endocannabinoid-binding receptors (CB2, CB1 and TRPV1), metabolic enzymes [NAPE-PLD and FAAH for anandamide (AEA); DAGLα/β and MAGL for 2-arachidonoylglycerol (2-AG)], and APP were quantified through Western blot, and were correlated when appropriate through linear regression. CB2 localization in the retinal layers was investigated by confocal microscopy and quantified as mean fluorescence intensity of immuno-stained cryosections. Gliosis was analyzed on retinal cryosections in terms of microglia number (IBA1+ cells) and astrocytes/Müller cells reactivity (GFAP immunostaing). Retinal thickness was quantified via bisbenzimide nuclear dye staining and imageJ software.
Results: Western blot analysis of the ECS revealed the up-regulation of CB2 in TG retinas (1.5 folds over WT; p=0.032) and this result was consistent with plot profile graphs and fluorescence intensity in anti-CB2 immuno-stained cryosections. CB2 expression was increased in all retinal locations except for the ganglion cell layer. No statistically significant differences were found for the other enzymes and receptors of the ECS. Intriguingly, linear regression analysis on individual animals showed a significant correlation between CB2 and FAAH (r=0.787; p=0.021), DAGLα/β (α:r=0.740; p=0.036 – β:r=0.927; p<0.001), and APP (r=0.788; p=0.020). CB2 up-regulation was also accompained by a significant increase of microglia cells in TG versus WT (p=0,002), and a trend towards increase in GFAP reactivity. Finally, the retinal architecture was not affected, and the retinal thickness remained the same in WT and TG mice.
Discussion: These findings indicate that the ECS may play a role in AD-associated retinal inflammation, resembling previous observations in the AD brain. CB2 appears to play a central role, and its positive correlation with increased APP, FAAH and DAGLα/β suggests decreased AEA and increased 2-AG levels in the retinas with a more severe AD phenotype.

Evaluation of endocannabinoid derivatives in the activation of colon epithelial cells. Different effects on different cell types

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Introduction: Inflammatory bowel diseases (IBD) are chronic inflammatory disorders of the gastrointestinal tract. While rarely lethal, IBD shows increasing prevalence and incidence profoundly affecting patients' quality of life. Available IBD treatments produce severe side effects and progressively lose their efficacy. Thus, there is a need to explore new ways to tackle IBD. Epithelial cells in the gastrointestinal tract form an impermeable barrier between the luminal content and the connective tissue. Moreover, epithelial cells can act as antigen-presenting cells to the immune system. However, their role in IBD is barely studied. Bioactive lipids are important modulators of inflammation. For instance, the endocannabinoids 2-arachidonoylglycerol (2-AG) and N-arachidonoylethanolamine (AEA) have anti-inflammatory and pro-apoptotic properties. Moreover, cyclooxygenase (COX)-2 can metabolize 2-AG and AEA, into glycerol ester (PG-G) and ethanolamide (PG-EA) derivatives, respectively, of the prostaglandins. Several PG-G and PG-EA have anti- or pro-inflammatory effects in some settings, but they remain poorly studied. To better understand the potential role of these bioactive lipids in colon inflammation, we studied their capacity to modulate inflammation and epithelial integrity in epithelial cell lines and in colon organoids.
Material and methods: We evaluated the capacity of PG, PG-G, and PG-EA to modulate the inflammatory status (cytokine production by ELISA and qPCR) on activated HT29 cells, CaCo2 spheroids, and colon organoids. Also, we evaluated the epithelium regeneration and integrity.
Results: Upon inflammatory activation, PGD2, PGD2-G, and PGD2-EA increased the expression of pro-inflammatory cytokines in HT29 cells, while in CaCo2 spheroids, only PGD2-G and PGD2-EA increased cytokine production. Conversely, when tested on activated colon organoids, PGE2 decreased the expression of pro-inflammatory markers. Interestingly, PGD2 and PGD2-EA increased stem cell signaling in colon organoids evaluated by the expression of specific markers.
Conclusion: These results support the interest in studying PG, PG-G, and PG-EA in colitis. Moreover, our results showed the diversity of effects that PG-G and PG-EA can exert depending on the condition and the cell type. Finally, several of these molecules showed interesting effects regarding colon stem cell regulation, posing them as potential therapeutic options to stimulate colon regeneration after inflammatory insults.

Regulation of SREBP1c transcriptional activity by oleate

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The transcription factor sterol regulatory element-binding protein 1c (SREBP1c) plays a crucial role in regulating lipid homeostasis. Although its regulation by various nutritional and hormonal stimuli is well-known, the specific molecular mechanisms underlying these adaptive responses are still unclear. However, preliminary findings indicate that a decrease in intracellular oleate concentration leads to reduced SREBP1c transcriptional activity, and experimental evidences suggest that SREBP1c may undergo acylation by oleate or its metabolites. Acylation is a post-translational modification that affects protein localization, stability, and interactions with other proteins.The objective of this study is to characterize the molecular mechanisms involved in the regulation of SREBP1c activity by oleate. Using a mass shift technique,we demonstrated that mature SREBP1c in mouse liver exists in three different acylation states: unacylated, monoacylated, and diacylated. Notably, acylated forms of SREBP1c were more prevalent in the livers of mice fed an obesogenic, high oleate diet compared to a non-obesogenic, low-fat diet. Through bioinformatics analysis, a cysteine residue on SREBP1c was identified as a potential acylation site. Mutation of this cysteine residue resulted in the prevention of SREBP1-c proteolytic cleavage, hampering the maturation of the transcription factor, and reducing its nuclear translocation and transcriptional activity in hepatocarcinoma cells. In addition, Alphafold modeling, predicted the interaction between SREBP1-c and a specific acylating enzyme, ZDHHC5. Interestingly, inhibition of this enzyme reduced SREBP1c transcriptional activity as well as lipid droplet formation in response to an oleate challenge. Taken together, our data suggest that SREBP1-c is acylated at least on one residue. The acylation by oleate appears to mediate the transcriptional activity of this factor as well as modulating downstream response implicated in lipid metabolism. This novel mechanism of SREBP1c regulation would represent a significant advancement in the field of lipid metabolism and contribute to a better understanding of metabolic disorders-associated diseases. The identification of the specific acylating enzyme involved in SREBP1c regulation suggests its potential as a novel drug target for therapeutic interventions in lipid metabolism and associated diseases.

Acute response to switch to high lipid diet predicts later susceptibility to obesity

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Introduction: Metabolic flexibility, i.e. the ability to switch between catabolism of glucose and lipids at both whole-body and tissue level, is associated with obesity resistance. Here we whether changes in metabolic flexibility induced by acute short term high-fat diet (HFD) feeding can predict later development of obesity in A/J mice and C57Bl/6J (B6) mice, a model of resistance and propensity to obesity, respectively.
Methods: Male A/J and B6 mice at the age of 8 weeks were exposed to standard (STD) or HFD containing 60% of calories as fat for 3 days. They underwent oral glucose tolerance test (OGTT), insulin tolerance test (ITT), lipid tolerance test (LTT) or 4-days indirect calorimetry (Somedic, Sweden) either at laboratory temperature or at thermoneutrality, after 2-week- acclimatization to the respective temperatures. Alternatively, food intake was characterized or mice were euthanized after or during the 3rd day of dietary treatment. Plasma parameters, such as glycemia, TAG, insulin and leptin and expression of selected genes were characterized in epidydimal white adipose tissue (eWAT).
Results: A/J mice after the short-term exposure to HFD as compared to B6 mice displayed healthier metabolic parameters, such as lower incremental AUC from OGTT and ITT and blunted increase in TAG levels after lipid load in LTT. The circadian rhythms in RQ and food intake were preserved after 3 days on HFD in A/J mice only. A/J mice also adapted to HFD by an increase in energy expenditure, resting metabolic rate (RMR) and body temperature (BT) both in mice pre-acclimated to laboratory temperature and thermoneutrality. As we did not detect any changes in heat losses through the tail as the main thermoregulatory organ in mice, we assumed higher body-temperature set point was set. We detected higher expression of genes for leptin and SOCS3 in eWAT and higher plasma leptin levels in A/J mice on HFD.
Conclusions: Our results document strain-specific difference in metabolic flexibility between A/J and B6 mice during short-term HFD-feeding. These difference predicts propensity to obesity later on during the life of the animals. Further studies are required to characterize contribution of various organs to the strain-specific metabolic phenotypes. Supported by Czech Science Foundation (22-07004S).

To Cleave or not to Cleave? Insights into HSD10's Promiscuity in Lipid Metabolism

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Introduction: A series of enzymes are involved in the homeostasis of cardiolipins, which are dimeric phospholipids crucial for inner mitochondrial membrane function. Recently, human 17β-hydroxysteroid dehydrogenase 10 (HSD10; EC 1.1.1.3; OMIM 3000256) has been described as novel cardiolipin metabolizing enzyme. HSD10 is a multifunctional mitochondrial protein that plays an important role as a structural component of the mitochondrial RNAse P complex and, additionally, catalyses one step of the isoleucine degradation pathway. Pathological variants of HSD10 are associated with HSD10 disease, a multi-systemic disorder primarily affecting males. The clinical presentations of HSD10 disease range from mild to severe, with a complete loss of the protein being incompatible with life. Here we investigated the newly described phospholipase C-like activity of HSD10 towards cardiolipins and its physiological relevance.

Material and Methods: To explore the lipidomic consequences of the proposed cardiolipin-cleaving activity, we conducted experiments using different cellular model systems with different genetic backgrounds and lipid environments. HSD10 knock-down cell lines were generated, and, along with patient fibroblasts, analysed using an LC-MS/MS workflow with a special analytical focus on cardiolipins.

Results and Discussion: In contrast to the postulated function, our experimental findings revealed that cardiolipin homeostasis is completely robust towards modulation of HSD10 expression. The analysis of HSD10 knock-down cell lines, as well as patient fibroblasts showed no measurable effect on cardiolipins, suggesting that the cardiolipin-cleaving activity of HSD10 may not be physiologically relevant. HSD10 is not new to controversy regarding its substrate specificity. Thus, we hypothesize that the essential role of HSD10 in the RNAse P complex directed its evolutionary trajectory towards optimizing its structural function, thereby increasing its catalytic promiscuity.

Conclusion: These findings highlight the importance of investigating the multifunctional roles of moonlighting enzymes such as HSD10 and their impact on cellular processes. Further discrepancies between activities and specificities found in enzyme assays and their actual physiological contribution to the lipid metabolism exist. These have to be resolved before a comprehensive understanding for the architecture of the lipid metabolic network and its regulation can be made.

Impact of arachidonic acid-containing phosphatidyl inositol on colonocyte stem and progenitor cell differentiation: implications in colon cancer epithelial subpopulations

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Introduction: The proper regulation of colonocyte proliferation and differentiation is crucial for maintaining tissue homeostasis, as disruptions in this process can lead to tumor initiation and cancer development. MALDI-IMS has revealed the sensitivity of the cell membrane lipidome to the pathophysiological state of the cell, particularly during differentiation and tumorigenesis.
Methods: To investigate the underlying regulatory mechanisms governing the differential lipid phenotype, researchers utilized FACS to isolate colonocyte subpopulations based on EPHB2 levels from healthy and tumor patient-derived biopsies. Lipidome and transcriptome of each subpopulation were analyzed using MALDI-IMS and gene expression microarray, and multiomic integrative analysis employing a systems biology approach was conducted. Additionally, the impact of prostaglandin metabolism on colonocyte differentiation was tested using mouse colon organoids and pharmacological inhibition and stimulation.
Results: The -omics profiles demonstrated coordinated regulation of PUFA- and MUFA-containing phospholipid species during colonocyte differentiation. In healthy subpopulations, stem cell-like colonocytes exhibited higher levels of PI 38:4 and PI 36:4, compared to differentiated colonocytes, which displayed an enrichment of PI 36:1 and 34:1. Gene expression profiles correlated with the lipidomic changes, showing an enrichment of eicosanoid metabolism in differentiated colonocytes. In tumor stem cell-like colonocytes, AA-containing PI levels were even higher, and gene expression was enriched in fatty acid biosynthesis, indicating a different metabolic program. Weighted gene co-expression analysis identified modules correlating with the levels of the specific PI species in each subpopulation, revealing metabolic hub genes and predicted transcription factors associated with the colonocyte subpopulations and pathological conditions. Organoid pharmacological assays highlighted the cell subtype-specific effects of prostaglandins and suggested differential regulation of membrane and nuclear prostaglandin receptors.
Conclusion: This study provides novel insights into the transcriptomic networks involved in the regulation of specific membrane lipids in healthy and tumor colonocyte differentiation states, underscores the versatility and reliability of MALDI-IMS, and emphasizes the need for a deeper understanding of lipid metabolism.

Leptin surge during early postnatal age as marker of body weigh trajectory in mice

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Introduction: In the perinatal period, a number of factors play an important role in influencing energy metabolism and the development of obesity in adulthood. Leptin is a key metabolic hormone secreted primarily by adipose tissue and plays an important role in the regulation of food intake and body weight (BW). Previous studies have suggested that circulating leptin levels during the postnatal surge could imprint/predict the propensity to obesity in both mice and rats, and have shown that leptin could be a potential programming factor. The aim of this study was to learn whether leptinaemia during its postnatal surge could predict the interindividual differences in BW development independent of dietary obesity in mice.

Materials and methods: In two complementary studies, two inbred strains, obesity-prone C57BL/6 (B6) and obesity-resistant A/J mice, were used. Plasma levels of leptin, adiponectin and lipid metabolism markers, gene expression and secretion of leptin (Lep) in various adipose depots were assessed at 2 and 4 weeks of postnatal age. The relationship to the development of obesity induced by feeding a high-fat diet (HFD lipids ~35% wt/wt,) was determined between 12 and 24 weeks of age.

Results and discussion: BW of newborn mice during lactation at week 2 was lower in A/J compared with B6 mice, while no difference was detected after weaning at week 4, unlike circulating leptin levels, which were lower in B6 mice than A/J mice in both 2 and 4 weeks. In general, the Lep expression was higher in A/J as compared to B6 mice in both 2 and 4 weeks. Leptin secretion from various tissues was higher at 2 weeks in comparisson with 4 weeks with no significant differences between strains. HFD-feeding resulted in a more pronounced accretion of BW in B6 as compared with A/J mice. Leptinaemia at week 2 but not week 4 correlated with BW of HFD-fed mice. Spearman´s correlation coefficients revealed that only leptin levels in 2-week-old pups correlated significantly with BW during the course of the feeding study and animal's growth.

Conclusion: Our results confirmed that the postnatal surge of leptin can imprint propensity to dietary obesity in mice. The circulating leptin levels during their postnatal surge represent a robust marker of BW development during aging in mice. This project is supported by Ministry of Health of the Czech Republic (AZV NU20-07-00026).

The Influence of Adipose Tissue on Colon Cancer Progression: A Transcriptomic Investigation of Tumor Microenvironment

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Introduction: The colon tissue directly interacts with two vital fat depots within the peritoneal cavity: the anterior omentum and posterior mesentery. This often results in adipocyte presence at the invasive margin of advanced colon tumors and within the tumor mass itself, raising questions about the implications of this close interaction for cancer progression and prognosis. In this context, we aim to examine the molecular crosstalk between the visceral adipose tissue VAT) and the colon cancer tumor microenvironment through transcriptomic analysis of colon cancer samples and neighboring VAT.

Materials and method: We conducted a transcriptomic analysis using the Clariom™ S Pico Assay platform on tissue samples from 13 CC patients at different TNM stages (pT2, pT3, and pT4). We analyzed the primary colon cancer (CC) tissue, as well as neighboring visceral adipose tissue (pVAT), from each patient. Additionally, distant mesenteric VAT (mVAT) samples were included for comparison.

Results and Discussion: Our study found that pVAT exhibited distinct gene expression patterns compared to mVAT, indicating its potential role in colon cancer progression. Notably, pVAT exhibited downregulation of pathways associated with lipolysis, lipogenesis, and adipogenesis. On the other hand, we observed transcriptional changes at different TNM stages in pVAT, with increased expression of fibrosis-related genes in later stages. Surprisingly, we also detected expression changes in mVAT, including upregulation of cytokines and chemokines in pT3 and pT4, indicating the potential for crosstalk through systemic circulation or across the peritoneal cavity. Finally, we conducted transcriptomic analysis on primary CC samples and categorized them based on their transcriptomic profiles using the consensus molecular subtype (CMS) classification system. Remarkably, pVAT associated with the CMS4 subtype (mesenchymal tumors characterized by prominent fibrosis) exhibited a higher number of differentially expressed genes compared to other subtypes. These genes were predominantly associated with endothelium and vascular smooth muscle, highlighting the distinct molecular characteristics of pVAT associated with this subtype.

Conclusion: In summary, our study underscores the importance of investigating the role of VAT in colon cancer progression. Additional research is needed to validate these findings and gain deeper insights into the underlying mechanisms driving this relationship.

Towards a better understanding of plasmanyl ether lipids and plasmalogens

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Introduction: Among the large variety of lipids, there is the class of ether lipids that fulfills many important functions including the support of brain structure and function, protecting the eye from cataracts, enabling male fertility and signaling. Ether lipids can be classified into 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. In humans, deficiency in ether lipids due to mutations in the early metabolic steps affects many tissues and results in cataracts, bone malformations, reduced fertility, neurodevelopmental abnormalities and a growth defect. Investigations to clearly dissect the importance of the two ether lipid subclasses that ultimately lead to the aforementioned defects were so far impeded by the fact that enzymes involved in this metabolic route were not yet assigned to their genetic information. Over the last decades, many of these enzymes have received their genetic identity, one of the most recent ones being plasmanylethanolamine desaturase (PEDS1). This enzyme introduces the vinyl ether double bond and is therefore indispensable for the biosynthesis of all plasmalogens.
Materials and methods: To better understand the contributions of plasmanyl lipids and plasmalogens, we are relying on two mouse models with knockouts in enzymes of the ether lipid biosynthesis pathway. The first one, Gnpat knockout, has a defect in the first enzyme and these mice are completely ether lipid-deficient. The second model, Peds1 knockout, was acquired from the EMMA consortium and presents with a selective plasmalogen deficiency. Results and discussion: In a first step, both mouse models were bred on the same background to allow a side-by-side comparison. Our first results from investigations of brain function, cataract formation, fertility and osteogenesis clearly show that Peds1 knockout results in a different phenotype than that of Gnpat knockout. This may point to the presence of plasmanyl lipids being beneficial for the mammalian body, as at least some of the investigated cellular processes are not hampered if only plasmalogens are missing.
Conclusion: Our study will shed light on the importance of plasmalogens and also dissect their role from that of the plasmanyl lipids, which comprise important biologically active lipids but have not received adequate attention so far in the field of ether lipid research.

Role of long-chain acyl-CoA synthetase 4 (ACSL4) in colitis-associated colorectal cancer

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Introduction: Long-chain acyl-CoA synthetase 4 (ACSL4) converts highly unsaturated fatty acids (HUFAs) such as arachidonic acid and eicosapentaenoic acid (EPA) into their acyl-CoAs and plays an important role in maintaining HUFA-containing phospholipids. We have previously found that ACSL4 is also involved in prostaglandin (PG) production by the regulation of arachidonate metabolism. It has been recently reported that the expression of ACSL4 is upregulated or downregulated in tumor tissues and is involved in carcinogenesis and cancer development. However, little is known about the role of ACSL4 in the pathogenesis of colorectal cancer and other colorectal diseases.

Materials and methods: To clarify the role of ACSL4 in colitis and colitis-associated cancer, we established dextran sodium sulfate (DSS)-induced colitis model and azoxymethane (AOM) /DSS-induced colorectal cancer model, and then investigated the effects of ACSL4 deficiency on the pathogenesis of these mouse models using wild-type (WT) and ACSL4 knockout (KO) mice.

Results and discussion: ACSL4 deficiency markedly alleviated DSS-induced colitis, as characterized by reduced weight loss, reduction in the incidence of diarrhea, and reduced amount of bloody stool. Histological analysis showed that colon mucosal atrophy was also suppressed in ACSL4 KO mice. AOM/DSS-induced colon tumorigenesis was also suppressed by ACSL4 gene deletion. In ACSL4 KO mice, the colon length was increased and the number of polyps was decreased compared to WT mice. We further found that ACSL4-villin-conditional KO mice which lack ACSL4 only in their intestinal epithelial cells exhibited similar phenotype to ACSL4 KO mice in both colitis and cancer models. Moreover, our LC-MS/MS analysis revealed that ACSL4 deficiency reduced the levels of HUFA-derived acyl-CoAs and HUFA-containing phospholipids in colon tissues. On the other hand, PG production in colitis was not significantly affected, whereas PGE2 and PGD2 levels in colon cancer were slightly increased by ACSL4 deficiency. These results suggested that changes not in PG production but in membrane phospholipid composition of intestinal epithelial cells by ACSL4 deficiency might suppress colitis and colitis-associated carcinogenesis.

Conclusion: ACSL4 expressed in intestinal epithelial cells plays a critical role in the exacerbation of intestinal inflammation and the progression of colitis-associated cancer.

Plasma and cerebrospinal fluid cholesterol esterification is hampered in Alzheimer's Disease

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Introduction: Several epidemiological studies indicate a strong inverse association between the risk of developing Alzheimer's disease (AD) and plasma HDL-C levels. The mechanism by which plasma HDL influence the pathogenesis and progression of AD is still unsolved and since cholesterol esterification is a crucial step in HDL metabolism it could be involved. The purpose of this study was to evaluate cholesterol esterification and HDL subclasses in plasma and cerebrospinal fluid (CSF) of AD patients.
Materials and Methods: The study enrolled 70 AD patients and 74 cognitively-normal controls comparable for age and sex. Lipids and lipoprotein profile, cholesterol esterification, and cholesterol efflux capacity (CEC) were evaluated in plasma and CSF using assays set for measurement in plasma, which were appropriately modified for CSF.
Results and Discussion: AD patients have normal plasma lipids, but significantly reduced unesterified cholesterol and unesterified/total cholesterol ratio. Lecithin: cholesterol acyltransferase (LCAT) activity and cholesterol esterification rate (CER), two measures of the efficiency of the esterification process, were reduced by 29% and 16%, respectively, in plasma of AD patients. Plasma HDL subclass distribution in AD patients was comparable to that of controls but the content of small discoidal preβ-HDL particles was significantly reduced. In agreement with the reduced preβ-HDL particles, cholesterol efflux capacity mediated by the transporters ABCA1 and ABCG1 was reduced in AD patients' plasma. The CSF unesterified to total cholesterol ratio was increased in AD patients, and CSF CER and CEC from astrocytes were significantly reduced in AD patients. In the AD group, a significant positive correlation was observed between plasma unesterified cholesterol and unesterified/total cholesterol ratio with Aβ1-42 CSF content.
Conclusion: Taken together data indicate that cholesterol esterification is hampered in plasma and CSF of AD patients, and that plasma cholesterol esterification biomarkers (unesterified cholesterol and unesterified/total cholesterol ratio) are significantly associated to disease biomarkers (i.e., CSF Aβ1-42).

Measuring lipid order to assess cell membrane permeability, lipid nanoparticle stability and membrane drug interaction

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Introduction: The state of synthetic and biological lipid systems can be characterized by the order of its lipid molecules. This phase state correlates with physical properties of the lipid system: For example, low lipid order goes along with low lipid packing density, low bending stiffness, high fluidity, high compressibility and vice versa.Our aim is to exploit the dependency of application relevant properties such as cell membrane permeability, lipid nanoparticle (LNP) stability and membrane drug interaction on the lipid order.
Materials and Methods: We use membrane embedded dyes such as Laurdan that are sensitive to the surrounding lipid order state and are incorporated either during the preparation of synthetic systems or in case of biological samples by dissolving them into the buffer using a solvent. The lipid order is quantified by measuring the fluorescent emission spectra and calculating the generalized polarization (GP).Furthermore, cell membrane permeability is quantified by the uptake of a fluorescent dye into the cytosol while simultaneously measuring GP using fluorescence microscopy. The change of lipid order in LNP suspensions is determined by determination of GP as function of time at different temperatures and serves as measure for LNP stability. The interaction of cell membranes and drugs is evaluated by measuring GP after short and long-time drug exposure.
Results and discussion: First, we found that the permeability of cellular membranes is directly related to the plasma membrane order determined by GP measurement. This finding can facilitate the optimization of permeabilization and transfection protocols. Second, irreversible structural changes within LNPs can be determined measuring GP as function of time or temperature demonstrating that LNP stability can be optically assessed in situ under varying conditions even in the frozen state far below 0°C.Third, lipid phase transitions within cellular membranes are strongly influenced by short and long-time exposure of the drug tamoxifen. These results open up a new perspective on the mode of action and long-time adaptation effects of membrane targeted drugs.
Conclusion: These observations might inspire researchers across different disciplines to include lipid order measurements in their studies. For this we provide detailed insight into the measurement procedure and introduce a custom-made device that facilitates this kind of studies.

Assessing the Therapeutic Functions of Ceramidase in Non-Alcoholic Fatty Liver Disease

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Non-alcoholic fatty liver disease (NAFLD) impacts around 24% of people worldwide. Without treatment, NAFLD can progress to non-alcoholic steatohepatitis (NASH) and cirrhosis. Finding effective therapeutic approaches is of the utmost importance. Excess lipids in the bloodstream are associated with NAFLD. Several lipidomics studies indicate that ceramide levels are substantially higher in the liver and plasma of NAFLD. Higher ceramide in hepatocytes leads to oxidative stress, pro-inflammatory cytokine release, and cell death. Neutral ceramidase (nCDase) is one of the enzymes responsible for hydrolyzing ceramide into sphingosine, a precursor of sphingosine-1-phosphate for cell survival. Thus, we aim to evaluate the therapeutic functions of nCDase for NAFLD.

The apolipoprotein E knockout (apoE-/-) mouse fed a high-fat diet was the primary animal model for NAFLD. HFD-fed apoE-/- mice were injected with bio-engineered nCDase through tail veins, three times a week for eight weeks. ApoE-/- mice fed with a normal chow diet were controls. We collected plasma and livers after the animals had been sacrificed to assess lipid components, biochemical profiles, and other pathological abnormalities. The Folch method was used to extract plasma lipids. We separated lipids using an ACQUITY® UPLC equipped with a CSHTM C18 column, and components were evaluated using a Xevo G2 QTOF (quadrupole time of flight mass spectrometry) in data-independent collection mode (MSE).

The C16:0, C18:0, C20:0, C22:0, and C24:0 ceramide levels were significantly elevated in the livers of HFD-fed apoE-/- mice ($p < 0.01$). While treated with nCDase, the ceramide levels were reduced ($p < 0.05$). In contrast, the sphingomyelin and high-density lipoprotein-cholesterol (HDL-C) were significantly enhanced in the mice livers of HFD-fed apoE-/- injected with nCDase. The oil red staining showed that HFD enhanced the lipid accumulation in the livers of apoE-/- mice; while treated with nCDase ($p < 0.001$), the effect was attenuated ($p < 0.05$). In the meanwhile, the inflammatory biomarkers for NAFLD such as tumor necrosis factor, interleukin 6, and the mitochondrial amidoxime reducing component 2 are under investigation. In summary, ceramide and accumulation of lipids in the liver were elevated in HFD-fed apoE-/- animals; these effects can be ameliorated by nCDase therapy. The preliminary results show that nCDase could be an effective approach for alleviating the progression of NAFLD.

Multifaceted crosstalk between the posttranscriptional axis hnRNPK/miR-7 and the lipidic metabolism

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Introduction: Dysregulation of lipid homeostasis is associated with the development of human pathologies from cardiovascular diseases and neurological disorders such as Alzheimer's disease (AD). Post-transcriptional regulators have emerged as key modulators of cellular components and pathways involved in the control of lipids and cholesterol, however, the functional crosstalk between posttranscriptional regulators and the stimuli that regulate them during the control of metabolic diseases remains incomplete.

Results: We previously have established the role of miR-7 in regulating LXR and amyloidosis, which represents one of the most common pathological hallmarks in AD. Our studies also demonstrate that miR-7-1 and its host gene, the RNA binding protein hnRNPK, are co-transcribed in response to LXR or insulin, participating in a regulatory feedbackloop by which miR-7 could potentially control its own expression. Furthermore, we analyzed additional metabolic functions of hnRNPK/miR-7 in the regulation of cholesterol metabolism. We found that miR-7 blocks the last steps of the cholesterol biosynthetic pathway by inhibiting relevant genes such as DHCR24 posttranscriptionally. We also found that cholesterol itself regulates endogenous levels of hnRNPK/miR-7 in vitro and in vivo, correlating with transcriptional regulation through SREBP2 binding to hnRNPK´s promoter region. Indeed, in parallel to SREBP2 inhibition, the levels of miR-7 and hnRNPK were concomitantly reduced in brain in a mouse model of Niemann Pick type C1 disease and in murine fatty liver, which are both characterized by intracellular cholesterol accumulation.

Conclusions: Taken together, these findings establish novel regulatory feedback loops showing the crosstalk between hnRNPK/miR-7 posttranscriptional pair and cholesterol metabolism, that might be taken in consideration for therapeutic interventions against important metabolic human diseases in the future.

Patatin-like phospholipase PNPLA6 regulates retinal homeostasis by regulating choline availability through phospholipid turnover

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Introduction: Although abnormalities in membrane phospholipids are often associated with retinal degeneration, the molecular mechanisms underlying retinal homeostasis and adaptive repair by phospholipid turnover (degradation and synthesis) remain unresolved. Mutations in human PNPLA6, a member of the phospholipase A2 (PLA2) family, cause retinitis pigmentosa associated with neurodegeneration. In this study, we analyzed the PNPLA6-driven regulatory mechanisms for lipid metabolism and retinal functions.
Materials and methods: Cell-based analyses in vitro were performed by PNPLA6 overexpression or knockdown in ARPE-19, a human retinal pigment epithelial (RPE) cell line, and 661W, a mouse photoreceptor cell line. Retinal degeneration in vivo was analyzed using tamoxifen-inducible conditional PNPLA6-deficient mice.
Results and discussion: PNPLA6 overexpressed in RPE cells released both sn-1/2 fatty acids from phosphatidylcholine (PC) leading to generation of glycerophosphocholine (GPC), suggesting that PNPLA6 acts as a phospholipase B. PNPLA6-knockdown RPE cells showed reduced intracellular GPC and choline levels, disturbed mitochondrial morphology and respiratory function, decreased ATP production, increased oxidative stress, and abnormal proliferation, phagocytosis and adhesion, all of which were rescued by choline supplementation. Knockdown of enzymes involved in de novo PC synthesis through the Kennedy pathway also showed similar abnormalities, suggesting that PNPLA6-driven PC turnover is critical for RPE homeostasis. Moreover, RPE cells released a pool of choline extracellularly, which was taken up and utilized for PC synthesis by neighboring photoreceptor cells. PNPLA6 knockdown in RPE cells resulted in growth inhibition and death of photoreceptor cells, suggesting that the transcellular supply of choline from RPE to photoreceptor cells is essential for photoreceptor homeostasis. Importantly, mice with tamoxifen-inducible, retina-specific deletion of PNPLA6 displayed retinal thinning, mitochondrial abnormality, degenerated photoreceptor disc structure, and impaired light responsiveness, which were ameliorated by choline supplementation via eyedrops.
Conclusion: PNPLA6 plays an essential role in retinal homeostasis by controlling choline availability for phospholipid turnover via RPE-photoreceptor interaction and provide a framework for the development of a novel ophthalmic drug target for retinal degeneration.

Group XIIA secreted phospholipase A2 promotes Th17 cell differentiation and psoriasis by mobilizing bioactive lysophospholipids

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Introduction: IL-17A-producing Th17 cells play an important role in pathogenesis of autoimmune diseases such as psoriasis and arthritis. Although aberrant lipid metabolism has been implicated in pathogenic Th17 responses, the roles of phospholipase A2 (PLA2) enzymes, which hydrolyze phospholipids to generate fatty acids and lysophospholipids, in Th17 defferentiation are unclear. In this study, we aimed to identify a PLA2 subtype and underlying lipid mechanism that contribute to Th17 differentiation and psoriasis.

Materials and Methods: We used global or CD4+ T cell-specific sPLA2-XIIA knockout (XIIA-KO) mice. Naïve CD4+CD62L+ T cells were polarized into Th17 cells by culturing with recombinant IL-1b, IL-6, IL-23 and TGF-b and plate-coated anti-CD3/CD28 antibodies for 3 days. Imiquimod was applied to mouse ears to induce psoriatic inflammation.

Results and Discussion: Of nearly a full set of PLA2 enzymes, sPLA2-XIIA showed the highest expression in Th17 cells. Ex vivo differentiation of IL-17A+ Th17 cells from naïve T cells was markedly impaired in global or T cell-specific XIIA-KO mice. In vivo, global or T cell-specific XIIA-KO ameliorated psoriasis-like skin inflammation and decreased IL-17A+ Th17 cells in the skin and draining lymph nodes (LNs). Lipidomics analysis of LNs showed that lysophospholipids, particularly LPA and LPE, were increased in imiquimod-treated WT mice, whereas this response occurred only partially in XIIA-KO mice. The defective Th17 differentiation by XIIA-KO was fully restored by supplementation with LPA or LPE. The restoring effect of LPE was abolished by inhibition of autotaxin (ATX), suggesting that the conversion of LPE to LPA by ATX is critical for this event. The impaired Th17 induction by XIIA-KO was rescued by LPA1 and LPA2 receptor agonists, and LPA1- or LPA2-deficient T cells failed to differentiate into Th17 cells. Importantly, sPLA2-XIIA hydrolyzed PE in extracellular vesicles (EVs) released from Th17 cells to generate LPE, thereby acting upstream of ATX and LPA1/2 receptors. sPLA2-XIIA-treated EVs, which enriched LPE, enhanced Th17 differentiation, and the impaired Th17 induction by XIIA-KO was restored by addition of EVs from WT, but not XIIA-KO.

Conclusion: In lymphoid tissues, sPLA2-XIIA is secreted from CD4+ T cells and generates LPE via hydrolysis of PE in EVs. LPE is then converted by ATX to LPA, which promotes Th17 differentiation via LPA1/2 receptors, thus contributing to exacerbation of psoriasis.

Role of microsomal prostaglandin E synthase-1 in chemical carcinogen-induced bladder carcinogenesis

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Introduction: Microsomal prostaglandin E synthase-1 (mPGES-1) is stimulus-inducible enzyme that functions downstream of cyclooxygenase (COX)-2 in the PGE2-biosynthetic pathway. Accumulating evidence indicated that COX-2-derived PGE2 participate in the development of various tumors. Furthermore, it was reported that a selective COX-2 inhibitor suppresses chemical carcinogen-induced bladder carcinogenesis. However, the involvement of mPGES-1 in chemically induced bladder carcinogenesis has not been fully elucidated. In this study, we investigated that the role of mPGES-1 in chemical carcinogen-induced bladder carcinogenesis.

Materials and methods: mPGES-1 knockout (KO) and wild-type (WT) mice were given drinking water containing 0.05% N-butyl-N-(4-hydroxybutyl) nitrosamine (BBN) for 8 weeks. All mice were killed 27 weeks after the cessation of BBN administration, and their bladders were subjected to pathologic examination and PG analysis.

Results and discussion: Bladder tumor was observed in 44% of WT mice and 29% of mPGES-1 KO mice. Furthermore, we analyzed the stages of bladder cancer. The stage T4, in which the tumor has spread outside the bladder was found in 33% of WT mice. However, mPGES-1 KO mice with T4 was not found. We analyzed PGE2 level in bladder using LC-MS-MS. PGE2 level was significantly decreased in bladder of mPGES-1 KO mice than that of WT mice. These results indicated that mPGES-1 deficiency suppressed PGE2 generation and chemical carcinogen-induced bladder carcinogenesis and cancer progression. There are many reports about COX-2 and mPGES-1-derived PGE2 promote cancer progression. However, this is the first report that mPGES-1 promote bladder carcinogenesis using mouse model. mPGES-1 inhibitors are now being developed as a new NSAID with less adverse effects. Inhibition of mPGES-1 would be a suitable therapeutic target to suppress tumor promotion with low risk in patients with bladder cancer.

Body Mass Index, Blood Pressure and Interictal Serum Levels of Cytokines in Migraine with and without Aura

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Introduction: Cytokines can act on neuronal receptors and cause neurovascular inflammation and contribute to pain. The aim of the study was to clarify correlations among body mass index (BMI), blood pressure (BP) and serum levels of cytokines in migraine female patients.

Materials and Methods: 14 migraineurs with aura, and 12 – without aura during their interictal period were compared with 25 controls. Interleukin-8 (IL-8), soluble intercellular adhesion molecule-1 (sICAM-1), soluble vascular cell adhesion molecule-1 (sVCAM-1), matrix metalloproteinase-9 (MMP-9), interferon gamma (IFN-γ), monocyte chemoattractant protein-1 (MCP-1), transforming growth factor alpha (TGF-α) and plasminogen activator inhibitor-1 (PAI-1) were measured in serum by ELISA method.

Results: Migraineurs had significantly increased levels of IL-8, but decreased serum levels of PAI-1 and sICAM-1 during the interictal period, regardless of aura. BMI correlated with BP, and also with IFN-γ and MMP-9 only in patients with aura. Conclusion. There were three correlations in migraine patients with aura that were absent in patients without aura: between IL-8 and PAI-1; MMP-9 and IL-8; IL-8 and sICAM-1. Migraineurs without aura, on the other hand, had correlations that patients with aura did not have (between PAI-1 and MCP-1, sICAM-1; between MMP-9 and sICAM-1, MCP-1; between TGF-α and PAI-1, MMP-9, sICAM-1; between sICAM-1 and MMP-9, PAI-1, MCP-1; as well as between sVCAM-1 and MCP-1). PAI-1, TGF and MMP-9 could be used as biomarkers to distinguish migraineurs from healthy individuals.

Malabaricone C derived from nutmeg inhibits arachidonate 5-lipoxygenase activity ameliorating murine psoriasis-like skin inflammation and OVA-induced allergic airway inflammation

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Introduction: Leukotrienes (LTs) exhibit a range of pathophysiological activities in chronic inflammatory diseases such as psoriasis and asthma. Since 5-lipoxygenase (5-LOX) is a key enzyme in LTs biosynthesis, the inhibitor zileuton is used clinically for LT-related chronic inflammatory diseases. However, it has effects such as hepatic toxicity and metabolic shift to the other pro-inflammatory lipid mediators. Therefore, we have been exploring food-derived 5-LOX inhibitors with reduced side effects and found malabaricone C (MLB-C) from nutmeg (Myristica fragrant) as a potent 5-lipoxygenase inhibitor with amelioration of imiquimod-induced psoriasis-like skin inflammation and ovalbumin-induced asthma-like airway inflammation in mice.

Materials and Methods: 5-LOX inhibitor was isolated from 50% EtOH extract of nutmeg (Myristica fragrans) by RP-HPLC and was identified as MLB-C by HR-MS and proton NMR. 5-LOX assay was performed using a partially purified rat 5-LOX under the optimal conditions containing 25 μM arachidonic acid and the products were analyzed by RP-HPLC. The ameliorating effects were confirmed in mouse models of imiquimod (IMQ, Beselna cream)-induced psoriasis, and ovalbumin (OVA)-induced asthma.

Results and Discussion: MLB-C competitively inhibited 5-LOX with IC50 of 0.2 microM, but showed only weak inhibitions of COX-1, COX-2, and mPGES-1. In IMQ-induced psoriasis-like skin inflammation, the topical application of 2 mM MLB-C significantly reduced hyperplasia and inflammatory cell infiltration and suppressed the expression of the psoriasis-associated genes S100a9, Krt1, Il17a, and Il22. MLB-C markedly decreased LTB4 but did not induce excessive synthesis of the other pro-inflammatory lipid mediators such as prostaglandin (PG) E2 and PGD2 in the psoriasis-like skin lesions. In OVA-induced asthma-like airway inflammation, MLB-C dose-dependently reduced the number of eosinophils in bronchoalveolar lavage fluid and suppressed inflammatory cell infiltration and mucus production in the lung tissue.

Conclusion: MLB-C derived from a commonly used spice nutmeg showed a novel effect of the potent 5-LOX inhibition and ameliorated LT-related inflammatory diseases such as psoriasis-like skin inflammation and asthma-like airway inflammation in mice. This suggests that MLB-C may be a potentially useful therapeutic agent for LT-related inflammatory diseases.

Apolipoprotein D's overexpression effect on systemic inflammation and metabolic syndrome development in mice on a high-fat, high-sugar diet

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Introduction: Apolipoprotein D (ApoD), a small lipocalin, has been shown to be able to modulate inflammation and oxidative stress in the context of neurodegenerative diseases such as Alzheimer and Parkinson. ApoD is is mainly expressed in the central nervous system (CNS), but the protein is able to cross the blood-brain barrier to accumulate in virtually every organ.

Methods: Since ApoD can reduce inflammation and oxidative stress in the brain, while also leaving the CNS, we looked at the effect of neuronal overexpression of the human form of this protein (HApoD) in mice challenged with a high-fat, high-sugar diet (HFHS) for 12 weeks. This diet induces chronic systemic inflammation, insulin resistance, and glucose intolerance. We assessed the metabolic parameters via glucose and insulin tolerance tests (GTT, ITT), anxiety and depression by open field and forced swim test, and verified various metabolic metrics by metabolic cages. Mice were then sacrificed and organs (liver, adipose tissues, brain, kidneys, heart, muscles, pancreas) were harvest.

Results: Both WT and transgenic mice gained weight in the same way and overexpression of human ApoD does not affect the development of insulin resistance and glucose intolerance. Data show that HApoD overexpression causes a decrease of Tumor Necrosis Factor Alpha (TNF-α) expression in the liver, brain and kidneys. A decrease of other inflammatory markers such as IL-6 and CCL2 was also notedAdecrease in the activation of ERK1/2 was also noted in the liver of transgenic mice. In conclusion, our data suggest that neuronal ApoD overexpression in a mice model challenged by HSHF to induce obesity cause a decrease in inflammation in liver, brain and kidneys suggesting a systemic effect

Benchmarking One-Phase Lipid Extractions for Plasma Lipidomics

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A key element of successful lipidomics analysis is a sufficient extraction of lipid molecules typically by two-phase systems such as chloroform-based Bligh and Dyer (B&D). However, numerous metabolomics and lipidomics studies today apply easy to use one-phase extractions to cover a broader range of analytes. In this work, quantitative flow injection analysis high-resolution mass spectrometry was applied to benchmark the lipid recovery of popular one-phase extraction methods for human plasma samples. The following organic solvents were investigated: methanol (MeOH), ethanol (EtOH), 2-propanol (IPA), 1-butanol (BuOH), acetonitrile (ACN) and the solvent mixtures BuOH/MeOH (3:1) and MeOH/ACN (1:1). The recovery of polar lysophospholipids was sufficient for all tested solvents. However, nonpolar lipid classes such as triglycerides (TG) and cholesteryl esters (CE) revealed extraction efficiencies less than 5% due to precipitation in polar solvents EtOH, MeOH, MeOH/ACN, and ACN. Sample pellets also contained a substantial amount of phospholipids, for example, more than 75% of total phosphatidylcholine and sphingomyelin for ACN. The loss of lipids by precipitation was directly related to the polarity of solvents and lipid classes. Although, lipid recovery increased with the volume of organic solvent, recovery in polar MeOH remains incomplete also for less polar lipid classes such as ceramides. Addition of stable isotope-labeled internal standards prior to lipid extraction could compensate for insufficient lipid recovery for polar lipid classes including lysolipids and phospholipids but not for nonpolar CE and TG. In summary, application of one-phase extractions should be limited to polar lipid classes unless sufficient recovery/solubility of nonpolar lipids has been demonstrated. The presented data reveal that appropriate lipid extraction efficiency is fundamental to achieve accurate lipid quantification.

PAF-induced local and systemic anaphylaxis depends on endothelial PAFR

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Introduction: Platelet activating factor (PAF) is a potent phospholipid mediator working at nanomolar concentrations. It has a wide variety of functions such as vascular hyperpermeability, hypotension, and neuropathic pain. In the context of anaphylaxis, the levels of PAF correlate to the severity of patients with anaphylaxis (Vadas et al., NEJM, 2008). It has been consistently reported that PAF receptor (PAFR) antagonist reduced anaphylaxis in both of IgE and IgG mediated passive systemic anaphylaxis (PCA) in mouse model (Strait et al., JACI, 2002), and PAFR deficient mice showed the attenuated phenotype of active systemic anaphylaxis (ASA) (Ishii et al., JEM, 1998). In addition, rupatadine, a dual antagonist of histamine receptor and PAFR, has been marketed in more than 80 countries worldwide for treatment of allergic rhinitis and urticaria. Although it has been proven that PAF had crucial pathophysiological functions, the molecular mechanisms of PAFR activation including its effector cells are not still well characterized.

Materials & methods: Cell-type specific PAFR deficient mice were generated using engineered PAFR-floxed mice. PAFR agonist, methylcarbamyl-PAF (mc-PAF, non-hydrolyzable PAF analogue), was injected into the ear skin and Evans blue dye was subsequently administered via tail veins. Vascular permeability was evaluated by the measurement of Evans blue dye amount. Additionally, mc-PAF was delivered intravenously as a systemic anaphylactic model, and the mortality was monitored.

Results & discussion: Single-cell RNA sequencing of the ear skin indicated limited PAFR expressions in endothelial cells, macrophages and mast cells. Macrophages specific PAFR knockout mice and mast cells specific PAFR knockout mice showed similar anaphylactic responses evoked by mc-PAF to their controls. In contrast, endothelial cells specific PAFR knockout (EC-KO) mice completely prevented mc-PAF induced responses both locally and systemically. We further investigated the role of PAFR in endothelial cells by the IgE-mediated PCA model. On the contrary, no apparent differences were observed between EC-KO and control mice, indicating PAFR in endothelial cells was not involved in the early phase of local allergic reactions.

Conclusion: Our findings clearly demonstrated that PAFR in endothelial cells are responsible for PAF-induced anaphylaxis, but not for the model of the local allergic reactions, which suggests the other mechanisms of PAFR activation.

Rapid and Sensitive Quantification of Bile Acids in Human and Murine Samples using LC-MS/MS

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Introduction: Bile acids are essential for intestinal lipid uptake, solubilising dietary lipids and enabling their efficient break-down and uptake in the small intestine. They are metabolised by the gut microbiota and act as potent signalling molecules influencing energy metabolism. Here, we present a rapid and sensitive LC-MS/MS based high-throughput workflow for bile acid extraction and quantification in mouse and human tissues, feces, and body fluids.

Materials and Methods: Samples including tissue homogenate, feces, bile, or plasma were subjected to acidic acetonitrile precipitation. 21 stable isotope labelled internal standards were added prior to extraction. Bile acids were separated using liquid chromatography (LC) with a Biphenyl Core-Shell column and a gradient elution with H2O/MeOH mobile phases containing 0.01 % NH4 and 10 mM ammonium acetate. The mass spectrometer was operated in negative electrospray ionisation (ESI) using scheduled MRMs. Data is analysed with Sciex OS and self-programmed Excel Macros, with quantification based on calibration curves.

Results and Discussion: Our method identified and quantified 30 bile acids: alpha, beta, gamma and omega MCA, UDCA, HDCA, CA, CDCA, DCA, and LCA, as well as their tauro- and glyco-conjugated versions. All isomers except beta, omega MCA, and their conjugated versions were baseline-separated. The total runtime per sample was below 6 minutes. Validation experiments based on plasma samples showed a broad linear range, high reproducibility with a CV < 10 % and a high sensitivity with a LOD < 5.5 nM.

Conclusion: Our method enables a versatile, robust and comprehensive quantification of bile acids in murine and human samples. Further, the short analysis time permits a throughput of 10 samples per hour with minute sample amounts due to high sensitivity mass spectrometric detection.

Examining the Influence of Ether Lipids and Tetrahydrobiopterin on Lipid Peroxidation

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Introduction: Ether lipids, a lipid subclass, can be categorized into plasmanyl and plasmenyl species depending on the absence or presence of a vinyl ether double bond. Ether lipids display high concentrations of polyunsaturated fatty acids (PUFAs) at their sn-2 position which are highly susceptible to lipid peroxidation and play a crucial role in ferroptosis, an iron-dependent programmed cell death. Recent research has shed light on the involvement of specific ether lipid metabolic enzymes in ferroptosis, although their exact roles remain unclear. Plasmanylethanolamine desaturase (PEDS1) has been shown to have a pro-ferroptotic effect, while the cofactor of alkylglycerol monooxygenase (AGMO), tetrahydrobiopterin (BH4), synthesized from guanosine triphosphate (GTP) by GTP-cyclohydrolase 1 (GCH1), appears to have an anti-ferroptotic effect. Our study aims to investigate the roles of these enzymes and BH4 in membrane homeostasis under both normoxia and hypoxia.

Material and methods: AGMO, PEDS1 and GCH1 activities were quantified using sensitive HPLC-based activity assays relying on fluorescent substrates. Enzyme knockouts were implemented using CRISPR/Cas9 technology and their genetic complementation will take place by means of plasmid transfection. Hypoxic conditions were induced in a hypoxic chamber and mitochondrial structure alterations were analyzed by immunocytochemistry.

Results and Discussion: In order to more clearly understand the role of these enzymes in ferroptosis and study their potential interconnection, we first had to find a cell line that displays all three enzymatic activities. From the tested cell lines, only the murine monocyte RAW264.7 cell line exhibited robust activities for all three enzymes. As a next step, we are now generating knockouts of the three enzymes and will then draw up rescued activities. In parallel, we are establishing tools to analyze the effect of normoxia and hypoxia on mitochondrial morphology in wild-type, knockout and rescued cell lines of the three enzymes using mitochondrial and hypoxic markers.

Conclusion: In the RAW264.7 cell line, we found an ideal model to explore the link between ether lipids and ferroptosis, as it expresses enzymatic activity for AGMO, PEDS1, and GCH1. By using our currently established knockouts and genetic complementation cell lines for these enzymes, we seek to delve deeper into the roles of ether lipids and BH4 in ferroptosis and their interplay in membrane homeostasis.

Altered lipidomic profile in an astrocytoma cell model of Alexander Disease

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Introduction: Alexander disease (AxD) is a rare neurodegenerative disease caused by mutations in the intermediate filament glial fibrillary acidic protein (GFAP) gene in astrocytes, causing its overexpression, destruction of white matter, loss of neurons and early death. Expression of AxD GFAP mutants in an astrocytoma cell model causes oxidative stress and increased susceptibility to lipoxidation, indicative of alterations in the generation and fate of electrophilic lipids. The main objective of the present work was to characterize the lipidome and epilipidome profiles in this model to disclose the role of lipid metabolism in AxD for the first time.

Material and methods: Cells expressing GFAP WT or R239C AXD mutant were obtained by stable transfection and cell sorting. Phenotype was confirmed by fluorescence microscopy. Targeted lipidomics were used to assess their lipid profile. We analyzed a total of 688 lipid species from the main lipid categories using an Agilent6495 LC/QC mass spectrometer. Univariate and multivariate statistics were applied.

Results and discussion: AxD cells showed the typical GFAP aggregation and bundles. Targeted lipidomics analysis showed that among 688 lipids quantified, 166 (24%) changed significantly between AxD and WT cells. Regarding specific lipid families, glycerophospholipids (involved in cell membrane structure and function), sphingolipids (signaling functions) and sterols were increased in AxD, whereas fatty acyls and glycerolipids (involved in cell bioenergetics) were decreased. Specifically, 4 acylcarnitines were decreased in AxD cells suggesting a dysfunction of mitochondrial β-oxidation, which is reinforced by the decrease of 63 triacylglycerol species. Levels of 32 glycerophosphopids and 10 lysophospholipids were increased in AxD cells suggesting increases in both de novo synthesis and remodeling of membrane phospholipids. 32 ceramides and 11 hexocylceramides were up-regulated in AxD cells, indicating a dysregulation of cell signaling. Notably, alterations in the sphingolipid–ceramide profile has been reported in neurological and neuroinflammatory diseases.

Conclusion: This AxD model presents a specific lipidomic profile that suggests that this mutation has sharp implications in the bioenergetic status, membrane remodeling and cell signaling. These results highlight new potential pathogenic pathways that could contribute to the physiopathology of this fatal disease. Grant LCF/PR/HR21/52410002 Fundación “la Caixa"

Physiochemical impact of the antitumoral 2-hydroxyoleic acid incorporated into lipid membranes.

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Introduction: Cancer cells present alterations in their phospholipid and fatty acids profiles, which consequently alters their membrane physicochemical properties, such as hydration, curvature, fluidity and surface charge, and cell signaling pathways. Significantly, these exceptional properties are correlated with their malignant potential, progression, and resistance to several anticancer drugs. Thus, manipulating cell membrane lipid composition and membrane physicochemical properties is presented as a good alternative to treat this condition. This is the principle of an original therapeutic approach: Membrane Lipid Therapy, or melitherapy, which may bring therapeutic tools to, still underserved, several types of cancer.2-Hydroxyoleic acid (2-OHOA), a natural analogue of oleic acid, is a melitherapeutic compound which presents a proven antiproliferative effect by changing the membrane lipid composition and structure of cancer cells. This molecule has showed pharmacological efficacy and safety in cellular and animal models, and it is currently in advanced clinical trials. This study seeks to focus on the biophysical effect of the incorporation 2-OHOA into the lipid cell membrane.

Material and methods: We explored membrane structure, hydration, curvature and fluidity by X-ray diffraction, differential scanning calorimetry and Laurdan fluorescent spectroscopy of lipid model membranes. In order to perform such experiments, we used synthetic phospholipids to precisely control the membrane composition and impurities.

Results and discussion: The incorporation of 2-OHOA into lipid membranes increase its lamellar repeat distance due to remarkably higher water layer thickness and interestingly, the thickness of the hydrophobic region is even thinner in presence of the aforementioned molecule. Moreover, 2-OHOA blocks the cooperativity between lipids and particularly, hinders the lipidic lamellar-to-hexagonal phase transition, which indicates a change in membrane curvature. Finally, fluorescent results indicates an increase in membrane fluidity in presence of 2-OHOA probably related to an increment of membrane disorder.

Conclusions: All the findings suggest that the incorporation of 2-OHOA into lipid membranes increases membrane mobility, hydration and fluidity by decreasing its order and curvature, which could partly explain changes in the localization and activity of signaling membrane proteins and ensuing its antiproliferative effect.

The study investigated the association between circulating lipids, inflammation, and cardiometabolic complications in a high-risk cohort of obese individuals

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Cardiovascular disease (CVD) is the leading cause of death globally. Obesity and low-grade inflammation are risk factors for atherosclerotic plaque formation and CVD. Yet, it is currently not known why some obese individuals develop cardiometabolic and cardiovascular complications, whereas others do not. There is mounting evidence that abnormalities in lipid metabolism caused by obesity may contribute to the development of inflammation and subsequently CVD. We aimed to explore the association between circulating lipids, inflammation and cardiometabolic complications in a cohort of 302 subjects with a BMI > 27 kg/m2. Given known sex differences, we aimed to perform sex-specific analyses. The purpose of this study is to look into the potential link between circulating lipids, inflammation, and cardiometabolic outcomes in this high-risk cohort. Using Liquid Chromatography/Mass spectrometry (LC/MS) we detected 17 lipid classes and 593 subclasses in the plasma samples of 292 individuals. Among all detected lipid classes phosphatidylserine levels were significantly reduced in individuals who developed carotid plaques. Furthermore, phosphatidylserine levels were negatively associated with the number of plaques and the maximal plaque thickness as well as levels of circulating pro-inflammatory cytokines: IL-18 and IL-18BP, acute inflammation marker alpha-1 antitrypsin (AAT), and resistin. This indicates a protective role of these lipids for CVD development in obese individuals. Further sex-specific stratification revealed elevated levels of various lipid classes in women compared to men including sphingomyelins, phosphatidylethanolamines, ether-linked lysophosphatidylethanolamines, phosphati dylcholines, ether-linked phosphatidylcholine, and cholesteryl esters. Interestingly, the levels of circulating lipids showed rather negative correlations with plaque presence in women while positive correlations with plaque presence in men. As such ether-linked phosphatidylethanolamine and phosphatidylcholine levels were negatively associated with the carotid plaques in females, but negatively, together with ceramides, hexocylsceramides, phosphatidylethanolamine, and sphingomyelin, associated in man. We also observed sex-specific associations of lipid composition with other clinical characteristics. For example, the HOMA1-IR score was negatively associated with sphingomyelin, hexosylceramide, and cholesteryl ester levels in men but not in women.

Hydroxylated fatty acids as potential therapeutic approaches to the treatment of neuropathic pain

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Introduction: Finding viable therapeutic approaches to neuropathic pain (NP) is one of the biggest challenges of modern medicine. The chemotherapeutic treatment with vincristine sulphate (VS) causes NP by destabilizing afferent nerve fibbers. The hydroxylated monounsaturated fatty acid, 2-hydroxioleic acid (NFX88, 2OH-C18:1), has shown therapeutic potential to treat spinal-cord-injury (SCI)-associated neuropathic pain in Wistar rats. In these previous studies, NFX88 has demonstrated a reduction in mechanical and thermal hypersensitivity in this SCI-based model. Also, the NFX88 is under research in Phase I clinical trials. On the other hand, in this work we also used 2-hydroxy-docosahexaenoic acid (DHA-H, 2OH-C22:6) as a potential treatment.
Methodology: In this work, we developed an animal model based on chemotherapy-induced peripheral neuropathy (CIPN) by administering VS intraperitoneally to Wistar rats at a dose of 0.1mg/Kg/day for 10 days, which then was treated with NFX88 for 28 days at a dose of 400mg/Kg. During the treatments with both vincristine and NFX88/DHA-H we performed behavioural tests to assess the mechanical and thermal hypersensitivity. After the treatment, the animals were euthanised, and the spinal cord tissue was gathered to perform RNA and protein extractions, in order to perform qPCR and SDS-PAGE experiments respectively. The Phase I clinical trial, conducted on chronic SCI patients, assessed the level of NP of patients with the Visual Analog Scale (VAS) and PainDETECT questionnaire.
Results: The Wistar rats showed a reduction of thermal and mechanical hypersensitivity in our model of CINP, after being orally treated with either NFX88 or DHA-H for 28 days. We also observed that VS reduces the GAD67 and VGAT expression in the spinal cord, as well as increase the KCC2 expression. The qPCR results showed that either VS or NFX88/DHA-H have an effect on inflammation and microglial activation in the spinal cord. On the other hand, NFX88 has shown a good safety profile, and a reduction of pain when compared to patients treated with placebo.
Discussion: These results suggest that both NFX88 and DHA-H are able to reduce the hypersensitivity caused by VS, by reverting the decrease of GABAergic synapsis markers. At the same time, NFX88 has shown potential for the treatment of SCI-induced NP in both animal models and humans.ConclusionNFX88 and DHAH are potential therapeutic approaches for NP arising from both physical and non-physical causes.

Targeting the Notch-Furin Axis with 2OHOA: A Promising Approach for Glioblastoma Therapy

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Introduction: Gliomas are the most common and aggressive cancer tumors of the central nervous system. 2OHOA, which is currently running a phase IIB/III clinical trial for newly diagnosed GBM patients, was developed in the context of melitherapy, a novel therapeutic platform based on the regulation of the membrane's structure and organization with the consequent modulation of certain cell signals to revert the pathological state in several disorders. These alterations trigger modifications in membrane-associated proteins, as Ras, inducing its translocation from the plasma membrane to the cytoplasm, followed by ER stress, differentiation and autophagy cell death. Notch signaling pathway is abnormally activated in gliomas and has been highly related to tumorigenesis and cell survival driving to the pathogenesis of GBM. The present work studies whether 2OHOA modulates the Notch pathway and its relevance in its mechanism of action as an antitumoral drug.

Methods: 2OHOA's effect was evaluated on different components of the pathway by W-blot, Q-PCR, and confocal microscopy. Notch receptor processing was analyzed by cell fractionation and colocalization studies. Results and discussion2OHOA inhibits Notch2 and Notch3 pathways in GBM cells by dual mechanism. On one hand, Notch3 is transcriptionally downregulated leading to a minor activation of the Notch3 pathway exhibiting less nuclear presence of NICD3. On the other hand, Notch2 signaling pathway is inhibited by repression of its processing in early step. This event is afforded by downregulating furin-like proteases activity through direct interaction with the bioactive lipid triggering the impairment of Notch2 trafficking in the Golgi compartment. Consequently, Notch2 and Notch3 signaling are disrupted, repressing NICD-dependent transcription genes and leading to malignant cell death. Finally, the relevance of this pathway was highlighted not only by the overexpression of the main target of this pathway, HES1, which partially inhibited 2OHOA's antiproliferative effect but also the reduction of its expression by the drug that correlated positively with their sensitivity to the molecule.

Conclusion: The inhibition of Notch pathway by 2OHOA plays a role in its antitumoral effect, and this event is unleashed by transcriptional repression for Notch3 or by direct inhibition of furin enzyme activity impairing Notch2 processing, identifying it as a novel target for this drug in the GBM treatment.

Synthetic Peptids as New Antimicrobial Treatment: Biophysical characterization

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Introduction: Antibiotic resistance causes approximately 33.000 death per year in Europe and although the number of multi-resistant bacteria is increasing, the number of effective antimicrobial therapies is gradually reducing. The antimicrobial peptides (AMPs), ancient evolutive agents of innate immune system, are a novel strategy to develop new antibiotics against pathogens. AMPs usually present a small size and have a broad spectrum of action related to their amphipathic properties. The design of antibiotics that attach to the cell membrane will contribute precluding pathogens to develop resistance since changes at membrane level suppose a too high metabolic expenditure. Materials and methods Peptide-lipid interactions were studied with specially designed AMPs in model membranes by three fluorescent assays: binding assay, leakage assay and quenching assay. Model membranes were made using lipid films and resuspending them to form lipidic vesicles. Tryptophan fluorescence was read for the lipid-peptide binding and quenching assays, while carboxyfluorescein (CF) fluorescence was scanned for the leakage assays.

Results and discussion: As shown by the binding assays, eukaryotic plasma model membrane shows a low peptide-membrane affinity compared with bacterial model membrane with similar lipid composition as plasma membranes from Escherichia coli, Salmonella and Staphylococcus aureus. Although, peptide affinity from bacterial model membranes differs between them, the three models have an unusual high affinity that could contribute to an antibiotic activity. Moreover, CF and quenching assays were performed to assess the peptide capacity to form pores on model lipid membranes and to find out the position of the peptide in the membrane, respectively. Remarkably, all biophysical studies show clear differences between bacterial and eukaryotic model membrane on their lipid-peptide interaction.

Conclusions: 1. Peptides discern the bacterial model membranes from the eukaryotic one and have a higher affinity for the different bacterial membranes than for the eukaryotic membrane. 2. Peptides induce the formation of membrane pores on bacterial model membranes by electrostatic interactions and embedding into the membrane. 3. These results emphasize the potential antibiotic activity of the peptides studied here, AMPs that could be used in a future against gram positive and gram negative multi-resistant bacteria.

Metformin protects pancreatic β-cells from palmitotoxicity via regulation of lipid droplet-mitochondria dynamics

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Introduction: The onset and progression of type 2 diabetes is crucially determined by the deterioration of lipid metabolism and loss of function of pancreatic β-cells. Lipotoxicity is known to promote β-cell failure by various mechanisms, among others, by impairement of mitochondrial dynamics and insulin release. It has been widely-recognized that antidiabetic drug metformin can reduce lipotoxicity-induced ectopic fat accumulation, apoptosis and oxidative stress in insulin-targeted tissues. However, the mechanisms of metformin action in pancreatic islets are still a matter of debate. In this study, we aimed to understand the direct effect of metformin on lipid storage and utilization in pancreatic β-cells, and on maintenance of β-cell function.

Materials and methods: The experiments were performed in INS-1E pancreatic β-cell line, treated independently or in combination with metformin and palmitate. The lipolysis, lipogenesis and β-oxidation enzymes were assesed by WB, qRT-PCR and kinetic tests. The ELISA assay, fluorescence labeling, transmission electron microscopy and gas chromatography-mass spectrometry (GC/MS) techniques were applied to evaluate lipid droplets (LDs), mitochondria morphology and β-cell functionality.

Results and discussion: Our results indicated that metformin administration can partially restore insulin secretion in β-cells overexposed on palmitic acid. Assessment of overall lipidome showed, that treatment of pancreatic β-cells with metformin decreases the number of LDs and prevents these cells from excessive lipid accumulation. Such an effect was linked to reduced lipogenesis and downregulation of the abundance of fatty acid translocase CD36 in response to palmitate. Metformin-induced adaptations of lipid metabolism in β-cells included also lowering the activity of lipolytic enzymes. Moreover, metformin stimulated expression of mitochondria fusion proteins MFN2 and OPA1, promoted mitochondria elongation, affected the physical interaction bewteen mitochondria and LDs and inhibited acetyl-CoA carboxylase (ACC). Therefore, metformin administration may lead to enhanced fatty acid β-oxidation in pancreatic β-cells.

Conclusion: Altogether, metformin can counteract palmitotoxicity by improving lipid turnover in pancreatic β-cells. These findings provide additional mechanistic insight toward better understanding the pleiotropic actions of metformin and its role in regulation of β-cell function.

Targeting tumour-microenvironment crosstalk to bypass resistance in HER2+ breast cancer patients

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Introduction: Breast Cancer (BrCa) represents a quarter of all cancers in women worldwide, and around 20% of BrCa overexpress the HER2 receptor (HER2+ BrCa). These patients undergo treatment with a combination of chemotherapy and HER2-targeted antibody (Trastuzumab), but primary and acquired resistance still represent an unmet clinical need. Non-responder HER2+ BrCa patients display a "lipogenic phenotype" and recent clinical data suggest a role for lipids in resistance to therapy. More, the tumour microenvironment (TME) has been reported to play a critical role in tumorigenesis, progression, invasion and therapy resistance in BrCa. Recently, lipid metabolism and signaling have started to be considered as a hallmark of aberrant cell proliferation and cancer progression. Moreover, lipids impact not only cancer cells, but also the immune and stromal components of the TME.We have previously identified Cyclophilin A (CyPA) as a key molecular switch in Trastuzumab-triggered HER2 downstream signaling and as a promising candidate to by-pass resistance to therapy in HER2+ BrCa.

Materials and methods: We generated a 3D HER2+ BrCa model exploiting a commercial system. We performed morphological analysis by means of immunohistochemistry, immunofluorescence and electron microscopy as well as transcriptomics, proteomics and metabolomics both at steady state and upon CyPA knock down by shRNA-mediated silencing. We also started co-culture experiments of BrCa mammospheres and stromal or immune cells.

Results and discussion: CyPA is a ubiquitously-expressed immunophilin that acts both as an intracellular isomerase and as an extracellular signaling molecule, when secreted upon hypoxia and/or oxidative stress. Here, we show that: 1. secreted CyPA modulates the phenotype and/or the activity of different components of the TME and 2. intracellular CyPA controls cancer cells lipid metabolism. Thus, we are working to dissect how CyPA affects the interplay between BrCa and its microenvironment, and, in particular, to clarify the role of the lipid classes that are affected most by CyPA.

Conclusion: Altogether, our preliminary results suggest a role of CyPA, and of cancer cells lipid metabolism, in the interplay between HER2+ BrCa and the immune and stromal components of the TME. Unravelling the molecular mechanism underlying this crosstalk might provide additional hits to be exploited as druggable targets in the therapeutic setting.

The novel antitumor compound 2-hydroxycervonic acid (HCA) induces apoptosis and endoplasmic reticulum stress in pancreatic cancer cells

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Introduction: Pancreatic cancer has a high mortality rate due to its aggressive nature and high metastatic rate. When coupled to the difficulties in detecting this type of tumor early and the lack of effective treatments, this cancer is currently one of the most important clinical challenges in the field of oncology. Melitherapy is an innovative therapeutic approach that is based on modifying the composition and structure of cell membranes to treat different diseases, including cancers. In this sense, we designed the compound 2-hydroxycervonic acid (HCA) which showed a high anticancer activity.

Material and methods: Human pancreatic cancer cells were treated with HCA and cell proliferation assays and cell DNA content analysis were performed. Detection of specific proteins were evaluated by Western Blot. For in vivo studies, nude mice were induced subcutaneous xenograft tumors.

Results and discussion: HCA is a melitherapeutic agent developed to combat pancreatic cancer cells, provoking the programmed cell death by apoptosis of these cells by inducing endoplasmic reticulum stress and triggering the production of Reactive Oxygen Species. The efficacy of HCA was demonstrated in vivo, alone and in combination with gemcitabine, using a MIA PaCa-2 cell xenograft model of pancreatic cancer in which no apparent toxicity was evident.

Conclusion: Given the unmet clinical needs associated with pancreatic cancer, the data presented here suggest that the use of HCA merits further study as a potential therapy for this condition.

Oleate promote triple negative breast cancer cell migration by enhancing filopodia formation in a Cdc42 dependent pathway

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Introduction: Monounsaturated fatty acids, specifically oleate (OA), which are synthesized by stearoyl-coenzyme A desaturase-1 (SCD1), play a fundamental role in metabolic diseases. Studies have shown a positive correlation between high levels of SCD1 and MUFA with cancer development and metastasis. Our recent study showed that inhibition of SCD1 or treatment with OA are both associated with changes in cellular migration properties of triple-negative breast cancer cells MDA-MB-231, including altered speed and direction of movement, as well as cell morphology. However, the underlying molecular mechanisms remain poorly understood. Here we studied the impact of OA on cell morphology change and the signaling pathways to provide new insights on tripe negative breast cancer (TNBC) therapy.

Materials and Methods: To investigate the impact of OA on the formation of cell membrane ruffling and cell protrusions, we used Phalloidin-TRITC to visualize actin-rich cell protrusions and localization in fixed TNBC cells (MDA-MB-231 and -468) by confocal microscopy. Anti-Cdc42 and anti-Arp2 antibody were also used to analyze the localization change upon OA treatment. ML141 and CK666 were used to Cdc42 and Arp2/3 complex inhibition analysis. Cell migration was analyzed by wound healing assay.

Results and Discussion: OA treatment induced rapid cell membrane ruffling of MDA-MB-231 and -468 cells with significant formation of filopodia. Cdc42, the key regulator of filopodia formation, was also found to be potentially activated by translocation change out of nucleus. The lamellipodia regulator, Arp2/3 complex, did not show significant change. Accordingly, inhibition of Cdc42, but not Arp2/3, abolished OA induced filopodia formation and cell migration. Bioinformatic data showed high expression Cdc42 in TNBC was associated with lower survival rate. In conclusion, our study showed that OA could promote TNBC cell migration by enhancing filopodia formation signaling in a Cdc42 dependent pathway. This morphology change may contribute to breast cancer cell migration and metastasis.

Interaction between menopause, obesity and insulin resistance in the development of fatty liver in a murine model of metabolic syndrome

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Introduction: The pathogenesis of non-alcoholic fatty liver disease (NAFLD), including non-alcoholic steatohepatitis (NASH), is not clearly known. Epidemiological studies observed that men display a higher risk of NAFLD and NASH than women, but this tendency reverts with the onset of menopause. In line with this, NASH has become the leading cause of liver transplantation in women worldwide. As the cause of this sexual dimorphism remains unclear, we investigated the impact of menopause on hepatic lipid metabolism using a well-established murine model of obesity.

Materials and methods: This study included 22 males and 57 females C57BL/6 mice ramdomized to standard chow diet (SD) or a high-fat diet (HFD, 60% fat, Brogaarden ApS, Denmark) for 6 months, where a subgroup of females were ovariectomized at day 60. Weight, intraperitoneal glucose tolerance and insulin tolerance tests were performed at 0, 3 and 6 months. Liver samples were subjected to lipid extraction and lipidomic analyses by thin-layer chromatography (HPTLC) and GC-MS to determine lipid classes and the total methylated fatty acids (FAMEs) profile as well as the FAMEs within TAG, PC, PE and PI classes. Additionally, the expression of different inflammation markers in the liver tissue were explored by western blotting.

Results and discussion: Both male and female animals on HFD developed a obesity, hyperglycemia and insulin resistance. Lipidomics analyses showed a marked increase in the hepatic triglyceride (TAG) from 20.1 +/- 4.6 to 54.1% +/- 11.6 (SD vs HFD) of total lipid in males and from 21.9 +/- 3.9 to 58.4% +/- 6.9 in females. Obese ovariectomized mice had a lower increase in TAG compared to non-ovarectomized obese female mice (HFD+OVX vs HFD, p=0.008). Also, obese ovariectomized female mice had higher levels of free fatty acids (FFA, HFD+OVX vs HFD, p=0.001) and diacylglycerides (DAG, HFD+OVX vs HFD, p=0.004) than obese non-ovariectomized. Concerning inflammation, the levels of NF-kB were higher only in obese males and obese ovariectomized females. Taken together, our data suggests that in the absence of estrogen there is a lower deposit of fat in the liver, mainly in the form of TAG, accompanied by an increase in cytotoxic metabolites as FFA and DAG, which per se are capable of initiate inflammation through NF-kB.

Conclusion: During menopause, the lack of estrogen may alter the hepatic lipidome in the context of obesity, promoting cellular damage, inflammation and the initiation of NASH.

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The interaction between menopause, lipids and inflammation in renal tissue of obese animals

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Introduction: Obesity and overweight are risk factors for chronic kidney disease (CKD). Epidemiological studies suggest that patients with obesity in the context of metabolic syndrome are at the highest risk of kidney damage. Furthermore, this damage may be exacerbated in women after menopause, but the pathogenesis of these associations is unknown. Lipotoxicity may play an important role in the pathogenesis of renal damage, however, few studies have performed a detailed lipidomic analysis of this matter. In this study, we evaluated the interaction between inflammation, lipotoxicity and menopause in a mice animal model with metabolic syndrome: the C57/BL6J fed with fat-enriched diet.

Material and methods: We studied the impact of obesity and metabolic syndrome in male and female C57BL6/J mice fed high fat diet for 6 months. Female mice (ovariectomized or not) and male mice were randomized to standard and hight fat diet. In a previous study, we found that obese animals developed insulin resistance, hyperglycemia and hyperfiltration. For this study, we studied the lipid metabolism in renal tissue and inflammatory markers were analyzed by immunohistochemistry in fresh tissue: NF-kβ, IL-1β, TNF-α and MCP-1. We carried out a complete lipidomic analysis: total lipid, lipid classes, total fatty acids profile and fatty acid profile in lipid classes. The analysis was performed by thin-layer chromatography (TLC), and gas chromatograph coupled to mass spectrometry.

Results: Obese animals had higher expression of two inflammatory markers in kidney tissue, TNF-alpha and MPC-1 and only obese males and obese-ovariectomized females had higher IL1β. The lipid profile of obese animals showed lower saturated and higher polyunsaturated fatty acid in renal tissue both in phospholipid and triglycerides. These changes in lipid and inflammatory profile was exacerbated in the castrated obese females.

Conclusion: Obesity induce inflammation and unbalanced lipid profile in renal tissue, with lower levels of proinflammatory fatty acid (16:0) and higher levels of anti-inflammatory fatty (22:6 n-3). These changes may be reflect a lipidomic adaptation pathway against inflammation and shear stress in renal tissue. Obese females on menopause showed more inflammation but not showed an increase in antiinflamatory fatty acids compared with non-castrated, possibly reflecting an impaired capacity for regulating the lipid profile when estrogen is absent.

Disease modeling of neurodegeneration with brain iron accumulation syndromes (NBIA) for the study of defective lipid metabolism

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Introduction: Neurodegeneration with brain iron accumulation (NBIA) is a rare, inherited, neurological disorder characterized by an abnormal accumulation of iron in the brain and progressive degeneration of the nervous system. NBIA-related genes are directly involved in iron metabolism, but are also involved in fatty acid metabolism. Several animal models have been created to study disease mechanisms, however these models fail to display iron accumulation. Therefore, it is important to find a suitable human research model of the disease, for which stem cells can be used.

Materials and methods: We have established a collection of primary dermal fibroblasts obtained from healthy subjects (n=4) and NBIA-MPAN patients (n=11). Fibroblasts were reprogrammed using a lentiviral vector, which resulted in five iPSC cell lines. The cells were fully characterized and the expression of markers characteristic for pluripotent cells was confirmed. Fibroblasts were also cultured in medium with glucose or galactose, which enhances oxidative metabolism. Cells were incubated in the presence of polyunsaturated fatty acids for 48h. Classes of neutral lipids and phospholipids in lipid extracts obtained from cells were determined by thin-layer chromatography (TLC). In addition, neutral lipids in the cells were stained with Oil-Red (ORO).

Results and discussion: The iPSC cells showed expression of genes and proteins characteristic for pluripotent cells. In vitro spontaneous differentiation analysis confirmed the potential to differentiate into various tissues, including adipose tissue. The analyses showed no significant differences between the levels of neutral lipid and phospholipid accumulation in NBIA-MPAN cells cultured in glucose medium. NBIA-MPAN fibroblasts cultured in medium with galactose showed increased accumulation of neutral lipids (including diacylglycerols, triacylglycerols, free fatty acids) compared to controls. NBIA-MPAN fibroblasts incubated with fatty acids also showed impaired lipid droplet formation.

Conclusion: In conclusion, we obtained five human iPSC cell lines from NBIA patients, which can become an excellent research model, accurately mimicking the conditions in the patients' bodies. In addition, we confirmed that NBIA patients exhibit impaired lipid metabolism, compared to control patients.

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The first step toward understanding the Malassezia virulence and lipid replacement: the genome-scale metabolic model and lipidomic data contextualization.

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Introduction: Malassezia, a lipid-dependent yeast genus, is essential for skin health as it breaks down sebum. However, it is also linked to skin disorders like atopic dermatitis, pityriasis versicolor and more dangerous conditions like fungemia. However, despite the relevance of this yeast's genus, important questions about its pathogenicity still need to be answered. Some studies examined the lipid profile of Malassezia yeast strains and their lipid droplets (LDs) to understand the impact of lipid metabolism on its virulence. Eighteen lipid classes have been identified, including triglycerides (TAG), sterol (CH), diglycerides (DG), and diacylglyceryltrimethyl homoserine (DGTS). Those last compounds have been found to substitute for phospholipids in pathogenic yeasts under phosphate deficiency conditions, impacting yeast pathogenicity. Therefore, this study aims to examine the lipid profile of several Malassezia species, as well as of their LDs, to resolve some of the questions about lipidomic metabolism and its impact on the virulence of this yeast.

Materials and methods: The strains M. pachydermatis CBS1879, M. globosa CBS7966, M. furfur CBS1878, M. sympodialis CBS7222, and M. restricta CBS7877 were included in this study, and lipid droplets were obtained following the protocol reported by Mantilla et al. 2021. Lipids were extracted using the method described by Bligh and Dyer in 1959, and lipid analysis was performed by liquid chromatography-mass spectrometry (LC-MS).

Results and discussion: This study identified 19 phospholipid classes, most found in whole yeast and LDs, including inositolphosphatidylceramides (IPC) and DGTS. DGTS was found to be inversely proportional to phosphatidylcholine (PC), a key component of eukaryotic membranes. This inversion was unvealed in different yeasts and demonstrated the replacement of phosphorylated by unphosphorylated compounds. Results in Galleria mellonella model suggest that DGTS suppresses the pathogenicity of the different strains and with cocurrent changes in their LD composition. Furthermore, at least one of the enzymes involved in DGTS synthesis was discovered in metabolic models of Malassezia species. The possibility that DGTS influences Malassezia's virulence is under current investigation.

Conclusion: This study provides evidence of phospholipid replacement in Malassezia under phosphate deficiency conditions. In depth analyses are needed to determine the effect of DGTS on the virulence of Malassezia

Understanding Malassezia communication: fatty acid metabolism-derived volatile organic compound-mediated interactions between Malassezia globosa and Staphylococcus aureus

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Introduction: Malassezia is a lipid-dependent yeast that inhabits the skin of humans and animals. It coexists with other microorganisms of the microbiota. M. globosa synthesizes volatile organic compounds as a result of its lipid metabolism, these compounds are involved in intra and interspecies communication. In addition, Staphylococcus aureus is a transitory bacteria of skin microbiota, and it is considered a causal microorganism of skin and systemic infections. For M. globosa, there are no reports on the role of its volatiles in the interaction with S. aureus, and these compounds may be involved in the growth of this bacteria, so understanding this interaction could represent an alternative to control the proliferation of this pathogen. This study aims to evaluate the effect of M. globosa VOCs on the growth of S. aureus when they are in interaction.

Materials and methods: Non-contact interaction tests were performed in a dual-plate, split-plate, and facing-plate model. One of the plates was inoculated with 100 µL of M. globosa (1*10*8 cells/mL) on Dixon aga or independent volatile compounds from lipid metabolism, and the other plate was inoculated with 20 µL of S. aureus (OD=0.1) in TSA. The plates were sealed with a double parafilm cover. The cocultures were incubated at 33 °C for 48 hours. The growth of S. aureus was evaluated by counting colony-forming units.

Results and discussion: The volatiles produced from the lipid metabolism of M. globosa have an inhibitory effect on the growth of S. aureus. These findings postulate Malassezia VOCs as a potential biocontrol alternative for S. aureus.

Conclusion: M. globosa can reduce the growth of S. aureus through releasing of volatile organic compounds derived from lipid metabolism.

Adaptation of microscopy for studying cell polarization

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Introduction: Cell polarization, i.e. a spatial difference in the structural organization such as of membrane molecules over the cell, plays an important role in cellular functions. An example is the polarization in epithelial cells, whose disruption by Ras gene mutations leads to cancerous developments. Still, the cell depolarization mechanism remains unknown.

Materials and methods: Here, we investigate polarized cells by means of advanced fluorescence microscopy and spectroscopy approaches, such as super-resolution STED microscopy, fluorescence correlation spectroscopy, and spectral imaging, to explore the differences between apical and basal membranes regarding lipid organization and Ras-lipid interactions. Results and discussion: We explored the differences between apical and basal membranes regarding lipid organization and Ras-lipid interactions. Importantly, we overcome the difficulties of measuring polarized membranes with light fluorescence microscopy due to the photoselection effect, i.e., the preferential excitation of membrane dyes depending on their orientation with respect to the excitation light polarization.

Conclusion: Our results are important to the study of polarized cell membranes using advanced fluorescence microscopy, and for the understanding of the role of Ras and its interaction with lipids.

Comprehending the impact of betaine lipids on Malassezia globosa, an analysis of lipid metabolism of pathogenic yeast

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Introduction: Malassezia, a lipid-dependent yeast genus, is essential for skin health as it breaks down sebum. However, it is also linked to skin disorders like pityriasis versicolor and seborrheic dermatitis and more complicated conditions like fungemia and Crohn's disease. But, despite the relevance of this yeast's genus, some questions about its pathogenicity still need to be answered. Previously studies characterized the lipid profile of Malassezia strains to understand the lipidomic metabolism and its impact on virulence. Eighteen lipid classes have been identified, including triglycerides (TAG) and diacylglyceryltrimethylhomoserine (DGTS). Those last compounds have been found to substitute for phospholipids in pathogenic yeasts under phosphate deficiency conditions, impacting yeast pathogenicity. The synthesis of DGTS is controlled by the BTA1 gene and regulated by the phosphate sensing and acquisition (PHO) pathway with Pho4 as a transcriptional factor. However, those genomic compound of PHO has not been characterized in Malassezia. Therefore, this study aims to examine the lipid metabolism and genome of Malassezia globose to analyze the brain lipids and their impact on the virulence of this yeast.

Materials and methods: The metabolic reconstructions of the M. globosa, carried out by Triana et al. in 2017, were incorporated and partially curated by including the reactions that were either obsolete or unnamed. We looked for the metabolic reaction involved in betaine lipid synthesis and phosphate homeostasis, and the reconstructions were contextualized through lipidomic and genomic data available.

Results and discussion: DGTS is found in M. globosa and is inversely proportional to phosphatidylcholine (PC), a key component of eukaryotic membranes. This proportion has been found in many yeasts, indicating that unphosphorylated ones might replace phosphorylated compounds. Furthermore, the gene homologs of Pho4 and BTA1 were found in the genome of this yeast, and at least one catalytic domain implicated in DGTS lipids was discovered in the M. globosa metabolic model. Therefore, the possibility that they can present in Malassezia and influence Malassezia's virulence cannot be ruled out.

Conclusion: This study provides evidence of the phospholipid replacement in Malassezia. However, more analyses are needed to determine its effect on Malassezia virulence.

Mechanisms regulating lipid-evoked proteostasis in the intestine

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Lipid-rich diets increase incidents of obesity, diabetes, inflammatory diseases, and cancer. Absorption of lipids in the intestine is a multistep process, initiated by the micellization and digestion of lipids in the lumen of the intestine, uptake of fatty acids (FAs) and glycerides by enterocytes followed by re-synthesis of triglycerides (TGs) at the endoplasmic reticulum. In enterocytes, TGs might be either directed into chylomicrons for the subsequent secretion or stored in lipid droplets (LDs). Dynamic processes of deposition and degradation of TGs stored in LDs buffer FAs levels in the cytoplasm at the postprandial and fasting states. Degradation of TGs (lipolysis) stored in LDs into FAs is mediated by multiple enzymes, like adipose triglyceride lipase (ATGL) or hormone-sensitive lipase (HSL). Since FAs at certain concentrations are toxic for cells, regulation of LDs dynamics is central to maintaining intestinal homeostasis. Our previous study showed that upon lipid ingestion protein kinase D2 (PKD2) promotes chylomicron-mediated TGs transport to the general circulation. Our new data show that PKD2 regulates also LDs dynamics in enterocytes and is a part of the signaling machinery regulating changes in enterocytes' proteostasis in response to lipid ingestion. Using mouse models in combination with intestinal organoid cultures, we demonstrated that ingestion of lipids promotes rapid changes in the proteome of enterocytes mediated by posttranslational mechanisms, also by PKD2. These changes include the degradation of LDs-associated lipases to prevent the toxic effects of FAs. These data suggest that enterocytes utilize a mechanism preventing excessive flux of FAs into the cytosol after consumption of lipids, which is required for preserving enterocytes' function.

In vivo evaluation of the impact of hypergravity on lipid phenotype and metabolism

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Introduction: The adverse conditions such as hypoxia, hypothermia and microgravity cause integrated alterations in lipid membrane composition, inducing a greater sensitivity to oxidative stress. Indeed, previous studies suggested that microgravity changes the permeability of plasma membrane and cell metabolism in erythrocyte, modifying cholesterol and phospholipid levels. In addition, also hypergravity affects the physiological functions of tissues and organs. The evaluation of the hypergravity effects on tissues is a fundamental step towards complete knowledge of the physiological response to adverse gravity conditions. Materials and Methods. The aim of this study was to investigate in vivo the effects of hypergravity on lipid phenotype and metabolism in erythrocytes and liver. We employed the Mice Drawer System (MDS) to expose mice to a 3G environment by means of the Large Diameter Centrifuge (LDC). The lipid phenotype were assessed by GC and LC chromatography; moreover, in order to analyse the impact on oxidative homeostasis, the haemolysed fractions were used to assay antioxidant enzyme activity.

Results and Discussion: The data collected suggested in erythrocytes isolated from mice exposed to hypergravity a significant increase of Arachidonic Acid/Eicosapentaenoic Acid Ratio (AA/EPA) ratio, marker of inflammatory state, and in liver a significant alteration of lipid profile and metabolism. Moreover, the hypergravity induced an increase of liver phospholipid level, in particular of phosphatidylethanolamine and phosphatidylserine. The biochemical evaluation of antioxidant enzymes indicated a decrease of superoxide dismutase, catalase and glutathione reductase activities but not of glutathione peroxidase.

Conclusion: This study demonstrates that hypergravity determinates modifications both in the lipid composition of erythrocytes and liver tissue and in the antioxidant system. In the future, further studies will be necessary to identify possible countermeasures to guarantee an adequate level of health and safety of the crew during long-duration space missions.

Impairment of adrenergically-regulated thermogenesis in brown fat of obesity-resistant mice is compensated by changes in lipid catabolism in skeletal muscle

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Introduction: Upon adrenergic stimulation in response to cold and diet, lipid stores are used to fuel non-shivering thermogenesis (NST) mediated by uncoupling protein 1 (UCP1) in brown adipose tissue (BAT) which contributes to both thermal and energy homeostasis. Other mechanisms, including metabolism of skeletal muscle, may also be involved in NST. However, relative contribution of these energy dissipating pathways and their adaptability remain a matter of long-standing controversy.
Materials and methods: We used warm-acclimated (30 °C) mice to characterize the effect of an up to 7-day cold acclimation (6 °C; CA) on thermoregulatory thermogenesis, comparing inbred mice with a genetic background conferring resistance (A/J) or susceptibility (C57BL/6 J) to obesity.
Results and discussion: Both warm-acclimated C57BL/6 J and A/J mice exhibited similar cold endurance, assessed as a capability to maintain core body temperature during acute exposure to cold, which improved in response to CA, resulting in comparable cold endurance and similar induction of UCP1 protein in BAT of mice of both genotypes. Despite this, adrenergic NST in BAT was induced only in C57BL/6 J, not in A/J mice subjected to CA. Cold tolerance phenotype of A/J mice subjected to CA was not based on increased shivering, improved insulation, or changes in physical activity. On the contrary, lipidomic, proteomic and gene expression analyses along with palmitoyl carnitine oxidation and cytochrome c oxidase activity revealed induction of lipid oxidation exclusively in skeletal muscle of A/J mice subjected to CA. These changes appear to be related to skeletal muscle NST, mediated by sarcolipin-induced uncoupling of sarco(endo)plasmic reticulum calcium ATPase pump activity and accentuated by changes in mitochondrial respiratory chain supercomplexes assembly.
Conclusion: Our results suggest that NST in skeletal muscle could be adaptively augmented in the face of insufficient adrenergic NST in BAT, depending on the genetic background of the mice. It may provide both protection from cold and resistance to obesity, more effectively than BAT. See also: Janovska et al. 2023, Mol Metab. doi: 10.1016/j.molmet.2023.101683Supported by Czech Science Foundation (22-07004S) and by the project National Institute for Research of Metabolic and Cardiovascular Diseases (Programme EXCELES, ID Project No. LX22NPO5104) – funded by the European Union – Next Generation EU.

Phenotypic and lipidomic characterization of A549 lung cancer cells exposed to indoor dust

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Introduction: It is well known that dust particles are directly related to potential health concerns such as cardiovascular and respiratory diseases. Indoor dust particles contain complex mixtures of many chemical families which can have a differential and additive behavior in lungs when they are inhaled, leading to alterations of lung homeostasis.

Materials and methods: This work explored the effects of dust samples collected from houses, libraries, and high schools on lungs, using the A549 lung cancer cell line. The chemical composition of these samples had been characterized previously. Three-dimensional alginate-encapsulated (3D) cells were used in this study to enhance the physiological relevance of the results. Cells were exposed to dust sample extracts for 72 hours, and cytotoxicity, reactive oxygen species, and interleukin-8 were measured. In addition, lipid cell extracts were analyzed using a UHPLC/Q-TOF-MS in positive ionization. Several chemometric methods were used to analyze lipidomics data, including principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA).

Result and discussion: The PCA of lipidomics revealed that changes in the lipidome occurred due to the exposure to dust extracts. The PCA of chemical composition revealed a clear separation between dust samples from houses from those of libraries and high schools, which were more similar to each other. Additionally, we used the chemometric method multivariate curve resolution alternating-least squares (MCR-ALS) to obtain the main profiles of chemical composition and lipid profiles of dust samples from houses, libraries, and high schools. These data was combined and MCR-ALS was applied again to associate chemical composition and the biological effects observed. As a result, six components representing 85% of the original data have been resolved. For instance, component 2 (12% of explained variance) was mainly distributed in a house, characterized by the presence of polyclorobiphenyls (PCB), poly aromatic hydrocarbons (PAHs), and nicotine, associated with an increase of monoacylglycerols and acyl carnitines, and a decrease of tryglycerides. This component was also related to increased cell death,ROS production and IL-8 release.

Conclusion: In this study, we combine the use of 3D cell cultures, lipidomics and chemometrics to identify connections between specific pollutants (and their combinations) and the potential harm they may cause to lung health.

Membrane lipids as source for free fatty acids fueling UCP1 activity in brown adipocytes

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Introduction and aim: The presence and activity of brown adipose tissue (BAT) in humans are associated with higher energy expenditure, lower adiposity and reduced risk of insulin resistance. BAT activation is mediated by uncoupling protein 1 (UCP1). To the current knowledge, UCP1 is fueled by free fatty acids (FFA) released from triacylglycerol (TAG) in lipid droplets during lipolysis by adipose triacylglycerol lipase (ATGL). Here, we propose that also FFA derived from membrane phospholipids by phospholipase A2 (PLA2) drive UCP1-activity in brown adipose tissue.

Materials and methods: Lipidomics was employed to quantify the phospholipidome of primary brown adipocytes after induction of UCP1 with beta-adrenergic agonists. mRNA and protein expression of phospholipid-metabolizing enzymes was investigated with qPCR and proteomics. Finally, PLA2 activity was modified by RNA interference and lentiviral overexpression, before quantification of UCP1 activity using microplate-based respirometry.Results and discussion: We found that beta adrenergic stimulation of murine primary brown adipocytes increases the expression of PLA2. As consequence, phosphatidylcholine (PC) and phosphatidylethanolamine (PE) are degraded to lyso-PC (LPC), lyso-PE (LPE) and FFA. Knockdown of PLA2 blocks LPC generation and reduces UCP1-activity, whereas its overexpression promotes UCP1-mediated respiration in primary brown adipocytes. Mechanistic investigations suggest that PLA2 induction is mediated via the β3-adrenergic receptor and that in addition to FFA liberated from PC, also saturated LPC species influence UCP1.

Conclusion: Free fatty acids and lyso-phospholipids released from membrane phospholipids by PLA2 stimulate and fuel UCP1 activity in brown adipocytes. Further studies with mice having a transient knockdown of PLA2 in UCP1-expressing cells will allow to characterize the physiological and pathophysiological relevance of membrane phospholipids for BAT activation.

The linoleic acid-derived leukotoxin 9,10-DiHOME drives immunosuppression in patients with acute-on-chronic liver failure

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Introduction: Acute decompensation (AD) of liver cirrhosis is characterized by smoldering systemic inflammation that favors the development of organ failure, a condition known as acute-on-chronic liver failure (ACLF) associated with high short-term mortality. In this context, inflammation-related immunosuppression is a crucial factor contributing to secondary infections and multiple organ dysfunction. In this study we explored the profile of immunomodulatory lipid mediators in patients with AD cirrhosis with and without ACLF and investigated their effects on neutrophil function.

Materials & methods: The profile of 101 lipid mediators was determined by targeted lipidomics using liquid chromatography coupled to tandem mass spectrometry in plasma from 84 patients with AD cirrhosis without ACLF (stratified in stable decompensated cirrhosis (n=53), unstable decompensated cirrhosis (n=10) and patients with high-risk of developing ACLF (pre-ACLF) (n=21)), and 9 patients with AD cirrhosis and ACLF. For comparison, 31 healthy donors were included. Bioassays determining degranulation, respiratory burst capacity and phagocytosis were performed to assess changes in neutrophil function. Gene expression for neutrophil immunocompetence markers were determined by real-time PCR.

Results & discussion: The exploratory analysis of the whole lipid dataset at baseline showed higher plasma levels of linoleic acid (LA)-derived lipid mediators in patients with AD cirrhosis as compared to healthy controls. Correcting for multiple hypothesis testing, we identified the LA-derived 9,10-dihydroxy-12-octadecenoic acid (9,10-DiHOME) as the only lipid mediator with discriminating power between AD patients with established ACLF from those without. In addition, 9,10-DiHOME levels were significantly higher at the time AD patients manifested an active infection. Moreover, expression of soluble epoxide hydrolase, the enzyme responsible for the biosynthesis of 9,10-DiHOME was upregulated in AD patients. In the neutrophil bioassays, 9,10-DiHOME impaired degranulation and respiratory burst capacity and induced a shift towards an immunosuppressed phenotype.

Conclusion: The leukotoxin 9,10-DiHOME induces neutrophil immunosuppression and therefore, increased levels of this lipid mediator might enhance susceptibility to bacterial infection and precipitate ACLF in patients with AD cirrhosis. These data positions soluble epoxide hydrolase as potential candidate for drug treatment in this condition.

Characterization of whole-body and tissue lipid metabolism of GPR10 x NPFF2R dKO mice

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Introduction: Energy homeostasis as a balance between energy intake and expenditure is controlled by brain centers, mainly hypothalamus. RF-amide peptides, such as PrPR, control numerous biological functions such as regulation of food intake. The aim of the study was to characterize the effect of deletion of two of receptors for PrRP, GPR10 and NPFFR2, on energy homeostasis and lipid metabolism during development of diet-induced obesity.

Materials and Methods: We used dKO mice on a hybrid B6J x B6N background and their non-littermate controls, both males and females. Mice were fed either standard (STD; Ssniff RMH) or high-fat diet (HFD; based on lard) for 16 weeks starting at 4 months of age. Indirect calorimetry was used to quantify energy expenditure (EE), energy intake, substrate partitioning, and physical activity at the beginning and end of this study. Gene expression was determined in hypothalamus and white adipose tissues using quantitative PCR analysis.

Results and Discussion: dKO male and female mice on STD diet displayed a shift towards lower RQ and a trend in the same direction at the end of the study and before dietary intervention, respectively, suggesting increased lipid oxidation. This shift could be explained in females by lower food intake of dKO mice; however, it was also significant in males, where no significant changes in food intake were detected. For a correct evaluation of EE we have assessed it both before and after dietary intervention and employed multilinear regression to be able to compare animals with different body weights. We have confirmed that the differences in EE between genotypes are not higher than the resolution of our indirect calorimetry system. Gene expression confirmed previously published gender differences in lipid metabolism in adipose tissues in dKO mice and no prominent changes in neuropeptide´s gene expression in hypothalamus.

Conclusions: To conclude, we have eliminated the possibility that increased body weights of dKO mice are due to changes in EE, so the signaling pathway from PrRP to GPR10 and NPFFR2 influences only food intake regulation. This is an important finding considering the pharmacological potential of lipidated analogues of PrRP. An increased whole-body lipid oxidation might be connected to higher obesity in dKO mice. Supported by the Czech Science Foundation from a grant 21-03691S.

Application of lipid bioinformatics in assessing the functional lipid composition in maternal breastmilk lipidome and influences on infant health outcomes

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Introduction: Complete resolution of the breast milk (BM) lipid molecular species during analysis is essential for understanding metabolism as a function of lactational programming since BM lipids have many functional roles in infants' health and development. Lipid bioinformatics is an emerging tool that could be useful in improving knowledge of how BM lipidome impacts infant health. This study aims to demonstrate the applications of lipid bioinformatics in showing the association between maternal body mass index (BMI) and BM lipidome with infant atopic disease and growth outcome during early life.

Materials and Methods: Study consisted of secondary analysis of 40 mothers (non-obese and obese) infant dyads of a randomized controlled trial of vitamin D supplementation during lactation. BM, Data regarding maternal diet, infant anthropometrics (weight, fat mass index (FMI), fat-free mass index (FFMI), and infant atopic disease data were collected at 1 and 4 months postpartum. BM lipids were assessed using ultra-high-performance liquid chromatography coupled with high-resolution accurate mass tandem mass spectrometry. Untargeted lipidomics workflow was applied to assess the BM lipidome and its association with infant health trajectories outcomes. Potential BM lipid biomarkers and metabolic pathways were evaluated using Receiver Operating Characteristic curves, networks, and pathway analysis.

Results and Discussion: Redundancy analysis was most suitable to reduce the dimensionality of the BM lipids data and show ordination with maternal status, visits postpartum, and infant health parameters. Sphingolipid(SM) and glycerophospholipid pathways were the high-impact pathways associated with the altered BM functional lipids (Phosphatidylcholine(PC), Phosphatidylethanolamine(PE), Phosphatidylserine (PS), Ceramides) that were significantly correlated with infant development and atopic disease outcome during the first year of life. We observed SM(d16:0/18:1), PE(18:0p/18:1), PE(18:0p/18:2), PC(18:0/16:0), PC(16:0/16:0), PS(18:0/20:3), PS(18:0/18:1), PE(18:0/16:0), PI(16:0/16:0) and SM(d22:0/18:1) are potential biomarkers of atopic disease (eczema) or growth outcomes (FMI and FFMI) and were significantly different in obese or non-obese mothers ($p<0.05$). **Conclusion**: The application of lipid bioinformatics workflows helped to better understand how maternal BMI alters the BM lipidome, associated metabolomic pathways, and its effects on infant health outcomes in early life.

Plasma lipidome analysis reveals a specific impact of Clostridioides difficile infection and treatment on sterol metabolism

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Introduction: Clostridiodies difficile infection (CDI) has been classified as an urgent public health threat and a major concern in hospital, outpatient, and extended-care facilities worldwide. Despite the correct clinical management of patients, the risk of CDI recurrence increases after multiple recurrences. Antibiotic treatment reduces gut microbiota diversity, providing optimum conditions for C. difficile spore germination and toxin production. Given the metabolic changes occurring at the gut level in CDI, our interest was focused on the effects at local and systemic levels, both during the infection and its treatment, paying particular attention to bile acids (BA) and cholesterol metabolism due to their close relationship with CDI pathogenesis.

Materials and methods: Participants were recruited at the University Hospital Son Espases (Palma, Spain). Stool and plasma samples were obtained from healthy volunteers and patients with CDI diagnosis, primary or recurrent, before and after treatment (antibiotic or FMT) to carry out gut microbiota, plasma bile acids, and plasma lipidome analysis. Total DNA was extracted from fecal samples and used for sequencing the V3 and V4 variable regions of the 16S rRNA gene (MiSeq, Illumina). Plasma BAs were quantified by liquid chromatography-tandem mass spectrometry (LC-MS/MS). The analysis of plasma lipids was performed by direct flow injection analysis using a hybrid quadrupole-Orbitrap mass spectrometer (FIA-FTMS).

Results and discussion: CDI and treated patients showed a significant increase in total plasma BA content, which was more pronounced in taurine-conjugated BA. Plasma lipidome analysis revealed a global decrease in circulating lipids in CDI patients, being the largest impact specifically on cholesteryl esters (CE). We also identified in CDI patients a specific and consistent decrease in the levels of the lipid species containing linoleic acid, an essential fatty acid, which recovered after treatment.

Conclusion: C. difficile infection impacted plasma BA, CE, and 18:2 content. Because CE and BA derive both from cholesterol, it is tempting to speculate that CDI somehow detours cholesterol channeling into BA synthesis. The decrease in 18:2-containing species is consistent with impaired absorption due to a malfunction of the bowels in CDI patients. Hence, these results would encourage a close follow-up on the nutritional state of patients after successful treatment.

How mitochondria functionally rely on the molecular composition of their membrane phospholipids

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Introduction: The regulation of the phospholipid composition in mitochondria is highly specific and strongly varies according to cell type, tissue, and species. In various human diseases the membrane lipid composition becomes disrupted, leading to detrimental effects on mitochondrial functions, such as oxidative phosphorylation, mitochondrial morphology, interactions with other subcellular compartments, metabolite transport, as well as intracellular signaling. In inherited metabolic disorders, such alterations play a crucial role in determining patient pathologies. This is also true for Barth Syndrome, a rare x-linked mitochondriopathy that is characterized by cardiomyopathy, skeletal muscle weakness, growth delays, and neutropenia. Here, we explored the impact of changes in mitochondrial membrane lipid architecture on its functions and vice versa.

Material and methods: We employed cell culture models for inherited disorders in mitochondrial membrane lipid homeostasis, combined with LC-MS/MS lipidomics approaches to structurally characterize lipid changes. Furthermore, cells were exposed to altered lipid environments, leading to a targeted reconfiguration of the lipid composition of different phospholipid classes. Results and discussion Here, we studied the dependency of lipid composition and membrane function in the genetic context of inherited metabolic diseases. Variable fatty acid pools affected specific lipid classes, and consequently modulated subcellular compartments with variable penetrance. While certain lipid compositions were able to suppress disease phenotypes, such as the accumulation of oxidative stress in mitochondria of Barth Syndrome model cells, others had less favourable effects on cellular functions. In contrast, we observed that the physiological, though not necessarily biochemical, capacity of the respiratory chain exhibits surprising resilience to changes in the mitochondrial membrane lipid composition.

Conclusion: In summary, a strong reciprocal relationship between membrane lipid architecture and mitochondrial function exists that extends across various layers of cellular physiology and crucially contributes to pathogenesis of mitochondrial disorders.

Transmembrane domain length as determinant of contact site localization and autophagic protein turnover

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Introduction: Eukaryotic membranes are compartmentalized into distinct micro- and nanodomains that rearrange dynamically in response to external and internal cues. This lateral heterogeneity of the lipid bilayer and associated clustering of distinct membrane proteins contributes to the spatial organization of numerous cellular processes. Here, the organellar organization of the endoplasmic reticulum and the distribution of lipid rafts within it were in the focus of the study. The endoplasmic reticulum fulfills a plethora of crucial cellular processes, such as protein and lipid synthesis as well as calcium homoestasis, and is composed of thinner and thicker regions, indicative of ER lipid rafts, with distinct functions.
Materials and Methods: Flow cytometric analyses, immunoblot analyses, cyclohexamide chase assay, and confocal microscopy was applied to obtain our results. Flow cytometry is a method that allows the determination of the GFP intensity to estimate the level of a GFP-tagged protein. It can also be used to evaluate the percentage of dead cells within a culture when staining cells with a red fluorophore. Immunoblot analyses enabled the visualization of proteins using an appropiate antibody to assess protein levels in cells. The cyclohexamide chase assay allowed us to determine the stability and thus turnover of a protein. Confocal microscopy served as a method to visualize proteins in living cells.
Results and Discussion: Here, we show that lipid rafts within the membranes of the endoplasmic reticulum (ER) of yeast cells change during metabolic reprogramming and aging. Using biosensors with varying transmembrane domain length to map lipid bilayer thickness, we demonstrate that in young cells, lipid rafts mainly exist within the nuclear ER, while progressing cellular age drives the formation of numerous lipid rafts specifically in the cortical ER. Partitioning of biosensors with long transmembrane domains into these lipid rafts increased protein stability and prevented autophagic removal. In contrast, reporters with short transmembrane domains progressively accumulated at the membrane contact site between the nuclear ER and the vacuole and were subjected to turnover via selective microautophagy occurring at these contact sites.
Conclusion: Our data reveal age-dependent rearrangement of the lateral organization of the ER and establish transmembrane domain length as a determinant of membrane contact site localisation and autophagic turnover.

Fuel Preferences and Metabolic Regulators of Cell Growth and Differentiation

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Introduction: Metabolic reprogramming is a hallmark of cancer cells and a key contributor to cancer progression. Recently, our and others work have demonstrated that acyl-CoA binding protein (ACBP) plays a fundamental role in cellular lipid metabolism by augmenting de novo lipogenesis, sphingolipid biosynthesis and fatty acid oxidation and is required for proliferation of tumour cells in vitro including glioblastoma and non-small cell lung cancer (NSCLC). Given the prominent expression of ACBP in a wide variety of human cancer types, we hypothesize that ACBP plays a critical role in tumour metabolism and growth by serving as a key regulator and driver of intracellular fatty acid flux.
Materials and methods: To obtain detailed biochemical insights into tumour metabolism in development and progression of NSCLC, we will first determine how nutrient preferences is altered in tumour using state-of-the-art in vivo tracer perfusion techniques combined with LC/MS-based metabolomics. We will examine how 13C-labeled substrates are metabolizxed in biochemical pathways in both tumor and healthy tissues. To specifically address the role of ACBP in tumour growth and metastatic colonization, we have generated human NSCLC cell lines by genetically ablating the gene encoding ACBP. We will do some initial in vitro characterization of these cells, including their proliferation, migration and respiratory capacity compared to control NSCLC cells. We will inject both control and ACBP-ablated cancer cells into the flank of immunodeficient mice to generate human xenograph models of NSCLC, and subsequently monitor the cells' ability to grow and form lung metastases. To explore the localization of metabolites on a cellular level in tumours from NSCLC mouse models, we will develop spatial metabolomics.
Results and discussion: We have confirmed that ACBP expression have successfully been ablated in the NSCLC cell line A549. Ongoing experiments will unravel how functional loss of ACBP affects proliferation and respiration in vitro.
Conclusion: Future experiments will provide valuable insights into tumour metabolism and metabolic regulation of cancer cells, contributing to development of improved treatment strategies.

Damage and repair of membrane lipids in mitochondrial β-oxidation disorders

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The constant repair of damaged lipids in biological membranes is a vital process for cellular integrity and function. Many metabolic diseases result in pathological alterations of the membrane lipid composition, which can contribute significantly to patient symptoms and can be considered as a damaging process. This is also true for inborn errors of mitochondrial lipid metabolism, such as very long-chain acyl-CoA dehydrogenase deficiency (VLCADD) and long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency (LCHADD), for which a reconfiguration of lipid metabolism has been reported. However, the extent of respective membrane damage is still largely uncharacterized and a mechanistic link to the mechanistic changes is missing. One reason for this is that it is still challenging to quantify and functionally interpret membrane lipid damage. Here, we used liquid chromatography - tandem mass spectrometry (LC-MS/MS) to describe the lipidomic changes in VLCADD and LCHADD patient-derived fibroblasts. First, the dysregulation of fatty acid metabolism in these cells was analysed by measuring acyl carnitine patterns. We observed an accumulation of long chain carnitine species in both disease models and increased levels of hydroxylated long chain carnitines in LCHADD cells, confirming the primary molecular defects. Next, we characterized alterations in membrane lipids by performing an untargeted lipidomics analysis, where we annotated approximately 600 lipids species from 16 different lipid classes. Indeed, disease-specific changes could be detected in a number of lipid species, especially in ceramides, as well as ether- and vinyl ether-linked phospholipids. Mitochondrial cardiolipins were not affected. Based on these observations, we were able to show that the disease related damage not only affects fatty acid metabolism, but also extends into the realm of complex membrane lipids. This now sets the basis for deconvoluting the individual contributions of these effects to the molecular phenotypes of VLCADD and LCHADD, allowing us to understand the contribution of membrane lipid damage to their pathomechanisms in detail.

Trimodality optical imaging for tracking subendothelial retention of electronegative low- density lipoprotein in vivo

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The fifth subfraction of low-density lipoprotein (L5 LDL) can be separated from human LDL using fast-protein liquid chromatography with an anion exchange column. L5 LDL induces vascular endothelial injury both in vitro and in vivo through the lectin-like oxidized LDL receptor-1 (LOX-1). However, no in vivo evidence shows the tendency of L5 LDL deposition on. vascular endothelium and links to dysfunction. This study aimed to investigate L5 LDL retention in vivo using state-of-art trimodality optical imaging, with Iodine-131 (131I)-labeled and injected into six-month-old apolipoprotein E knockout (apoE-/-) mice through tail veins. Besides, we examined the biodistribution of L5 LDL in tissues and analyzed the intracellular trafficking in human aortic endothelial cells (HAoECs) by confocal microscopy. The impacts of L5 LDL on HAoECs were analyzed using electron microscopy for mitochondrial morphology and western blotting for signaling. Results showed I-labeled-L5 was preferentially deposited in the heart and vessels compared to L1 LDL. Furthermore, L5 LDL was co-localized with the mitochondria and associated with mitofusin (MFN1/2) and optic atrophy protein 1 (OPA1) downregulation, leading to mitochondrial fission. In summary, L5 LDL exhibits a propensity for subendothelial retention, thereby promoting endothelial dysfunction and the formation of atherosclerotic lesions. Keywords: Electronegative low-density lipoprotein (L5 LDL); trimodality optical imaging system; detection of L5 LDL retention in vivo; propensity for subendothelial retention; formation of atherosclerotic lesions; endothelial dysfunction.

SS-31 treatment improves mitochondrial function in heart of TAFAZZIN knockdown mice without affecting the cardiolipin fingerprint

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Introduction: Barth Syndrome (BTHS) is a rare, X-linked recessive disease, which mainly causes early-onset cardiomyopathy and skeletal myopathy, but also growth delay, neutropenia, organic aciduria, mitochondrial dysfunction. BTHS results from loss-of-function mutations of the TAFAZZIN (Taz) gene, encoding for an acyltransferase enzyme, which is required for remodeling of the mitochondrial phospholipid cardiolipin (CL) towards its highly symmetrical acyl composition. Although the development of an effective therapy for BTHS patients remains challenging, one of the most promising therapeutic approaches includes the CL-targeted tetrapeptide, named SS-31 (or elamipretide). Here we describe the beneficial effects of SS-31 on cardiac mitochondrial disfunction in tafazzin-knockdown mice.
Materials and Methods: We isolated mitochondria from heart of tafazzin-knockdown mice (Taz-KD, at 4 months of age) after administration of SS-31 peptide (3 mg/Kg/day) for 10 weeks. Lipidomics analysis was performed by MALDI-TOF/MS (both positive and negative ionization modes), using 9-aminoacridine as a matrix. Respiratory activity of isolated mitochondria was measured polarographically with a Clark-type oxygen electrode. Electrophoretic procedures (BN-PAGE and SDS-PAGE) and Western blotting were performed to study the effects of SS-31 on respiratory chain supercomplex organization and mitophagy. Mitochondrial ultrastructure and morphology were analysed by TEM.
Results and Discussion: In this study, we show that treatment of Taz-KD mice, an animal model of BTHS, with SS-31 peptide resulted in promotion of supercomplexes organization and improvement of respiratory capacity in heart mitochondria, although the MALDI phospholipid profile (and the monolysocardiolipin/ cardiolipin ratio) remained unaffected. Furthermore, we demonstrate that the positive effects on mitochondria are associated with the pharmacological amelioration of mitochondrial ultrastructural morphology and restoration of defective mitophagy in tafazzin-deficeint mice.
Conclusion: SS-31 peptide have beneficial effects on mitochondrial dysfunction of Taz-KD mice by improving inner membrane ultrastructure and restoring defective mitophagy, suggesting the small peptide as future pharmacological agent for BTHS.

Analysis of the Kinase and Phosphatase Activities of Undecaprenol Kinase from Gram-positive Bacteria Using In Surfo and In Meso Assays

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Introduction: Novel antibiotics and therapies are urgently required to combat antibiotic-resistant bacteria. Some bacterial lipid-processing enzymes have been proposed as potential drug targets. Undecaprenol kinase (UdpK) is one such enzyme. It is involved in the pathogenicity of Streptococcus mutans by converting undecaprenol into undecaprenyl phosphate, which plays a vital important role in cell wall synthesis. UdpK also has phosphatase activity in the presence of ADP, although its physiological relevance is still unknown. In this study, we use two assays (direct and indirect) to investigate the activity of 5 UdpK orthologs in order to guide future structure and mechanistic enzymology studies and for inhibitor library screening.
Materials and methods: For the direct assays, pure recombinant UdpK was incubated in a reaction mix containing all the necessary substrates and cofactors and the mix was subsequently analysed by thin layer chromatography. The direct (discontinuous) assay allows both the kinase and phosphatase activities to be analysed by in surfo (the enzyme is in a surfactant micelle) and in meso (the enzyme is reconstituted in a lipid cubic mesophase) methods. The indirect assay uses pyruvate kinase and lactate dehydrogenase to couple the production of ADP by UdpK to the conversion of NADH to NAD+ which is quantified by absorbance at 340 nm. The indirect assay can also be performed in surfo and in meso. It allows for continuous and high-throughput measurements.
Results and discussion: UdpK from 2 orthologs showed activity in both assays, while the other 3 require further investigation. The indirect assay can now be used in functional studies to characterise the kinetics and mechanism of the enzymes, and for high-throughput inhibitor screening. The direct assay can be used to quantify the kinase and phosphatase activities of UdpK. Importantly, both assays can be performed in surfo and in meso. This means that functional characterisation of UdpK can be carried out under conditions akin to those used for crystal growth and subsequent structure determination by X-ray crystallography.
Conclusion: The activity assays presented here have been validated for the functional characterisation of UdpK for use in structural studies to guide the design and development of inhibitors.

Subcellular lipidomics in neurodegeneration: TDP-43 and mitochondria-associated membranes

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Introduction: TDP-43 may contribute to ALS pathogenesis in different cellular compartments like mitochondria. Mitochondria show intimate contact with the particular endoplasmic reticulum (ER) membrane subdomains termed mitochondrial-associated membranes (MAMs). MAM lipid composition is essential for its proper function. Consequently, changes in their lipidome could compromise the activity of proteins residing in MAMs. Our previous finding that TDP-43 alterations in animal and cellular models are related to changes in MAM activity led us to assess the possible relationship between TDP-43 (dys)function and MAM lipidome.
Objective: evaluate the potential changes in MAM lipidome secondary to TDP-43 alterations and establish a relationship between TDP-43 and lipid metabolism.
Material and methods: We evaluated the lipidome of MAM and ER in the human frontal cortex (n=5 control group; n=6 ALS group) and tissue from transgenic B6N-Cg-Tg(Prnp-TARDBP*Q331K)103Dwc/J (TDP-43 Q331K) mice, both in brain and spinal cord samples. After subcellular fractionation, we used liquid chromatography-mass spectrometry (LC-MS) to perform both an untargeted and targeted lipidomic approach. To assess the potential alterations in MAM-controlled lipid metabolism related to TDP-43 dysfunction, we have also evaluated phospholipid metabolism in a human cellular model of TDP-43 loss of function (HeLa-PLKO cells).
Results and discussion: Untargeted lipidomics analysis shows that MAM and ER lipid composition is different in humans and animals, suggesting that although MAM is an ER domain, it has a unique lipid composition. In addition, we also observed that alterations in TDP-43 affect part of MAM and ER lipidome in all of our models. These results indicate that TDP-43 may play an important role in lipid metabolism and MAM function. In contrast, the decrease of TDP-43 levels in HeLa-PLKO cells does not lead to global changes in phospholipid metabolism, suggesting that extra-MAM lipid metabolism (Kennedy pathway), could compensate for potential loss of TDP-43 controlled pathways.
Conclusion: Abnormalities in TDP-43 can affect the lipid composition of MAM and ER, both in human and animal models of mutated TDP-43. Changes in MAM composition may affect proteins that reside in them, which would partly explain previous results in which we have observed alterations in MAM activity in models of TDP-43 dysfunction.

The lipidomic profile of the human extreme longevity

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Introduction: Lipids play crucial roles in regulating aging and longevity. It has been described in our group that centenarians have a specific plasma lipidome, which distinguishes them from adults and the elderly. Therefore, plasma lipidomic profile is an optimized feature associated with longevity, and centenarians are a model of healthy ageing and extreme longevity. In this context, offspring of centenarians are a promising model for the genetic and phenotypic characterization of the ageing process. Thus, we hypothesize that centenarians genotype is projected to the lipid profile to their descendants.
Objective: evaluate and describe specific lipid biomarkers of extreme longevity.
Material and methods: We use targeted lipidomics approach to assess plasma lipid profile from centenarians (100 years old), offspring of centenarians (70 years old) and a control group (70 years old). Lipid analysis of samples extracts was performed on an Agilent 6495 LC/QC mass spectrometer. A total of 688 lipid species and univariate were analyzed, and multivariate statistics was applied.
Results and discussion: Targeted lipidomics analysis shows that among a total of 688 lipids quantified, 196 are different between groups. Main differences (167 species) are observed between centenarians and the other groups indicating that these lipids are biomarkers of aging. Interestingly, 19 lipid species are statistically different between control group and centenarian/offspring groups indicating that these lipids can be biomarkers of extreme longevity. Furthermore, 10 lipids were specific of centenarians' offspring. The extreme-longevity lipidomic fingerprint suggest a stress-resistant lipidome profile and a better preservation of β-oxidation and membrane structure and function that protect these individuals since adulthood and could be crucial for determining their lifespan.
Conclusion: The lipidome profile of centenarian and descendants shows 19 markers of extreme longevity that indicate increased protection against lipid peroxidation, increased preservation of cell membranes and changes in bioenergetic metabolism.

The protective effects of E2 on lipid metabolism in palmitic treated HepG2 cells are associated to AMPK

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Introduction: Non-alcoholic fatty liver disease (NAFLD) is the leading cause of liver illness and liver transplantation worldwide. NAFLD shows a sex-specific prevalence with men having a higher incidence of NAFLD than women, at least until menopause. This suggests that estrogens could play a pivotal role in the control of fatty acid (FA) metabolism that led to a lower ectopic fat accumulation in women. On the other hand, the adenine monophosphate activated protein kinase (AMPK), which acts as an energy sensor in the cell, has been proposed as a key molecule against hepatic steatosis due to its ability to activate catabolic processes and to inhibit the anabolic ones. Our hypothesis states that estradiol (E2) exerts a protective role against the development of NAFLD in women favoring FA oxidation and reducing FA synthesis through the activation of AMPK.

Material and methods: To evaluate the role of E2 in the regulation of the FA metabolism in the hepatocytes, HepG2 cells were treated with palmitate (PA, 0,75 mM) to induce lipotoxicity and with E2 (100 nM) for 24h. The role of AMPK as a mediator of the cell signaling induced by E2 was tested treating HepG2 cells with the AMPK inhibitor compound C (10µM). Results and discussion: Our results show that despite inducing the expression of genes involved in FA oxidation (PPAR, CPT1A, MACD and ACOX) and VLDL secretion (MTP), PA caused an increase in lipid accumulation in HepG2 associated to an increase in the expression levels of genes involved in their uptake (CD36), transport (FATP2, FABP1) and synthesis (SREBP and FAS). E2 treatment reduced PA-induced lipid accumulation by inducing the expression of the main markers of FA oxidation (PPARalpha, CPT1A, MCAD and ACOX) and VLDL secretion (MTP), as well as through the reduction of the levels of SREBP-1c, marker of lipogenesis. E2 also induced an increase in the expression of genes involved in FA uptake and transport (CD36, FATP2 and FABP1), although it was not enough to counteract FA mobilization and oxidation. Finally, the inhibition of AMPK reversed the effects of E2 and led to a rise in lipid accumulation by increasing the expression levels of SREBP-1, while decreasing the expression levels of PPARalpha, MTP, CPT1A, ACOX and MCAD.

Conclusion: Our results show that E2 improves liver steatosis by inducing FA metabolism through the activation of AMPK and explain, at least in part, why women are protected from NAFLD until menopause.

Plasma oxylipins as biomarkers to differentiate between Non-Alcoholic Fatty Liver Disease severity grades

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There is a lack of conclusive biomarkers for the non-invasive monitoring of Non-Alcoholic Fatty Liver Disease (NAFLD). Diagnosis of Non-Alcoholic SteatoHepatitis (NASH) progression is based upon the NAFLD Activity Score using liver biopsies. The abdominal magnetic resonance imaging-estimated proton density fat fraction (MI-PDFF) measuring the intrahepatic fat content (IFC) represents the non-invasive standard gold reference for grading “Not NASH” NAFLD in four stages: without-, mild-, moderate- and severe-NAFLD. Oxylipins plasma levels such as 11,12-diHETrE, dhk PGD2 and 20-COOH AA has been proposed as biomarker to differentiate NAFLD from NASH; however, no oxylipins have been proposed to differentiate the stage of “Not NASH” NAFLD. We propose that the oxylipin plasma levels correlating with the IFC could have diagnostic value to range NAFLD steatosis. Ninety 40–60-year-old adults recruited in the Balearic Islands participating at the FLIPAN trial, Spain, with NAFLD diagnosed by MI-PDFF were selected. The trial number NCT04442620 registered at ClinicalTrials.gov. NAFLD diagnosis was performed by abdominal MI-PDFF. Participants were grouped considering their IFC in four stages of NAFLD: IFC0 (stage 0, control group without steatosis) IFC < 6.4%; IFC1 (mild steatosis) 6.4% ≤ IFC < 17.4%; IFC2 (moderate steatosis) 17.4% ≤ IFC < 22.1% and IFC3 (severe steatosis) IFC≥ 22.1%. Plasma oxylipin levels were determined by an adaptation of our method for the determination of oxylipins in immune cells by HPLC-MS/MS technology after solid phase extraction of plasma oxylipins, using d4-PGF2α as internal standard. The discriminatory capability of plasma oxylipin concentrations for different steatosis grades was tested by the area under the receiver operating characteristic curve (AUCROC) using the steatosis grade determined by MI-PDFF as standard gold reference. The plasma levels of 12-hydroxy-stearic acid (12HEST), prostaglandin-2α (PGF2α), 15-hydroxy-eicosatetraenoic acid (15HETE) showed significant diagnostic accuracy to distinguish patients with different NAFLD grades. The oxylipin 12HEST is the most sensible biomarker to diagnose severe NAFLD (IFC3), but with low specificity. The PGF2α plasma levels exhibit similar sensitivity but higher specificity than 12HEST. The 15HETE plasma levels have a similar diagnostic value as PGF2α to diagnose severe NAFLD (IFC3).

Mitochondrial lipidome's fatty acid profile is a tissue-specific adaptation

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The main source of damaging intracellular reactive species is mitochondria. Since unsaturated fatty acids (UFA) are the more easily oxidable biomolecules, optimization of mitochondrial membrane FA composition is essential to ensure its integrity. The main objective of the present work was to characterize the lipidome's FA profile of mitochondria from different rat tissues. We have isolated mitochondria from brain, heart, muscle, liver and kidney, and analysed its FA profile using a gas chromatography system. We have detected a total of 20 different FA, and univariate and multivariate statistics were applied. Our results revealed that: 1) Mitochondrial FA composition differs among tissues, and clusters in three groups: brain, heart and muscle, and liver and kidney; 2) brain mitochondria FA have the highest content of monounsaturated FA (MUFA), and the lowest content of polyunsaturated FA (PUFA; mostly ω-6), double bond content and susceptibility to oxidation compared to other tissues; 3) heart mitochondria is enriched in FA with long acyl chain length and have a lower content of PUFAω-6 compared to that of muscle; 4) liver mitochondria is enriched in FA with long acyl chain length and have a higher content of PUFA compared to that kidney; 5) heart mitochondria is enriched in FA with short acyl chain length, high content of SFA and low UFA (mainly ω-6) compared to that of liver; and 6) FA mitochondrial composition from heart and kidney is similar. Globally, our results suggest that the mitochondrial lipidome's FA profile is a tissue-specific trait probably programmed during development and regulated to ensure and maintain the energetic needs of each tissue.

Circulating lipidome's fatty acid shift in Alzheimer's disease

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Introduction: The role of lipidome's fatty acid (LFA) profile, as building blocks of more complex lipids and signaling compounds, in Alzheimer's disease (AD) progression remains unclear.

Subjects and Methods: The LFA composition of plasma and cerebrospinal fluid (CSF) samples of 289 participants (103 AD, 92 mild cognitive impairment (MCI), and 94 control) was determined by gas chromatography-flame ionization detector (GC-FID). The MCI subjects were followed up for a median of 58±12.5 months. Statistical analyses were adjusted for age, sex, MMSE, and APOE ε4 allele. Results: In healthy adults, the LFA of CSF is more peroxidation-resistant and neuroprotector than plasma profile. In CSF, higher anti-inflammatory index was associated with decreased risk of AD diagnosis vs MCI, and higher content of docosahexaenoic acid (DHA, 22:6n3) was associated with reduced risk of MCI to AD progression. In plasma, higher oleic acid (OA, 18:1n9) content was associated with reduced risk of AD and MCI, and MCI to AD progression; whereas higher levels of DHA were linked to a higher rate of progression.

Discussion: Our results suggest that circulating LFA are associated with the pathogenesis and progression of AD.

Stearoyl-CoA desaturase 1 deficiency in perivascular adipocytes induces phenotypic switch in vascular smooth muscle cells

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Introduction: Perivascular adipose tissue (PVAT) surrounds most blood vessels in the body and has been shown to regulate vascular homeostasis through secretion of vasoactive factors. Under pathological conditions, PVATs secretory profile becomes dysregulated and may therefore contribute to vascular complications by targeting vascular smooth muscle cells (VSMC) and switching their phenotype from contractile to synthetic one. Stearoyl-CoA desaturase 1 (SCD1) is an enzyme that converts saturated fatty acids into monounsaturated fatty acids. SCD1 has also been implicated in inflammation and atherosclerosis. Therefore, we hypothesized that SCD1 deficiency in adipocytes may affect VSMC function.

Materials and methods: Stromal vascular cells were isolated from periaortic PVAT of wild-type (WT) and SCD1-/- mice and differentiated into adipocytes. Adipocyte conditioned medium (FCCM) was collected. Rat aortic A7r5 VSMCs were treated with FCCM from WT and SCD1-deficient PVAT adipocytes. VSMC protein markers were measured by Western blot. VSMC migration was measured by transwell assay. Proliferation was measured by flow cytometry using iodium propide. ERK1/2 nuclear translocation and actin filament organization were assessed by confocal microscopy. Cell contraction was analyzed by xCELLigence RTCA CardioECR. Adipokine/cytokine mRNA levels were analyzed by RT-qPCR.

Results and discussion: Expression of the contractile marker caldesmon was decreased in A7r5 cells treated with SCD1-/- FCCM when compared to WT FCCM-treated and control cells. Alignment of actin fibers was impaired in both WT and SCD1-/- FCCM-treated VSMCs. However, in SCD1-/- FCCM-treated cells, the impairment it was more severe than in WT cells. Cellular contractility measurements confirmed that SCD1-/- FCCM exerts inhibitory effects on AngII-induced VSMC contraction. Synthetic phenotype marker levels such as fibronectin, osteopontin and PCNA expression were increased in SCD1-/- FCCM group compared to WT control. SCD1-/- FCCM-treated A7r5 cells also showed the highest level of transwell migration as well as S/G2-M cell cycle phase transition. The phosphorylation level of ERK1/2 as well as its nuclear translocation were increased in SCD1-/- group compared to WT.

Conclusion: We conclude that SCD1 deficiency in perivascular adipocytes leads to increased secretion of factors inducing dedifferentiation of VSMCsFunded by NCN (Poland) grant UMO-2016/22/E/NZ4/00650.

Untargeted Lipid Profiling Discovered Five Selective Markers Associated with Lung Cancer

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The World Health Organization (WHO) 2020 statistics show that lung cancer has the highest mortality rate globally. In the localized stage, the 5-year relative survival rates are 64% and 29% in non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC), respectively. Once distance metastasis happened, the 5-year relative survival rate was only 8% in NSCLC; 3% in SCLC. Pathologic biopsy is the golden standard for the diagnosis of lung cancer. However, the invasive properties and difficulty in early detection are significant limitations. Due to the above reason, finding a new marker for diagnosis, staging, and differentiation from normal tissue and tumor is imperious and essential. Several studies have shown that lipid synthesis, metabolism, and storage are associated with cancer. Thus, we aim to investigate the lipid metabolism of lung cancer tissue and find the biomarkers for tumor identification. In addition, we analyze the potential of markers for different stages of lung cancer. The 35 study participants were recruited at Tamsui Mackay Memorial Hospital from August 2021 to November 2021. Participants collected tumor and distant normal tissue, extracted total lipids, and analyzed untargeted lipid profile using liquid chromatography-mass spectrometry (LC-MS). Screening the data with p value < 10e-5, fold change > 2, and raw abundance > 3000, selecting 60 lipid components as biomarkers by Progenesis QI, and choosing five markers by (Area under curve) AUC finally. The AUC value of the five identified markers were 0.8287, 0.8114, 0.8037, 0.7981, and 0.7978; the mass-to-charge ratio (m/z) were 1367.23, 906.61, 601.87, 367.34, and 1004.62; retention time (min) were 16.64, 7.68, 0.78, 14.88, 0.86. respectively. Moreover, one out of the selected markers, 1367.23 m/z, was significantly decreased in stage 4 compared with stage 1 lung cancer ($p < 0.05$). On the contrary, 367.34 m/z was significantly elevated in stage 4 compared with stage 1 lung cancer ($p < 0.05$).Due to mentioned above, we suggested that five lipid markers can differentiate tumor tissue from normal tissue. Furthermore, 1367.23 m/z and 367.33 m/z can distinguish the early stage from the late stage.

Subcellular distribution of 2-hydroxyoleic and civetic acid after 2-OHOA treatment in glioblastoma cell line and its effect on membrane fusion

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Introduction: 2-OHOA (2-hydroxyoleic acid, LAM561) is a synthetic hydroxylated fatty acid derived from oleic acid acting via melitherapy, an innovative therapeutic approach targeting membrane lipids. Granted orphan designation for the treatment of glioma in both the European Union and the United States, 2-OHOA changes membrane lipid composition consequently modulating different cellular processes and exerting an antiproliferative effect. LAM561 completed a phase I/IIA study on advanced solid tumours in adults (NCT01792310) showing pharmacological efficacy and safety. It is currently undergoing a phase IIB/III clinical trial on glioma patients (NCT04250922) in combination with the standard of care for glioma and a paediatric study on glioma and other solid tumours (NCT04299191).We investigated the effect of 2-OHOA on membrane lipid composition by lipidomic and chromatographic techniques. 2-OHOA was incorporated to the structure of different lipid families and cell membranes and also metabolized via alpha-oxidation to cis-8-heptadecenoic acid (C17:1n-9, or civetic acid). C17:1n-9 has shown antiproliferative effect and different mechanism of action to 2-OHOA (PCT/ES2021/070068).
Materials and methods: Human U118-MG glioblastoma cells were treated with 400 µM of 2-OHOA for 24h. Cell homogenization was followed by isolation of nuclei, mitochondria, cytosol and membrane by differential centrifugation and lipid extraction by Folch method. Lipidomics analyses of membrane compartments were performed by electrospray ionization. Additionally, gas chromatography analyses of the same fractions were carried out. Finally, membrane fusion of model membranes containing 2-OHOA and C17:1n-9 was assessed by spectrophotometry at 600 nm.
Results and discussion: Lipid analysis showed that both 2-OHOA and C17:1n-9 are incorporated into all membrane compartments, preferentially accumulating in nucleus and mitochondria. Both fatty acids preferentially bind to phospholipids, while only C17:1n-9 is incorporated into sphingolipids. 2-OHOA is consistently more abundant than its metabolite. Presence of both fatty acids in model membranes showed inhibition of fusion, suggesting membrane fusion may have a role in their mechanism of action.Conclusion2-OHOA and its metabolite show a differential distribution pattern in glioma subcellular membranes. Additionally, both compounds inhibit membrane fusion, indicating a possible mechanism of action for their antitumor effect.

Surface Plasmon Resonance as a tool to determine binding affinity of lipid drugs

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Introduction: Surface Plasmon Resonance (SPR) is a label-free analytical tool to analyze and quantify biomolecule interactions. But the study of Protein-Lipid interactions using this technology has been mostly limited to interactions of Lipids forming a membrane over the SPR chip or in whole cells, due to the significant challenges that lipids molecules in solutions poses. Nevertheless, when lipids substances are used as a small molecule's drugs, a steady state affinity of monomeric lipid molecules over the mobile phase needs to be achieved to perform accurate quantifications and affinity determinations of discrete interactions between lipids and proteins. Here, we present several attempts to determine the affinity constant (Kd) during the interactions of several hydroxylated and non-hydroxylated lipids drugs with immobilized target proteins related with neurodegenerative (PPARg) or cancer pathways (Furin).
Materials and methods: PPARg was obtained by PCR amplification of the CDS from Human total cDNA samples, a Flag-tag was fused in frame by SOE-PCR and cloned into a pcDNA expression vector. The protein was obtained by transfection of HEK293T cell lines and purified using Antiflag-M2 magnetic beads. Furin protein was purchased from Preprotech Inc. SPR measurements were performed in a Biacore X100 using PBS EP+ Buffer as a mobile phase at 20mL/min and 25ºC as standard temperature unless otherwise noted. Mobile phase was solvent-corrected as needed depending on the nature of the sample.
Results and discussion: Firstly, PPARg interactions with 2-OHDHA, DHA and HPA are analyzed in various conditions over and under the CMC point of each lipid, determining the best experimental setup. Temperature and solvent screening were performed, and affinity constants were determined for each compound. Secondly, we used SPR to determine if the noticed inhibition of Furin by 2-OHOA is mediated by a direct protein-lipid interaction. We found 2-OHOA to interact with Furin with a Kd of (xuM), whereas Oleic acid doesn't show any noticeable interaction with the protein.
Conclusions: SPR can be used to analyzed direct Lipid-protein interactions of lipid drugs candidates if the adequate assay conditions are found. SPR can be a useful tool to confirm the direct nature of these interactions and to obtain quantitative and qualitative affinity data, but optimization work should be performed in a case-to case basis.

α-Hydroxy-Docosahexaenoic Acid (DHA-H) exerts neuroprotection against Alzheimer's disease, putatively through its metabolic derivative Heneicosapentaenoic Acid (HPA)

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Introduction: α-Hydroxy-Docosahexaenoic Acid (DHA-H) is a molecule under development for Alzheimer's disease (AD) therapy, based on the concept of the membrane lipid therapy (melitherapy). Once entered the cell, DHA-H is metabolized via α-oxidation to the fatty acid HPA (Heneicosapentaenoic acid; C21:5 n-3). DHA-H has widely demonstrated neuroprotective effects against this disease in animal and cell models. Chronic oral administration for 4 months of DHA-H to 5xFAD mice, a transgenic mouse model of AD, prevents cognitive decline as well as synaptic and neuron degeneration. This neuroprotection is also mediated by restoration of neuronal proliferation up to healthy levels in the hippocampus. At molecular level, DHA-H reduces both β-amyloid accumulation and tau protein phosphorylation as compared to untreated 5xFAD controls.

Methods: Cognitive evaluation was assessed by Radial Arm Maze. Synaptic degeneration and β-amyloid accumulation and tau phosphorylation were evaluated by western blot. Immunohistochemical experiments were performed with a single immunolabeling for NeuN or with a double immunolabeling for pHH3 (phospho-Histone H3) and GFAP (Glial Fibrillary Acidic Protein). DHA-H and HPA neuroprotection against NMDA-induced excitotocity was tested using neurons differentiated from SH-SY5Y using retinoic acid and hBDNF.

Results and discussion: 20 mg/kg of DHA-H treatment prevented spatial cognitive decline showed in 5xFAD control group. Synaptic degeneration and neuronal death were prevented by DHA-H in the hippocampus of 12-month-old 5xFAD mice. In addition, DHA-H treatment promoted hippocampal cell proliferation restoration and decreased β-amyloid accumulation and tau protein phosphorylation as compared to untreated 5xFAD controls. Moreover, DHA-H and HPA showed a neuroprotective effect against NMDA-induced excitotoxicity. Interestingly, when such an effect is mediated by DHA-H, this can be prevented by oxythiamine, an inhibitor of DHA-H conversion into HPA by α-oxidation.

Conclusion: DHA-H is a plausible therapeutic approach for AD therapy in animal and cell models.

Sphingomyelin is a critical component of the lipid environment affecting the unconventional secretion of FGF2

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Introduction: FGF2 is a tumor cell survival factor that lacks a signal peptide and gets secreted from cells following an unconventional secretory pathway. FGF2 forms ternary complexes with heparan sulfate proteoglycans and FGF high affinity receptors on cell surfaces. In this way, it supports tumor growth and metastasis, triggering tumor-induced angiogenesis and mediating chemoresistance. These effects can be mediated in either an autocrine or a paracrine manner. FGF2 secretion from cells is mediated by direct translocation across the plasma membrane. It involves sequential interactions of FGF2 with the α1 subunit of the Na,K-ATPase, Tec kinase and PI(4,5)P2 at the inner plasma membrane leaflet. The interaction with PI(4,5)P2 favors FGF2 oligomerization, leading to membrane pore formation. Membrane-inserted FGF2 oligomers are then disassembled at the outer leaflet by membrane-proximal heparan sulfate chains on heparan sulfate proteoglycans, which compete against PI(4,5)P2 for binding to FGF2. This competition always results in FGF2 translocation to the extracellular space, as heparan sulfate chains affinity for FGF2 is much higher than the one of PI(4,5)P2.
Results and discussion: In a recent publication, the unconventional secretion of FGF2 has been shown to be highly affected by cellular cholesterol levels. This has been shown combining in vitro reconstitution experiments, in silico approaches, and cell-based experiments focusing on both recruitment and translocation of FGF2 from cells. Starting from these findings, sphingomyelin, another important lipid component of liquid-ordered domains has been put forward for a potential role in the unconventional secretion of FGF2. Cellular sphingomyelin manipulation has been shown to affect both recruitment and translocation of FGF2 from cells, using different cell-based readouts. Interestingly, sphingolipids have been also shown to interact with the α1 subunit of the Na,K-ATPase. As already discussed, α1 is an important component of this secretion machinery that has been also shown to interact with other lipid components known to be enriched in liquid-ordered domains [PI(3,4,5)P3, palmitic acid, and cholesterol].
Conclusion: Combining these various findings, we put forward a possible role for liquid-ordered nanodomains in the unconventional secretion of FGF2. This hypothesis will be further challenged in future experiments combining DRM fractionation, super resolution microscopy, and in silico approaches.

A role for PEDS1 at the crossroad between ether lipid metabolism and immunology

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Introduction: Among the glycerophospholipids are lipids containing an ether bond. A prominent subclass of these is constituted by plasmalogens, of significance e.g., for membrane architecture. Plasmanylethanolamine desaturase 1 (PEDS1) is responsible for the final step in plasmalogen synthesis, by inserting a distinctive vinyl ether double bond. Our laboratory recently characterized the coding gene for PEDS1 and a PEDS1-deficient mouse model is here available. In the mouse, disturbances in the first enzymes of ether lipid metabolism result in symptoms resembling phenotypes frequently reported in human patients suffering from ether lipid deficiencies. This includes neurological and behavioural symptoms and also bone, eye, and sperm development is severely affected. Just recently, investigations in the framework of inflammation and the immune system highlighted significant alterations depending on plasmalogen levels.

Materials and methods: Primary and secondary lymphoid organs from PEDS1-deficient mice and controls were analysed employing the flow cytometry technique. Blood analysis was performed via complete blood count tests and complemented by ELISA examination of blood serum.

Results and discussion: Our examination on the impact of PEDS1 loss on the haematopoietic system revealed abnormalities in diverse blood parameters. Interestingly, we found a significantly increased number of B cells in the bone marrow and a trend to impaired humoral immunity, hinting at a crucial role for plasmalogens in antibody-mediated immune response. Further, putative defects in T cell development and myeloid cell compartment will also be investigated.

Conclusion: Our study will give first insights into the function of the metabolic enzyme PEDS1 in the hematopoietic and lymphoid system.

Chemometric discrimination of small and large intestinal tissue using a chlorofrom-free total lipid extraction method

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Introduction: Lipid profiling from selected tissue samples is a promising strategy for developing targeted therapies and biomarkers in different clinical settings. Accurate lipid profiling relies on the unbiased recovery of lipid species from the sample of interest. Lipid recovery using a given extraction system highly depends on the sample matrix. However, little attention is generally given to lipid extraction, and most studies continue using classical protocols based on carcinogenic agents (i.e., chloroform) or rely on alternative chloroform-free methods not optimized for their biosample of interest.
Materials and methods: We developed a modified chloroform-free total lipid extraction protocol for intestinal tissue using methyl tert-butyl ether (MTBE). We evaluated the performance of our approach for lipid profiling of mice intestinal tissue vs. the Folch method ("gold standard" for tissue lipid extraction) regarding total lipid extraction, lipid profiles generated for main lipid classes (i.e., fatty acids, triacylglycerols, diacylglycerols, monoacylglycerol, free fatty acid, and phospholipids), and reproducibility. We separated and purified the different lipid fractions, except for the fatty acids, by solid phase extraction (SPE) using different cartridges and analyzed them using conventional chromatography techniques.
Results and discussion: Our strategy provided higher total lipid recovery than the Folch method and similar or better recoveries of species of most all major lipid classes. The reproducibility of both protocols was also similar for most lipid species and better for diacylglycerols using our method. In addition, based on the profiles generated with our protocol for the different lipid fractions, we were able to discriminate mice small from large intestinal tissue with high accuracy, thus supporting the utility of our method for lipid profiling of intestinal tissue. Importantly, compared to the original MTBE approach, we have reduced the total protocol time and reinforced the elimination of water content from the final product to avoid interference with downstream applications.
Conclusion: We developed a modified chloroform-free total lipid extraction method that enables chemometric discrimination between mice small and large intestine. These results support the utility of our method for lipid profiling in intestinal disorders.

Enhancing targeted delivery in glioblastoma nultiforme via radiation-induced redox stress

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Introduction: Glioblastoma multiforme (GBM) is the most common primary malignant brain tumor in adults. It is highly resistant to its current standard-of-care regimen, which includes surgical resection followed by adjuvant ionizing radiation and temozolomide. Therapy resistance can be attributed to the abundance of genomic alterations in GBM tumors that evolve over time, which contribute to intra-and inter-tumoral heterogeneities that render targeted therapies as inadequate treatment options. Thus, there is an urgent unmet need for effective therapies targeting primary and recurrent GBM. Here, we investigated the impact of ionizing radiation on the transcriptome of patient derived GBM tumor spheres to identify key alterations and biological pathways involved in therapy resistance.
Materials and methods: We acquired four independent human GBM tumor sphere cell lines, which were derived from patients and cultured in serum-free media. Tumor spheres were treated with either a sham radiation or a single dose of 10Gy. Using poly-A enrichment whole transcriptome sequencing, we evaluated transcriptional alterations occurring in GBM tumors spheres at 96h post-radiation. To do so, we performed differential expression analysis and gene set enrichment analysis(GSEA)to identify top differentially expressed genes and significant changes in biological phenomena. Additionally, we evaluated the cell viability response of these GBM tumor spheres to radiation.
Results and discussion: PCA analysis of the four human GBM tumor sphere cell lines demonstrates that cell type has a substantial impact on variation among samples compared tonon-irradiated and irradiated treatment conditions. Upon analysis of genes in common that are either upregulated or down regulated followed by GSEA, we identified enrichment of genes associated with inflammatory responses and ferroptosis repression(e.g., NUPR1, PTGS1, AOX1), and depletion of genes involved in fatty acid metabolism (e.g., INSIG1, PLA2G3, ACAT2). Furthermore, the GBM tumor sphere cell viability responses allowed us to stratify our models into radiosensitive and radioresistant subgroups.
Conclusion: Radiosensitive human GBM tumor spheres exhibit transcriptional alterations in genes linked to inflammation and fatty acid metabolism to a greater extent compared to radio resistant umor spheres. Our study characterizes the radiation response of patient derived GBM models, which is to be leveraged for combating therapeutic resistance.

LAM362C and LAM363C induce ferroptosis via a peroxisome-dependent pathway

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LAM262C and LAM363C are lipid based molecules with potent antitumor activity that have as a target the cell membranes composition. LAM362C and LAM363C promote ferroptosis in pancreatic adenocarcinoma (PDAC) cell lines evidenced by the inhibition of the RAS-Nrf2-GPX4 pathway and the induction of lipid peroxidation. Activation of ferroptosis induced by LAM362C and LAM363C doesn't require upregulation of acyl-CoA synthetase long -chain family member 4 (ACSL4) but involves the formation of lipid droplets and activation of autophagy. Targeting the membrane-lipid droplet-autophagy axis may represent a strategy to treat PDAC.

Phospholipid metabolism is altered in abdominal aortic aneurysm

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Introduction: Over the last 15 years, our research focused on characterisation of enzymatically-oxidized phospholipids (eoxPL) generated by platelets and leukocytes. We showed these are pro-coagulant, through supporting PS to bind and activate circulating factors at membrane surfaces. Our current studies in humans and murine models aim to determine the generation and action of these lipids in abdominal aortic aneurysm (AAA), a disorder where the aorta develops an inflammatory thrombotic lesion which is at risk of sudden devastating rupture.

Materials and Methods: Human cohort tissue from the OxAAA cohort was analysed using lipidomics mass spectrometry (MS), while coagulation parameters were determined using ELISA. AAA development was measured (AngII/ApoE-/-, and elastase/anti-TGFb) in mice genetically lacking Alox12 or Alox15.

Results and Discussion: Mice lacking either 12/15-LOX (leukocytes) or 12-LOX (platelets) were found to be protected against AAA in the angII/ApoE-/- model. Numerous eoxPL were detected in AAA lesions from wild type mice and the disease was associated with coagulopathy. Lesion development could be prevented using a FX inhibitor, showing that coagulation is causally driving disease. eoxPL were also detected in human AAA lesions. Currently, the role of eoxPL in elastase-induced AAA, which involves local damage to the aorta, instead of the systemic inflammatory challenge of angII/ApoE-/- is being characterised. Preliminary data shows that this model maybe less dependent on both coagulation and eoxPL. In a human AAA cohort, eoxPL and native phospholipids (PL) are being profiled in plasma and AAA tissue, to examine which lipid species may be associated with altered coagulation. When stratifying AAA subjects genetically via a known AAA risk SNP in Chr9p21, many correlations in plasma PL metabolism are seen for the risk SNP that are completely reversed in the non-risk SNP.

Conclusions: Further studies aim to understand the underlying biochemical changes responsible. In this presentation, recent data on both the mouse model and human cohort will be presented. Funded by British Heart Foundation

Analysis of histamine receptor activation over lipid fingerprint using cell membrane microarrays and MALDI-MS

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Introduction: The aim of this study is to find a way to detect specific drug binding in a simple and fast way. The detection of drugs incubated directly on membranes is not able to reach the required sensitivity to ensure that there is no nonspecific binding. In this work, we explore the possibility that specific binding to certain GPCR coupled to phospholipases induces changes in the lipid fingerprint of cell membranes and, that these changes are significant enough to differentiate whether the drug has bound.

Materials and Methods: Microarrays of cell membrane homogenates isolated from brain cortex, spinal cord and two cell lines, were incubated with a set of histamine receptor agonists and antagonists (histamine, cetirizine, atropine) using a GPCR receptor activation protocol variant with three different buffers. The incubated microarrays of cell membrane samples were analyzed in negative (MS-) ionization mode after being coated with 1,5-Diaminonaphthalene using a sublimator. Samples were measured using a Thermo LTQ XL Orbitrap spectrometer.

Results and discussion: Certain drugs show specific differences with respect to basal samples within each protocol-tissue set. Mainly differences are seen between samples with histamine and histamine+cetericin and the rest, appearing to be more significant when using the incubation protocol with PI buffer.

Palmitate-derived changes in pancreatic β-cells cardiolipin composition and remodeling destabilize mitochondria in a stearoyl-CoA desaturase 1-dependent manner

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Introduction: Type 2 diabetes is a metabolic disorder in which insulin signaling is impaired from reaching its effectors. Ultimately, the accelerating accumulation of surplus lipids in the pancreas triggers mitochondrial dysfunction and imbalanced dynamics as have been observed in diabetic patients and animal models. In fact, insulin secretion in pancreatic β-cells is modulated by the bioenergetic state of mitochondria which undergo dynamic and often reversible physiological recalibrations. Mitochondria contain signature phospholipids - cardiolipins (CL) which composition is modulated by fatty acyl availability. Stearoyl-CoA desaturase 1 (SCD1) is critical for producing unsaturated fatty acyl moieties and affects the overall rate of β-cell survival. Here, we investigated the molecular effect of SCD1 deficiency on the regulation of mitochondrial biogenesis, architecture and function in pancreatic β-cells undergoing lipid stress.
Materials and methods: The mitochondrial structure and remodeling were explored in pharmacologically and genetically SCD1-deprived INS-1E cells, in pancreatic islets in SCD1-/- mice and wildtype littermates in the lipotoxic milieu. The morphology of mitochondria was evaluated by staining with MitoTracker and transmission electron microscopy imaging. The GC-MS analysis were applied to assess the fatty acid composition of the intracellular CL fraction.
Results and discussion: Deprivation of SCD1 led to an impairment in bioenergetic function, indicated by more depolarized mitochondria and a decrease in ATP production compared with INS-1E cells that were independently treated with palmitate. This effect occurred in parallel with higher amounts of smaller mitochondria and we observed changes in I, III, IV and V OXPHOS complexes which directly bind with CL. The CL fraction was enriched in 16:1, 18:0, 20:3n-6 and 24:1 fatty acids in β-cells and pancreatic islets. The aforementioned rearrangements coincided with abnormal CL synthesis and significantly affected the abundance of the protein effectors linked to CL remodeling/fatty acyl moieties distribution (TAZ, LCLAT1, PLSCR3, ACSL5) and cristae shaping (Prohibitin1, MIGA2, Mic10).
Conclusion: The mechanistic link between the Δ9 desaturation and the CL acyl side chain composition requires an additional remodeling process which is then crucial for establishing mitochondrial lipid homeostasis, holding promise for novel therapeutic modalities to fix lipotoxicity-derived mitochondrial impairment.

Lipidomic analysis of M. globosa at different growth stages and the dynamics of uptake and secreted lipids with growth media

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Introduction: Malassezia is one of the most abundant genera found on human skin; specifically, M. globosa is one of the species dominant in human skin as it has been associated with several skin diseases. Malassezia cannot synthesize fatty acids; in response, the yeast cell intakes external fatty acids from the host or the growth media for survival. Several studies have focused on investigating the identity of lipids and enzymes in M. globosa to understand its lipid metabolism and the biology of the interaction cell- host.

Material and Methods: In this work, we performed a supernatant lipidomic analysis on the mDixon media and the supernatant and on the M. globosa at early and late stationary phase (72h and 90h, respectively) to determine the lipid dynamics (lipids consumed vs. lipids secreted) between the growth media and the two stages of growth. Results: We were able to identify 85 lipids within 17 classes of lipids; during the analysis, the concentration of several lipids increased throughout time with respect to the growth media suggesting a secretion pattern from the cell to the media; some lipids found in this group were conjugated Sterols (ST) such as Glycochenodeoxycholic acid (GCDCA), Glycerophospholipids (GP), specifically phosphocholine's (PCs), Cardiolipins (CL), in particular those with chains of (47 to 54 carbons) and Sphingolipids (SP) such as Cer-PI which might have some role in pathogenicity. Likewise, the concentration of some lipids decreased, but some only decreased at the late stationary phase (90h) only when the concentration of nutrients available was minimal. Finally, we observed a third pattern in which the concentration of some lipids decreased throughout time (starting in the early stationary phase and finishing in the late stationary phase), hinting at a distinctive consumption pattern. The principal lipids consumed were: Sterols (ST) bile acids, cholic acid and its derivates, some phosphocholines (PCs), Fatty acyls (FA), and cardiolipins (CL).

Conclusion: The consumption of these lipids was associated with different metabolic roles of the lipids in the cell as it lacks production of these lipids in Malassezia globosa.

Analysis of lipid profiles and identification of potential biomarkers in a mouse model of breast cancer using MALDI-TOF spectrometry

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Introduction: Breast cancer is a common neoplasm affecting one in twelve women. Lipid metabolism plays a major role in cellular phenotypic changes. Our study focused on obtaining lipid profiles from healthy and tumour cell lines and compare them in order to find a differential lipid fingerprint, with a special interest in the sphingomyelin (SM) class.

Material and Methods: We used a triple-negative breast cancer cell line, MDA-MB-231, and 184B-5 as a nontumorous control. In addition, lipid analysis of athymic nude-Foxn1nu mouse sera were done prior to (S0), and after (S1) implantation of MDA-MB-231 xenografts. Lipidomic analyses were done using cellular and serum samples, extracted with isopropanol, analyzed by MALDI-TOF under negative and positive polarities.

Results and Discussion: A total of 197 differential lipid peaks were detected in MDA-MB-231 and 184B-5 cells. In mice, 73 differential peaks were detected in sera. In all cases, the differences between groups were significant at p≤0.05. In comparison with the control cell line, 55.8% of the identified lipid species in MDA-MB-231 are either absent or show reduced levels, while 44.2% are only present in the tumour cell line or display greater signal intensities. In mouse serum samples, 67.1% of differential lipids show decreased or no signal in S1 sera and 32.9% are only present in S1, or show increased relative signals, compared with S0 sera. Principal Component Analysis enabled the differentiation of lipid profiles using the two main components in both cells and sera. As for SM, an increase in signal intensity was observed in SM4(13.45±4.10%), SM8(85.0±10.1%), SM12(90.8±9.9 %), in a significant reduction in SM5(-34.7±4.0%),SM6(-46.3±3.0%), SM10(-44.9±11.9%) and SM11(-36.7±4.1%) in MDA-MB-231 cells compared with 184B-5 cells. Conversely, in S1 sera, no signals were observed for SM14-17-21, and a reduction in SM4(-71.2±3.7%), SM8(-67.5±4.1%), SM17(-71.2±4.1%). Lastly, the signal of SM15 and SM21 were a new appearance in these sera, while increased relative intensities in the signals of SM5(191.1±49.7%) and SM11(154.9±55.7%) were also observed, compared with S0.

Conclusion: MALDI-TOF proved an effective method to obtain lipid profiles from our samples. The spectra made possible the discrimination between cancer samples and controls. Opposite SM signal patterns were found in MDA-MB-231 and mouse serum samples. Causes for this will be discussed in relation to lipid metabolism and organism physiology.

A preliminarily analysis of Malassezia furfur lipidome during host-pathogen interaction in the Galleria mellonella systemic infection model.

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Introduction: Malassezia furfur is a lipid-dependent yeast that has been found to be the main yeast, belonging to the genus Malassezia, that may cause fungemia in neonates with low birth. About this genus, a high lipid versatility has been reported in the stationary growth phase with a high amount of neutral lipids. However, more knowledge about lipid composition and metabolism is required to understand better these microorganisms, their interaction with their host, and to propose therapeutic targets. The lipid metabolism has been reported to be important for the host-pathogen interaction of viruses, bacteria, fungi, and parasites. For this reason, in this study, the infection model Galleria mellonella has been used to understand better the host-M. furfur interaction through a lipidomic analysis. Here, the methodology to obtain the cells of interest for the study, reproducibility analysis, and preliminary analysis to identify differences between interacting and non-interacting M. furfur yeast with the host will be presented.
Materials and methods: G. mellonella larvae were inoculated with 20□L of a 1.5*109 CFU/mL. After 24 hours, larvae hemolymph was extracted and processed to isolate M. furfur yeasts from the hemolymph. Samples of free hemocytes hemolymph, whole cell package, which include hemocytes from G. mellonella and M. furfur yeasts, isolated M. furfur, m-Dixon agar cultured M. furfur, and m-Dixon agar were analyzed using HPLC-QTOF-MS.
Results and discussion: M. furfur yeast were partially isolated from hemolymph and the full scan lipid analysis permitted to discriminate between phases of yeast isolation, with a high amount of triacylglycerides, as previously reported. Also, reproducibility was observed between the three evaluated replicates. These results showed that the isolation methodology was a suitable method to continue with the final host-M. furfur interaction study. In that way, the five replicates for each sample were collected and analyzed. As a result, differences in the lipid profiles of M. furfur and G. mellonella, associated with the host-pathogen interaction, were found. The next step is to identify these lipids and their role during the pathogenesis of M. furfur.
Conclusion: The proposed methodology used to evaluate Host-Malassezia interaction using the infection model G. mellonella may allow us to understand the role of the lipid metabolism in the pathogenesis de M. furfur. Likewise, this may allow to propose therapeutic target.

Study on the senescence-associated secretory phenotypes induced by cholesterol imbalance in human primary hepatocytes

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Introduction: Cellular senescence is a state of cell cycle arrest, with two major types, replicative and stress-induced senescence. The senescence-associated secretory phenotype (SASP) is characterized in senescent cells, secreting biological molecules into the extracellular space, including cytokines, chemokines, growth factors, and extracellular vesicles (EV). This study explored SASP components induced by cholesterol imbalance in primary human hepatocytes (PHH) and the associated mechanisms to regulate inflammation, which might lead to the progression of hepatic steatosis to steatohepatitis.

Materials and methods: Cellular senescence was evident by SABG staining and western blot for senescence hallmarks. SASP gene expression was evaluated by qPCR, western blot as well as ELISA. EVs were isolated by ultracentrifugation and confirmed by Transmission Electron Microscopy (TEM) and western blot. Macrophage polarization was evaluated by analyzing M1 and M2 markers mRNA expression.

Results and discussion: Our results showed PHH treated with TMP-153, a sterol O-acyltransferase inhibitor, could induce cellular senescence by inhibiting the conversion of cholesterol to cholesteryl ester and caused cholesterol imbalance. The SASP mRNA levels of IL-1B, IL-6, IL-8, MCP-1 and MMP1 were increased in PHH after treated with TMP-153 that the IL-8 mRNA was with the highest fold-of-change among them. Importantly, the secreted protein and mRNA levels of IL-6, IL-8, MCP-1 were increased parallelly in the conditioned medium as well as in EVs fraction of TMP-153-treated PHH. In addition, the upstream signaling NF-κB pathway (including p-NF-κB, p-IKKβ, p-IκBα) and JAK/STAT pathway (including p-JAK1 and p-STAT3) were elevated in PHH after treated with TMP-153. Subsequently, the EV and the conditioned medium of TMP-153 group significantly stimulated M2 polarization of macrophages.

Conclusion: In conclusion, the senescence of PHH induced by cholesterol imbalance promoted SASP secretion (both free forms and EV-associated ones) that stimulated M2 macrophage polarization which could lead to macrophage-associated inflammation and might play an important role in MAFLD progression.

Reciprocal regulation between very long-chain sphingolipids and phosphatidylserine maintains the plasma membrane lipidome

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Introduction: The hydrophobic acyl-chains affect how lipids behave in membranes and interact with other lipids. Sphingolipids are abundant lipids in the outer leaflet of the plasma membrane, and in contrast to glycerophospholipids, they are rich in very long-chain acyl-chains. It is not fully understood how very long-chain sphingolipids differ in functions from long-chain sphingolipids. Phosphatidylserine (PS) is mainly localized in the inner leaflet of the plasma membrane, and has the characteristic of being enriched in stearic acid at the sn-1 position, with oleic acid often seen at the sn-2 position. Consequently PS 18:0/18:1 is a major inner leaflet lipid in many cells, but it remains to be established how these characteristic acyl-chains are acquired, and for which purpose. In this study, we found that very long-chain sphingolipids and 18:0/18:1 PS are regulating each other, which is likely to affect the plasma membrane lipidome.
Materials and Methods: Using CRISPR-Cas9, we generated mutant HeLa cells lacking CERS2, the enzyme required for very long-chain sphingolipid synthesis, together with cells lacking phosphatidylserine synthesis. Untargeted lipidomics was done in these cells to characterize lipid co-regulation, and lipid localization was monitored using lipid-binding proteins fused to fluorescent proteins.
Results and Discussion: Lipidomics revealed that CERS2 mutants have drastic reductions in very long-chain sphingolipids. Among abundant PS species, 18:0/18:1 was specifically reduced in these mutants. We successfully generated cells lacking PS synthesis by mutating PSS1 and PSS2, the two enzymes required for PS synthesis in human cells. These cells could be maintained in the presence of PS liposomes, while the omission of liposomes reduced PS levels and killed the cells. Lipidomics analysis revealed that the blockade of PS synthesis specifically reduces saturated very long-chain sphingolipid levels, thus suggesting a reciprocal regulation between very long-chain sphingolipids and 18:0/18:1 PS. Finally, we found that PS localization at the plasma membrane is impaired in CERS2 mutants, suggesting that this reciprocal regulation maintains the lipidome of the plasma membrane.
Conclusion: Lipid acyl-chains are not only important for the functions of the lipid itself, but also affect how other lipids behave. Lipid co-regulation might be seen in other lipid species too, which is worthy to investigate in the future.

A comprehensive panel of cells lacking lysophospholipid acyltransferases reveal multiple regulators of glycerophospholipid acyl-chains

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Introduction: Differences in acyl-chains found in glycerophospholipids (GPLs) are critical to obtain the required physicochemical properties of membranes in various cell types. On the other hand, phosphatidylinositol (PI) acyl-chains are relatively similar between cell types, consisting mainly of an 18:0/20:4 combination. An understanding about how differences or similarities in GPL acyl-chains are acquired would allow us to manipulate lipid composition in cells to unveil how acyl-chains affect biological functions. In this study, we investigated how GPL acyl-chains are regulated by lysophospholipid acyltransferases (LPLATs), which are enzymes that incorporate acyl-chains in GPLs. We also compared the contribution of LPLATs and other metabolic pathways to regulate the typical 18:0/20:4 acyl-chains of PI.
Materials and Methods: Using CRISPR-Cas9, we generated a library of mutant HeLa cells lacking individual LPLATs or glycerol 3-phosphate acyltransferases (GPATs), which are the acyltransferases that incorporate sn-1 acyl-chains during de novo GPL biosynthesis. The mutant cells were analyzed by untargeted lipidomics, using LC-MS/MS on a qExactive mass spectrometer.
Results and Discussion: We successfully generated a library of polyclonal mutant HeLa cells lacking LPLATs or GPATs, using a recently developed CRISPR-Cas9 approach. Using this library, lipidomics analysis confirmed previous results, such as the importance of LPLATs in the regulation of arachidonic acid levels. Novel regulators were also found, for example in the regulation of phosphatidylglycerol acyl-chains. Finally, we focused on the acyl-chains of PI, and compared the contribution of LPLATs and another metabolic pathway, named the PI cycle, for its enrichment in arachidonic acid. While we confirmed the importance of the LPLAT MBOAT7 in arachidonic acid incorporation into PI, we found no evidence of contribution the PI cycle, at least in our system.
Conclusion: Our results highlighted the importance of LPLATs in the regulation of GPL acyl-chains, opening the possibility of targeting them to manipulate lipid composition of cells and study how lipids regulate biological functions.

Intestinal PKD2 couple chylomicron and LD metabolism to optimize postprandial lipid absorption

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Introduction: Absorption of all macronutrients, including dietary lipids, is largely limited to the small intestine. Epithelial cells lining the organ and mediating calorie uptake (enterocytes) are regularly exposed to high fluctuations in nutrients abundance with each meal ingested. Adaptation to rapidly changing nutritional cues requires an equally fast and precise cellular response to maintain epithelial homeostasis. Primarily, lipids are secreted from the intestinal epithelium as lipoproteins (chylomicrons) into the lymph while a pool of taken taken-up lipids is transiently stored in newly formed lipid droplets (LDs) in enterocytes. However, the factors that determine the distribution of lipids between secretion and storage are largely unknown.
Results and Discussion: Previously we showed that protein kinase D2 (PKD2) promotes intestinal fat absorption via increasing chylomicron size. Recently, we found that PKD2 is implicated also in LD turnover. In the absence of the kinase, LDs are enlarged and the trafficking of LD-degrading enzymes(ATGL, HSL) to LDs is impaired. Moreover, we identified that lipid challenge triggers proteolysis of lipases both in vivo and in mouse intestinal organoids but it is prevented in enterocytes depleted from PKD2. Similarly, pharmacological inhibition of chylomicronsynthesis prevents the removal of a targeted protein.
Conclusion: Our data indicate that PKD2 integrates lipid secretion and storage pathways in the postprandial periodand such a co-regulation is required to optimize lipid uptake. In addition, a newly identifiedevent of lipid-induced proteolysis of LD-associated lipases suggests a potential fine-tuning mechanism of free fatty acids released from intracellular pools in response to dietarylipids load with a limiting role of PKD2.

Development of HILIC-MS/MS method for acyl-CoAs covering short- to long-chain species in a single analytical run

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Introduction: Acyl-CoAs are a group of thioesters compound and have a pivotal role in various metabolic processes such as fatty acid beta-oxidation, biosynthesis of lipids, signalling, xenobiotics metabolism etc. The most important biological function of acyl-CoA is in the metabolism of fatty acids via beta-oxidation. Since acyl-CoA are involved in numerous physiological and pathophysiological pathways, it is important to develop analytical methods for their identification and quantification. The physicochemical properties of acyl-CoA vary greatly depending on carbon chain length, degree of saturation, and functional groups. Various efforts have been made to cover the full range of acyl-CoA in a single analytical run, although with limitations for application in routine clinical analysis. The goal of the current research is to develop an analytical method that can cover short to long chain acyl-CoA in one single analytical run
Materials and Methods: The hydrophilic interaction chromatography (HILIC) coupled with mass spectrometry as detector was used for method development. The neutral loss of 507 is the diagnostic fragment that has been used for the identification of acyl-CoA. Various other chromatographic parameters such effect of buffer, injection solvents, pH etc. were determined. We have applied this method in wildtype HepG2 cells cultured in supplemented and starved state.
Results and discussion:With of help of our HILIC-MS/MS method, we were able to cover entire range of acyl-CoA compounds in one analytical run. We observed an increase in the profile of acetyl-CoA, medium- and long-chain acyl-CoA while decrease in the level of free CoA in HepG2 cells cultured in starved state.
Conclusion: We have developed a HILIC-MS/MS method covering short- to long-chain acyl-CoA species in one analytical run. This HILIC-MS/MS method was assessed on various validation parameters. The increase in the profile of acetyl-CoA, medium- and long-chain acyl-CoA while decrease in the level of free CoA in HepG2 cells cultured in starved state suggests activation in the fatty acid oxidation process in starved state**.**

Visceral adipose tissue in colon cancer microenvironment: a multi-omic approach

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Introduction: Colon cancer (CC) is the 3rd most common cancer. Worryingly, the incidence in people <50 years has increased for reasons still not well established. One of the main risk factors of CC is obesity, defined as an excess of adipose tissue (AT). Compared to subcutaneous AT, visceral AT (VAT) is considered the culprit of causing health disturbances, including cancer. Adipocytes can be part of the tumor microenvironment (TME), especially in tumors tightly associated with adipose tissue. In colon, the interaction of tumor cells with adipocytes can be either indirect via production of hormones and inflammatory mediators that can reach the TME (as in T2 stage) or caused by cell-to-cell contact once the tumor mass extends to the VAT (as in T3 and T4 stages). Thus, we aimed to characterize, using a multi-omic approach, the CC-associated human AT at several stages.

Material and methods: Anthropometric data, CT scans, blood samples, peritumoral (pVAT) and mesenteric VAT (mVAT), and tumor biopsies from CC patients undergoing elective surgery were collected. Pathologists established three study groups according to the TNM classification. We analyzed the: 1) cytokine and adipokine levels in plasma and AT (Luminex); 2) fatty acid methyl esters (FAMEs) profile in plasma samples by GC-MS; 3) lipid profile of adipocytes isolated from pVAT and mVAT by UHPLC/MS; 4) gene expression profile of VAT and tumor tissue (Affymetrix).

Results and discussion: The results showed that patients <50 years and higher body mass index (>25 kg/m2) presented a more aggressive tumor (stages T3-T4). In plasma, FABP4 levels decreased when tumor aggressiveness increased, becoming a biomarker worth following. VAT transcriptomic data revealed significant differences between mVAT and pVAT, even at early CC stages. Furthermore, genes involved in adipocyte differentiation and lipid metabolism were decreased in pVAT, while genes related to a fibrotic phenotype were increased. Interestingly, secreted phospholipase A2 (PLA2G2A) was the lipid enzyme showing the highest impact on the expression: 40-fold increase in pT2 and 18-fold in pT3 and pT4, compared to the mesenteric counterpart. We are currently studying the mechanisms.

Conclusion: These results reinforce the concept of crosstalk between adipocytes and cancer cells, highlighting the importance of studying the TME.

A female-specific fatty acid elongase in *Drosophila erecta*

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Introduction: Sexually dimorphic cuticular hydrocarbons (CHs) as sexual pheromones are involved in courtship behavior in many Drosophila species, including the model organism D. melanogaster and its closely related species, D. sechellia and D. erecta. Females of these three species carry more very-long-chain CHs than males. Previous studies have shown that a female-specifically expressed fatty acid elongase gene, eloF, is responsible for the sexual dimorphism of CH length in D. melanogaster and D. sechellia. However, in D. erecta, the specific elongase gene underlying the sexual dimorphism of CH lengths is still unknown because there is no eloF ortholog in D. erecta.
Materials and Methods: To identify which elongase gene is responsible for the sexually dimorphic CH length in D. erecta, we first surveyed the gene expression of 19 elongases in D. erecta adults and found that a recent tandem duplicate gene of eloF was the only female-biased gene. By knocking out the candidate gene, we showed that the mutant flies show a dramatic decrease of very-long-chain (29-33 carbons) CHs accompanied by an increase of shorter-chain (≤27 carbons) in females but not in males.
Results and Discussion: Mating choice experiments showed that when given a choice, males preferred wild-type females over knockout females. This difference in mating success suggested that the lack of those female-specific very-long-chain CHs (29-33 carbons) could reduce the females' attractiveness. Perfuming experiments showed that the knockout females perfumed with wild-type females' CHs could increase their mating success.
Conclusion: These findings indicated that this female-biased gene plays an important role in Drosophila reproduction.