



ESTABLISHED 2001

XVI SPHINGOLIPID CLUB MEETING

September 22–26, 2026
Supetar, island of Brač, Croatia



BOOK OF ABSTRACTS

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MEETING ORGANIZERS

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SPHINGOLIPID CLUB

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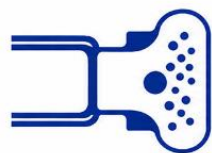
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PROGRAM

Tuesday, Sept. 22, 2026

From 14:00 *Welcoming and registration*

17:00 – 18:40 *Poster display*

19:00 *Welcome reception*

Wednesday, Sept. 23, 2026

9:00 – 9:05 *Welcome*

Riccardo Ghidoni (Milan, ITA)

9:05 – 9:15 *Opening remarks*

Dragana Fabris and **Ivana Karmelic** (Zagreb, CRO)

9:15 – 9:45 *Keynote Lecture*

★ **Yusuf Hannun** (Stony Brook, USA)

Bioactive Sphingolipids: Principles, Approaches, Case Studies, and Advice

SESSION 1 The Complex Network of Sphingolipids

Chairs: Liana Silva (Lisbon, POR) and *Massimo Aureli* (Milan, ITA)

9:45 – 10:05 ★ **José Carlos Fernández-Checa** (Barcelona, ESP)

Mitochondria and sphingolipids in metabolic liver diseases

10:05 – 10:25 ★ **Giovanni D'Angelo** (Lausanne, SUI)

Sphingolipids as molecular codes for supracellular architectures

10:25 – 10:45 ★ **Annarita Di Lorenzo** (New York, USA)

How sphingolipids shape the heart's fate

10:45 – 11:15 *Coffee break*

11:15 – 11:30 **Lauren Ashley Cowart** (Richmond, USA)

Atypical sphingolipids at the crossroads of health and disease

11:30 – 11:45 **Thorsten Hornemann** (Zurich, SUI)

CYP4F2-mediated ω -hydroxylation of 1-deoxysphingolipids reveals a new hepatic detoxification pathway

11:45 – 11:55 **Lucia Centofanti** (Milan, ITA)

Distinct canonical and atypical sphingolipid signatures differentiate type 1 and type 2 diabetes

11:55 – 12:15 ★ **Christian Metallo** (La Jolla, USA)

Quantifying sphingolipid fluxes that influence skin health and disease

12:15 – 12:25 **Camillo Morano** (Milan, ITA)

Sphingolipidomics of women with anorexia nervosa and obesity: signatures at opposite ends of energy balance

12:25 – 12:40 ★ **Marija Heffer** (Osijek, CRO)

Sex bias in cell membrane lipidomes

12:40 – 14:20 *Lunch break*

★ *Invited Speaker*

SESSION 2 Ceramide: At the Center of the Stage

Chairs: **Caterina Bernacchioni** (Florence, ITA) and **Antonio Gomez-Muñoz** (Bilbao, ESP)

- 14:20 – 14:40 ★ **Reijo Laaksonen** (Espoo, FIN)
Ceramides as cardiovascular biomarkers: from lipidomic discovery to personalized prevention and treatment
- 14:40 – 14:55 **Alexey Glukhov** (Madison, USA)
Neutral Sphingomyelinase Couples Mechanical Stress to Cardiac Excitability and Remodeling
- 14:55 – 15:10 **Eric Hajduch** (Paris, FRA)
Intracellular remodeling of LDL-derived ceramides as a key regulator of muscle insulin resistance
- 15:10 – 15:20 **Nassri Abdoush** (Erlangen, GER)
Differential effects of ceramide chain length on alcohol consumption and behavioral phenotypes in mice
- 15:20 – 15:35 **Liana Silva** (Lisbon, POR)
Ceramide Couples TNF- α Signaling to Organelle Biophysical Remodeling
- 15:35 – 15:55 ★ **Scott Summers** (Salt Lake City, USA)
The bond that breaks the heart—and the liver, and the kidney, and the...
- 15:55 – 16:20 *Picture time*
- 16:20 – 16:50 *Coffee break*
- 16:50 – 17:10 ★ **Pilar Martínez-Martínez and Beatriz Gonçalves Arede** (Maastricht, NED)
Identifying and validating ceramide transfer protein binding partners
- 17:10 – 17:20 **Arianna Dall'Ara** (Zurich, SUI)
Disease-associated SPT variants drive distinct sphingolipid remodeling states
- 17:20 – 17:30 **Federico Fiorani** (Perugia, ITA)
The potential role of nuclear sphingomyelin in non-canonical exosome biogenesis
- 17:30 – 17:40 **Xian-Cheng Jiang** (Brooklyn, USA)
Total sphingomyelin synthesis deficiency promotes lipodystrophy and prevents adipogenesis
- 17:40 – 18:00 ★ **Besim Ogretmen** (Charleston, USA)
Targeting sphingolipid metabolism regulates tumor growth, metastasis, and anti-cancer therapy
- 18:00 – 18:15 **Miroslav Machala** (Brno, CZE)
Multiple mechanisms converge on deregulation of sphingolipid and glycosphingolipid metabolism in colorectal carcinoma
- 18:15 – 18:25 **Charlotte Dandine** (Toulouse, FRA)
Targeting ELOVL1 impacts sphingolipid metabolism, reduces the aggressiveness of Triple-Negative Breast Cancer and enhances the efficacy of immunotherapy
- 18:25 *Free evening time*

Thursday, Sept. 24, 2026

SESSION 3 Sphingosine-1-Phosphate: The Most Bioactive Sphingolipid

Chairs: Marco Presta (Brescia, ITA) and Miroslav Machala (Brno, CZE)

- 9:00 – 9:20 ★ **Chiara Donati** (Florence, ITA)
Sphingosine-1-phosphate orchestrates inflammation and fibrosis in benign uterine disorders
- 9:20 – 9:35 **Nadja Engel** (Rostock, GER)
Resolving the expression–function paradox of sphingosine-1-phosphate lyase reveals a key regulator of metastasis in head and neck squamous cell carcinoma
- 9:35 – 9:45 **Aimie Fauré** (Toulouse, FRA)
S1P-induced $\beta 8$ integrin: a driver of primary melanoma progression?
- 9:45 – 10:00 **Zoltán Benyó** (Budapest, HUN)
Sphingosine-1-phosphate-induced relaxation of human coronary arteries and its impairment in ischemic heart disease
- 10:00 – 10:20 ★ **Markus H. Gräler** (Jena, GER)
The adaptive role of sphingosine 1-phosphate in red blood cells to hypoxic conditions in respiratory diseases
- 10:20 – 10:50 *Coffee break*
- 10:50 – 11:05 **Timothy Hla** (Boston, USA)
Sphingosine 1-phosphate bound to HDL promotes of cerebrovascular health and cognitive functions
- 11:05 – 11:20 **Webster Santos** (Blacksburg, USA)
Molecular pharmacology of blocking sphingosine-1-phosphate transport
- 11:20 – 11:35 **Dagmar Meyer zu Heringdorf** (Frankfurt, GER)
Photoactivatable sphingosine reveals distinct target profiles in SphK1/2-overexpressing cells and uncovers HN1 and HN1L as regulators of sphingosine-induced Ca^{2+} signaling
- 11:35 – 11:45 **Matteo Prisinzano** (Florence, ITA)
Beyond the brain: sphingosine 1-phosphate dysregulation and skeletal muscle impairment in Huntington's disease
- 11:45 – 12:05 ★ **Michele Vittorio Dei Cas** (Milan, ITA)
Modelling genetic defects in sphingolipid biosynthesis using CRISPR-Cas9-edited HEK-293T cells
- 12:10 – 13:40 *Lunch break*

SESSION 4 When Sphingolipids Become Complex: Glycosphingolipids and Gangliosides

Chairs: Thorsten Hornemann (Zurich, SUI) and Marcelo Einicker-Lamas (Rio de Janeiro, BRA)

- 13:40 – 14:00 ★ **Ronald L. Schnaar** (Baltimore, USA)
Probing the ganglioside interactome using synthetic bifunctional gangliosides
- 14:00 – 14:15 **Kristina Mlinac Jerković** (Zagreb, CRO)
Glycosphingolipid engineering in pursuit of ganglioside-protein interactions

- 14:15 – 14:30 **Svjetlana Kalanj Bognar** (Zagreb, CRO)
Multimodal MRI reveals structural and microstructural brain alterations in GD3 synthase-deficient mice
- 14:30 – 14:50 ★ **Simonetta Sipione** (Edmonton, CAN)
Role of gangliosides in extracellular vesicle secretion and fate: Implications for misfolded protein diseases
- 14:50 – 15:05 **Tsaffir Zor** (Tel-Aviv, ISR)
The endogenous sphingolipid sulfatide affects inflammation via multiple receptors – TLR4/MD-2, TLR2 and the Inflammasome
- 15:05 – 15:15 **Luca Mignani** (Brescia, ITA)
The sphingolipid-metabolizing enzyme β -galactosylceramidase promotes melanoma growth by activation of a tumor cell pro-inflammatory signalling
- 15:15 – 15:25 **Henrik Nurmi** (Turku, FIN)
The glycolipid transfer protein GLTP as a modulator of COPII vesicle export from the ER and of cellular GSL homeostasis
- 15:25 – 15:55 *Coffee break*
- 15:55 – 16:05 **Laurane Payet** (Toulouse, FRA)
Selective impact of glycosphingolipids on melanoma progression and response to immune checkpoint inhibitors
- 16:05 – 16:15 **Sara Penati** (Milan, ITA)
From enzyme dysfunction to altered sialylation: defining the biochemical signature of ST3GAL3-CDG
- 16:15 – 16:25 **Maria Fazzari** (Milan, ITA)
The crucial role of sphingolipid glycan code: the GM1 oligosaccharide
- 16:25 – 16:45 ★ **Alina-Diana Zamfir** (Arad, ROU)
Ion mobility mass spectrometry: A new dimension in brain ganglioside research

16:45 – 18:00 Flash Poster Presentations (2 slides in 2 minutes)

Chairs: Michele Vittorio Dei Cas (Milan, ITA) and Laurane Payet (Toulouse, FRA)

- Poster numbers: 1-24

19:30 *Gala dinner*

Friday, Sept. 25, 2026

ESN Session Sphingolipid Signalling and Metabolism in the Nervous System

Chairs: Dagmar Meyer zu Heringdorf (Frankfurt, GER) and Svjetlana Kalanj Bognar (Zagreb, CRO)

- 9:00 – 9:10 **Mia Jurilj Sajko** (Zagreb, CRO)
Presentation of the ESN and acknowledgements
- 9:10 – 9:30 ★ **Floriana Della Ragione** (Naples, ITA)
Sphingolipid imbalance as a novel therapeutic target in Rett syndrome: a drug repositioning approach

- 9:30 – 9:50 ★ **Christian Müller** (Erlangen, GER)
Sphingolipid control of brain serotonergic signaling and its consequences for behaviour and mental disorders
- 9:50 – 10:00 **Sara Grassi** (Milan, ITA)
Role of sphingolipids in fractalkine mediated myelin repair
- 10:00 – 10:15 **Massimo Aureli** (Milan, ITA)
The two faces of glucosylceramide catabolism in neuronal life
- 10:15 – 10:35 ★ **Vladimir Parpura** (Hangzhou, CHN)
Glutamate release by regulated exocytosis in human glioblastomas
- 10:35 – 11:05 *Coffee break*
- 11:05 – 11:20 **Hervé Le Stunff** (Saclay, FRA)
Role of hypothalamic de novo ceramides synthesis in resistin-induced hypothalamic inflammation and insulin resistance: impact on glucose homeostasis
- 11:20 – 11:30 **Linda Montavoci** (Oxford, GBR)
Glycosphingolipid dysregulation in Parkinson's disease
- 11:30 – 11:45 **Giuseppe Pepe** (Pozzilli, ITA)
Targeting sphingolipid metabolism rescues cognitive deficits and hippocampal integrity in Huntington's disease mouse models
- 11:45 – 12:00 **Daan Van Kruining** (Maastricht, NED)
Sphingolipid dysregulation across neurodegenerative disease: genotype-dependent lipid alterations in Alzheimer's disease mice and GBA-associated Parkinson's disease
- 12:00 – 12:10 **Giulia Lunghi** (Milan, ITA)
Lysosomal dysfunction in GCase-deficient dopaminergic neurons induces plasma membrane alterations and metabolic rewiring
- 12:10 – 12:20 **Adriana Lipovčić** (Zagreb, CRO)
Glycosphingolipid mapping reveals a unique ganglioside signature of murine cortical mitochondria

12:30 – 13:00 ESN Flash Poster Presentations (5 slides in 5 minutes)

Chairs: Mia Jurilj Sajko (Zagreb, CRO) and Aleksandar Blivajs (Zagreb, CRO)

- Poster numbers: 25-30

13:00 – 14:30 *Lunch break*

14:30 *Island excursion*

20:30 *Dinner*

Saturday, Sept. 26, 2026

- 9:30 *General Meeting of the Sphingolipid Club – Report, evaluation, and comments*
- 10:30 **Riccardo Ghidoni**
Appointment to the XVII Sphingolipid Club Meeting and conclusions

A horizontal banner with a light purple background. On the right side, there are several vertical sprigs of lavender flowers. The text "ABSTRACTS OF TALKS" is centered in a bold, dark purple font.

ABSTRACTS OF TALKS

KL1

Bioactive sphingolipids: principles, approaches, case studies, and advice

Yusuf A Hannun

The Stony Brook Cancer Center and the Department of Medicine, Stony Brook University, New York City, NY, USA

Significant have consolidated the appreciation of the many critical roles of key bioactive sphingolipids, especially ceramide, ceramide 1-phosphate, sphingosine, and sphingosine 1-phosphate, in cell biology, pathophysiology, and therapeutics. These functions include several key cellular processes: cell growth, cell death, migration, and inflammatory responses. However, specific properties and complexities of bioactive sphingolipids have emerged that require particular attention: sphingolipid metabolism constitutes a network of connected bioactive metabolites that often exert distinct or even competing functions. Moreover, enzymes of sphingolipid metabolism show very specific subcellular localization, emphasizing the local metabolism and action of their substrates and products.

Here, we will discuss these concepts. I will focus on one key enzyme of sphingolipid metabolism, neutral sphingomyelinase 2, which generates ceramide from the hydrolysis of sphingomyelin. Results will be presented on the regulation, structure, and mechanisms of this enzyme. We will also discuss results on defining the plasma membrane as a specific compartment for action of this enzyme for specific functions of ceramide in regulating cancer cell migration and adhesion. This will help illustrate specific approaches. I will briefly discuss some specific hazards in the study of the cell biology of bioactive sphingolipids.

Mitochondria and sphingolipids in metabolic liver diseases

Carmen García-Ruiz^{1,2,3,4}, and José Carlos Fernández-Checa^{1,2,3,4}

¹*Department of Cell Death and Proliferation, Institute of Biomedical Research of Barcelona (IIBB), CSIC, Barcelona, Spain; Liver Unit, Hospital Clinic I Provincial de Barcelona, Barcelona, Spain*

²*Instituto de Investigaciones Biomédicas August Pi i Sunyer (IDIBAPS), Barcelona, Spain*

³*CIBEREHD, Madrid, Spain*

⁴*Department of Medicine, Keck School of Medicine, Division of Gastrointestinal and Liver disease, University of Southern California, Los Angeles, CA, United States*

Metabolic-dysfunction associated liver disease (MASLD) is currently the most prevalent form of chronic liver disease due to its association with the obesity pandemic, affecting up to 45% of the general adult population and about 70% of people with insulin resistance and type 2-diabetes. As a multifactorial disease, MASLD pathogenesis involves multiple players, with alterations in mitochondria playing a crucial role. In addition, sphingolipids and ceramide homeostasis contribute to the development and progression of MASLD towards advanced stages, such as metabolic-dysfunction associated steatohepatitis (MASH). Mitochondria are not only a source but also a target of sphingolipids, including ceramide and ganglioside GD3, and this interplay orchestrates MASH pathogenesis. Acid sphingomyelinase (ASMase) and alterations in methionine metabolism engage in a self-regulate cycle that mediates diet-induced MASH. Moreover, ceramide metabolism by acid ceramidase (ACDase) regulates mitochondrial cholesterol homeostasis through steroidogenic acute regulatory protein (STARD1) and impacts liver and brain function in the lysosomal storage disorder, Niemann-Pick type C disease. Ganglioside GD3, which plays a proapoptotic function in part by targeting mitochondrial and disabling survival pathway via NF- κ B, regulates hepatic stellate cell biology and GD3 acetylation contributes to HSC activation and liver fibrogenesis. Thus, exploiting the interplay between mitochondria and sphingolipids may be an important approach for the treatment of chronic liver disease.

T2

Sphingolipids as molecular codes for supracellular architectures

Giovanni D'Angelo

Swiss Federal Institute of Technology, Lausanne, Switzerland

Lipids are fundamental constituents of living matter. In addition to being structural components of cellular membranes and serving as energy reserves, lipids encode essential spatial information across scales. Within cells, membrane lipid diversity underlies organelle identity and function via the organization of lipid domains, gradients, and membrane contacts between subcellular compartments. Beyond intracellular aspects, heterogeneous lipid composition tracks with fine-grained tissue architecture. Here I will discuss how recent advances in spatial lipidomics and lipid are beginning to capture sphingolipid signatures that define cellular states, cell identities, and tissue domains.

T3

How sphingolipids shape the heart's fate

Annarita di Lorenzo

Department of Pathology and Laboratory Medicine, Cardiovascular Research Institute, Brain and Mind Research Institute, Weill Cornell Medicine, New York City, NY, USA

Cardiometabolic diseases are major drivers of cardiovascular morbidity, yet the molecular mechanisms linking metabolic dysfunction to vascular injury remain incompletely understood. In this talk, I will discuss our work uncovering a central role for endothelial sphingolipid metabolism in maintaining vascular homeostasis and determining cardiovascular health. Using complementary approaches spanning genetic mouse models, lipidomics, single-cell technologies, and disease-relevant models of obesity and coronary artery disease, we have identified key mechanisms driving the dysregulation of endothelial sphingolipid *de novo* biosynthesis and defined how these alterations promote endothelial dysfunction, hypertension, and the progression of coronary artery disease. These findings establish endothelial sphingolipid metabolism as a critical regulator of vascular homeostasis and highlight its therapeutic potential for preventing and treating cardiovascular complications of cardiometabolic disease, including coronary artery disease.

Atypical sphingolipids at the crossroads of health and disease

Lauren Ashley Cowart

*Virginia Commonwealth University Department of Cellular, Molecular, and Genetic Medicine,
Richmond, VA, USA*

SPTLC3 is an inducible catalytic subunit of the serine palmitoyltransferase (SPT) complex that redirects substrate utilization from palmitoyl-CoA to alternative substrates (e.g., myristoyl-CoA), thereby generating a suite of atypical sphingolipids bearing alternative carbon number sphingoid bases in place of the canonical 18-carbon backbone. Unlike the constitutive SPTLC1/SPTLC2 heterodimer, SPTLC3 is expressed at low levels in most normal tissues and is upregulated in response to pathological and metabolic stimuli, making its atypical sphingolipid products conditionally deployed rather than homeostatic. We have previously demonstrated that SPTLC3 is induced in both human and mouse ischemic cardiomyopathy, where it governs the lipid composition of the inner mitochondrial membrane and is required for complex I activity of the electron transport chain; cardiomyocyte-specific ablation confers cardioprotection following myocardial infarction [1]. In the liver, high-fat diet induces SPTLC3, and hepatocyte-specific knockout mice exhibit impaired hepatic glucose production; mechanistically, SPTLC3-derived d16sphingomyelins accumulate at the plasma membrane and regulate glucagon-stimulated adenylate cyclase/cAMP/CREB signaling to sustain gluconeogenic gene expression [2]. Here we report an additional role for SPTLC3 in normal mitochondrial energetics in hepatocytes. In a hepatocyte-specific SPTLC3 knockout mouse model, transcriptomic analysis reveals broad disruption of mitochondrial biology, attributable to remodeling of mitochondrial membrane lipid composition caused by loss of atypical sphingolipids, thereby establishing SPTLC3 as an essential regulator of hepatic mitochondrial function. We further present evidence that SPTLC3 expression is elevated in hepatocellular carcinoma (HCC) cell lines, patient datasets, and a cMyc-induced mouse HCC model. Sptlc3 is required for HCC cell survival and malignant behavior, as demonstrated by cell viability, proliferation, wound-healing/scratch, migration, and colony-forming assays. This extends prior observations linking SPTLC3 to the progression from NAFLD to HCC. Taken together, these findings establish atypical sphingolipids as an emerging class of conditionally induced, physiologically consequential metabolic regulators. Their upregulation in cancer appears to be required for tumor progression rather than merely incidental to it. Because constitutive SPTLC3 expression is low in most normal tissues, these studies nominate it as a compelling and tractable therapeutic target in HCC and highlight the broader importance of inducible sphingolipid remodeling in metabolic and oncological disease.



CYP4F2-mediated ω -hydroxylation of 1-deoxysphingolipids reveals a new hepatic detoxification pathway

Adam Majcher¹, Essa M Saied², Zoltan Kutalik³, Maftuna Shamshiddinova¹, Andreas J. Hülsmeier¹, Par Bjorklund^{4,5}, Elkhan Yusifov¹, Irina Alecu¹, Christoph Arenz², and Thorsten Hornemann¹

¹*Institute of Clinical Chemistry, University Hospital Zurich, Zurich, Switzerland*

²*Humboldt Universität zu Berlin, Berlin, Germany*

³*University Center for Primary Care and Public Health, University of Lausanne; Swiss Institute of Bioinformatics, Lausanne, Switzerland*

⁴*Department of Medicine Huddinge, Cardio Metabolic Unit, Karolinska Institute, Stockholm, Sweden*

⁵*Clinical Department of Endocrinology, Karolinska University Hospital Huddinge, Stockholm, Sweden*

1-deoxysphingolipids (1-deoxySLs) are atypical, cytotoxic sphingolipids (SL) formed by the serine palmitoyltransferase through the alternative use of L-Alanine over its canonical substrate L-Serine. Elevated plasma levels of 1-deoxySLs have been implicated in metabolic and neurodegenerative diseases. Due to the missing C1 hydroxyl group, 1-deoxySLs cannot be converted into complex sphingolipids nor degraded via the canonical SL catabolic pathways. However, previous reports suggested a cytochrome P450 mediated ω -hydroxylation of 1-deoxySLs as a potential detoxification mechanism although the exact downstream metabolism of these lipids remained unclear. We combined genome-wide association analysis with targeted lipid analysis to identify genes involved in 1-deoxySL metabolism. Functional validation was performed in cell culture models, enzyme assays, and through quantitative high-resolution mass spectrometry using isotope labelled synthetic standards. We identified a strong association between the CYP4F2 rs2108622 variant and plasma 1-deoxySL, implicating CYP4F2 is involved in 1-deoxySL metabolism. We demonstrated that CYP4F2 catalyzes the ω -hydroxylation of 1-deoxysphinganine, forming a previously uncharacterized hydroxylated sphingoid base. In liver cells, this metabolite was further metabolized via three distinct pathways: one forming the N-acyl, a second involving omega acylation and third resulting in omega carboxylation. All reactions generated a new spectrum of 1-deoxysphingolipids that are based on ω -hydroxylated 1-deoxySA as a precursor. The metabolic steps were confirmed by structural validation using synthetically prepared external standards. Importantly, ω -hydroxylation significantly attenuated the acute cytotoxicity of 1-deoxySLs in liver cells, indicating that this modification is the initiating step of a multi-branched metabolic clearance pathway. This study identifies CYP4F2 as a key enzyme initiating the hepatic clearance of atypical 1-deoxySLs, mitigating their cellular toxicity and revealing multiple downstream metabolic fates. Our findings highlight a previously unrecognized clearance mechanism for atypical sphingolipids with relevance to metabolic disease.

Distinct canonical and atypical sphingolipid signatures differentiate type 1 and type 2 diabetes

Lucia Centofanti¹, Camillo Morano¹, Michele Dei Cas¹, Paola Zermiani¹, Loredana Bucciarelli², Monica Bignotto¹, Monica Miozzo¹, Pier Maria Battezzati¹, Rita Paroni¹, and Franco Folli¹

¹*University of Milan, Department of Health Sciences, Milan, Italy*

²*University of Milan, Department of Biomedical and Clinical Sciences "L. Sacco", Milan, Italy*

Deoxy- and deoxymethyl- sphingolipids (m18:1 and m17:1) are two classes of sphingolipids (SLs) resulting from the condensation of palmitoyl-CoA with alanine or glycine respectively, instead of serine. Due to their structure, they cannot be phosphorylated to sphingosine-1-phosphate (S1P), and thus they evade normal catabolic routes, favoring cellular accumulation and lipotoxicity. Recent studies have highlighted the role of 1deoxySLs in the pathophysiology of type 2 diabetes (T2D). The aim of this study was to compare the concentrations of circulating 1-deoxySLs in type 1 diabetes (T1D), impaired fasting glucose (IFG, or pre-diabetes) and T2D as compared to healthy controls (HC). Sphingolipidomic profiles of plasma samples from T1D, IFG and T2D patients, and HC (age, sex and BMI matched, n. 185 subjects) were analyzed by targeted LC/MS-MS. Total ceramides concentrations were significantly reduced in T1D as compared to IFG and T2D ($p < 0.05$). Glycosylated species (HexCer and LacCer) were reduced in IFG and T2D as compared to T1D and HC (all, $p < 0.001$). Total content of 1-deoxyDHSLs and 1deoxySLs was elevated in T2D, compared to T1D and HC ($p < 0.05$). 1-deoxymethylSLs showed a similar trend as 1-deoxySLs, although their changes were not as marked. Sphingosine-1-phosphate (S1P) levels were elevated in T1D ($p < 0.05$), IFG and T2D ($p < 0.001$) compared to HC. The ratio Cer 18:0/Cer 24:0, a metabolic and cardiovascular risk marker, was significantly higher in both IFG and T2D compared to T1D and HC (all, $p < 0.001$), while there were no significant differences between T1D and HC. There were differences in canonical SLs, particularly in glycosylated classes, between T1D and IFG and T2D. Interestingly, T1D and T2D had very different SLs signatures, evidencing a differential regulation of the biosynthesis and the degradation in both canonical and atypical SLs.

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Quantifying sphingolipid fluxes that influence skin health and disease

Christian M. Metallo^{1,2}

¹*Molecular and Cell Biology Laboratory, Salk Institute for Biological Studies, La Jolla, CA, USA*

²*Department of Bioengineering, University of California, La Jolla, CA, USA*

Metabolism is central to virtually all cellular functions and contributes to disease states. My laboratory applies stable isotope tracers, mass spectrometry, and metabolic flux analysis (MFA) to quantify metabolism in various biological systems and improve our understanding of health. Recent efforts have aimed to expand MFA frameworks to encompass sphingolipid homeostasis, leveraging high-resolution mass spectrometry data and mechanistic network modeling. Given the diverse lipids present and high rates of recycling within the sphingolipid network, MFA-based approaches traditionally used for studying the tricarboxylic acid (TCA) cycle are needed to decipher how these pathways operate. A central focus of our lab is the function of serine palmitoyltransferase (SPT), which integrates in health and disease. We are particularly interested in understanding how amino acid and lipid metabolism are coordinated in the context of familial and age- metabolic disorders. The SPT complex initiates the de novo synthesis of bioactive sphingolipid, integrating metabolic signals (i.e. amino acids and fatty acids) into the “sphingolipid cycle” to shape the membrane lipidome. Here I will detail how we apply MFA to decipher how dysregulation of serine and SPT metabolism contribute to pathologies of the skin, including impaired wound healing and atopic dermatitis (AD). Using mouse models of diabetic wound healing, we have observed that modulation of serine levels supports wound healing and vascular function, in part through support of SPT flux driving the sphingolipid cycle. We have also applied tape stripping and lipidomics to profile the outputs of SPT flux in human skin and engineered tissue models. We identified very-long-chain sphingoid bases (VLCBs) that constitute a major sphingoid base pool in human stratum corneum. We have characterized the roles of SPTSSB and SPTLC3 in synthesizing these species, which have distinct fates, biochemistry, and biological impacts compared to long-chain sphingoid bases (LCBs). These lipids are altered in lesional skin of AD patients, suggesting potential links between VLCBs, keratinocyte differentiation, inflammation and cutaneous barrier function. Overall, these data provide mechanistic insights into the roles of SPT flux and lipid diversity in health and disease.



Sphingolipidomics of women with anorexia nervosa and obesity: signatures at opposite ends of energy balance

Camillo Morano¹, Aldijana Sadikovic¹, Michele Dei Cas¹, Adele Bondesan², Diana Caroli², Francesca Frigerio², Laura Abruzzese³, Sandra Savino⁴, Silvano G. Cella⁵, Antonello E. Rigamonti⁵, Alessandro Sartorio², and Rita Paroni¹

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Anorexia nervosa (AN) and obesity (OB) represent opposite extremes of chronic energy imbalance and are characterized by profoundly different metabolic adaptations. To characterize sphingolipid remodeling across these contrasting nutritional states, we performed targeted plasma sphingolipidomics by LC–MS/MS in 114 women (normal-weight healthy controls [NWH], $n = 49$; AN, $n = 28$; OB, $n = 37$). Clinical and metabolic phenotyping was performed in all participants.

Women with AN were characterized by increased plasma concentrations of ceramides (Cer), sphingomyelins (SM) and gangliosides (GM3), together with an enrichment of very-longchain fatty acids across multiple sphingolipid classes. Plasma levels of S1P and DHS1P were also significantly increased. Conversely, women with OB exhibited higher concentrations of selected DHCer species and ceramide ratio indices, accompanied by depletion of several very-long-chain glycosphingolipids, together with reduced levels of several very-long-chain glycosphingolipids. Supervised PLS-DA accurately discriminated the study groups (overall accuracy = 0.851), identifying DHCer 24:1, DHCer 18:0, HexCer 22:0, Cer 24:1, SM 24:1, and DHCer 18:1 as the strongest discriminant variables. Ageadjusted regression analyses demonstrated significant associations between sphingolipid species and body mass index (BMI), insulin resistance (HOMA-IR), blood pressure, Creactive protein (CRP), triglycerides, and HDL cholesterol.

Women with AN and OB exhibit distinct, yet partially convergent, plasma sphingolipidomic signatures, indicating coordinated remodeling of interconnected sphingolipid pathways. AN was primarily associated with SM- and glycosphingolipid-enriched profiles, whereas OB displayed a remodeling pattern dominated by alterations in DHCer- and Cer-related pathways. These findings identify sphingolipid remodeling as a central metabolic feature of chronic energy imbalance and highlight plasma sphingolipidomics as a promising approach for investigating metabolic adaptation in nutritional disorders.

Sex bias in cell membrane lipidomesMarija Heffer*Faculty of Medicine Osijek, Department of Medical Biology and Genetics, Osijek, Croatia*

Sex is a biological variable reinforced by sex chromosomes, hormones, and genomic imprinting. Viable embryos are generated exclusively through the fusion of dimorphic germ cells, and the resulting organism must be capable of producing either male or female gametes to ensure propagation. The differences between the sexes are most pronounced during the reproductive period, when sex hormone levels are at their peak. These hormones do not merely generate distinct germ cells; sex-based differences are pervasive across all tissues, as sex hormone receptors act as transcription factors with thousands of binding sites across the genome. Consequently, processes such as fatty acid oxidation, lipogenesis, and the transport and remodeling of cellular membranes are directly or indirectly regulated by these hormones. The identification of sex-specific differences is facilitated by big-data approaches such as matrix-assisted laser desorption/ionization time-of-flight imaging mass spectrometry (MALDI-TOF IMS). Using two m/z ranges (300–700 Da and 700–1000 Da), we analyzed the metabolomic profiles of the liver, kidney, and skeletal muscle in young (3-month-old) and aged (10-month-old) gonadally intact and gonadectomized Sprague–Dawley rats of both sexes. Partial least squares discriminant analysis (PLS-DA) score plots demonstrated clear sex-based separation in all three tissues and across age groups. The largest number of statistically significant differentially expressed signals was detected in the liver of young animals, whereas the smallest number was observed in the skeletal muscle of aged animals. Age and gonadectomy contributed to the observed differences across all three tissues. Among the tentatively annotated signals associated with sex-specific separation, compounds related to diacylglycerols, phosphatidylserines, and sphingomyelins were particularly prominent. Overall, membrane-associated lipids represented the most pronounced sex-specific metabolic differences and may contribute to distinct molecular landscapes involving membrane receptors, ion channels, transporters, and other membrane-associated proteins. These differences may help explain clinically relevant sex-related variation in the pathophysiology, diagnosis, and treatment of common diseases.



Ceramides as cardiovascular biomarkers: from lipidomic discovery to personalized prevention and treatment

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Introduction: Cardiovascular risk assessment remains incomplete, particularly in patients with established coronary artery disease, in whom substantial residual risk persists despite contemporary preventive treatment. Lipidomic studies have identified specific circulating ceramides as strong predictors of cardiovascular events and mortality. These findings led to the development of clinically applicable ceramide-based risk scores.

Results: The first ceramide risk scores combined concentrations and ratios of selected ceramide species associated with atherosclerosis, plaque instability, and adverse cardiovascular outcomes. Further analytical and clinical development resulted in CERT2, which integrates ceramides and phosphatidylcholines into a single risk score. CERT2 has shown consistent associations with cardiovascular death and major adverse cardiovascular events in different cardiovascular patient populations. Its prognostic value is partly independent of conventional risk factors and commonly used biomarkers, including LDL cholesterol and markers of inflammation. In patients with established coronary artery disease, CERT2 may therefore provide clinically relevant information on residual risk that is not adequately captured by traditional lipid measurements. Standardized mass-spectrometry-based measurement and a simple scoring system facilitate its translation from lipidomic discovery to clinical testing.

Conclusions: Ceramide-based biomarkers have progressed from exploratory lipidomic findings toward practical tools for cardiovascular risk stratification. The next step is to move beyond risk association and establish how testing can influence clinical decisions. CERT2 may help identify patients who need more intensive preventive treatment, closer follow-up, or additional cardioprotective therapies. It may also support the selection of patients that are most likely to benefit from distinct interventions. Thus, ceramide testing may contribute to a new era of more personalized cardiovascular prevention and treatment

T11

Neutral sphingomyelinase couples mechanical stress to cardiac excitability and remodeling

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Neutral sphingomyelinase (nSMase) is a membrane-associated hydrolase that converts sphingomyelin into ceramides, bioactive signaling lipids implicated in cardiovascular and other chronic diseases. Chronic mechanical overload is a major driver of cardiac remodeling, heart failure, and arrhythmias, pathological conditions associated with ceramide accumulation. However, whether mechanical stress directly regulates sphingolipid metabolism and contributes to disease progression remains unknown. Our studies identify nSMase as a previously unrecognized mechano-transducer that translates mechanical stress into sphingolipid signaling through ceramide generation in the heart. We demonstrate that acute mechanical activation of nSMase contributes to physiological mechano-chronotropic and mechano-inotropic responses through cAMP-dependent protein kinase A activation and Ca^{2+} -dependent stimulation of mitochondrial reactive oxygen species production. To determine the pathophysiological significance of this pathway, we examined mice subjected to 8 weeks of transverse aortic constriction. Pharmacological inhibition of nSMase preserved left ventricular systolic function and markedly reduced ventricular electrical remodeling by preventing action potential prolongation and conduction slowing. These findings are supported by increased production of short-chain ceramides in chronically stretched human engineered cardiac tissues and are consistent with elevated ceramide levels in patients with chronic hypertension and experimental models of chronic cardiac overload. Together, our findings establish nSMase as a mechanosensitive regulator of sphingolipid metabolism that couples mechanical stress to adaptive cardiac function under physiological conditions and maladaptive remodeling during chronic overload. This work uncovers a mechanistic link between cardiac mechanobiology and ceramide signaling, highlighting nSMase as a promising therapeutic target for hypertension, cardiac hypertrophy, heart failure, and arrhythmias.

Intracellular remodeling of LDL-derived ceramides as a key regulator of muscle insulin resistance

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Insulin resistance (IR) is a major feature of type 2 diabetes (T2D). In obesity, lipid overload promotes ceramide accumulation in skeletal muscle, where these bioactive sphingolipids impair insulin signaling. Circulating ceramides carried by low-density lipoproteins (LDL) have emerged as biomarkers of metabolic dysfunction and may also contribute to muscle IR. C24:0-ceramide is one of the main LDL-associated ceramide species, but the mechanisms by which LDL-derived C24:0-ceramide alters insulin action still remain unclear.

Here, we investigated whether LDL-C24:0-ceramide impairs insulin signaling through intracellular ceramide remodeling. C2C12 myotubes were incubated with human LDL enriched in either C24:0- or C18:0-ceramide, and insulin-stimulated Akt phosphorylation, used as a readout of insulin signaling activation, was analysed together with ceramide species composition and subcellular distribution.

LDL-C24:0-ceramide increases intracellular C24:0-ceramide levels and promotes the accumulation of endogenous shorter-chain ceramides, particularly C16:0- and C18:0ceramide species. These remodeled ceramides are associated with reduced insulin stimulated Akt phosphorylation. In contrast, LDL-C18:0-ceramide produces a distinct ceramide profile and does not induce the same remodeling pattern or comparable inhibition of insulin signaling. Inhibition of ceramide synthases with fumonisins B1 prevents the conversion of LDL-derived C24:0-ceramide into shorter-chain ceramides and restores insulin-stimulated Akt phosphorylation.

These results suggest that LDL-C24:0-ceramide impairs insulin signaling not through direct cellular accumulation, but through ceramide synthase-dependent remodeling into bioactive C16:0/C18:0 ceramides. This species-specific mechanism supports a direct role for circulating LDL-C24:0-ceramide in skeletal muscle insulin resistance.



Differential effects of ceramide chain length on alcohol consumption and behavioral phenotypes in mice

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Introduction: Ceramides are bioactive sphingolipids that regulate membrane organization, cellular stress signaling, and neural function. Although ceramide pathways have been implicated in neuropsychiatric disorders and are emerging as modulators of alcohol-related behaviors, whether these effects depend on acyl-chain length remains poorly understood.

Results: In independent cohorts of female C57BL/6 mice, repeated iv injection of C16:0, C18:0, C24:0, or vehicle were administered during a two-bottle choice alcohol paradigm. C18:0 produced the most robust increase in voluntary alcohol intake, which persisted throughout the two-week post-injection period; C24:0 elicited a modest increase, whereas C16:0 failed to alter consumption. In separate conditioned place preference testing, C18:0 did not enhance alcohol reward; instead, vehicle-treated mice showed a stronger baseline corrected response, suggesting dissociation between drinking and reward learning.

Behavioral profiling revealed domain-specific effects: all ceramides increased sucrose preference; C16:0 reduced anxiety-like behavior in open field and light–dark tests; C16:0 and C18:0 impaired object recognition; and C16:0 selectively disrupted nest-building. Targeted sphingolipidomics in prefrontal cortex, ventral striatum, and dorsal hippocampus revealed no broad increases in absolute sphingolipid levels. Instead, lipid changes were predominantly compositional: C16:0 induced extensive remodeling of cortical glycosphingolipid/ceramide ratios and dorsal hippocampal pools, whereas C18:0 produced a more restricted profile.

Conclusions: These findings demonstrate that ceramide acyl-chain length is an important determinant of domain-specific behavioral and lipidomic outcomes. C18:0 selectively increased alcohol consumption without enhancing conditioned reward, while C16:0 was associated with anxiolytic-like effects, cognitive impairment, and pronounced brain sphingolipid remodeling. Collectively, these data suggest that discrete compositional shifts in brain sphingolipids, rather than global lipid accumulation, may contribute to alcohol related and comorbid neurobehavioral phenotypes.

Ceramide couples TNF- α signaling to organelle biophysical remodeling

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Ceramides are essential bioactive lipids that regulate cell physiology and stress responses, yet how their biophysical effects on membranes translate into coordinated cellular responses remains poorly understood. Here, we demonstrate that endogenous ceramide generation during TNF- α -induced stress rapidly remodels the plasma membrane and initiates a cascade of intracellular events. Ceramide accumulation increases membrane order and promotes the formation of ceramide-enriched internalizing vesicles that traffic through the endolysosomal system. Acting as carriers of remodeled membrane domains, these vesicles propagate ceramide-driven biophysical changes from the plasma membrane to intracellular compartments, altering their membrane properties and function. In parallel, ceramide production reprograms lipid droplet (LD) metabolism, decreasing LD polarity and modifying the expression of genes involved in lipid storage. Together, our findings provide direct evidence that ceramides act as biophysical integrators of the TNF- α stress response, connecting plasma membrane remodeling, vesicular trafficking, and lipid metabolic adaptation. These results uncover a mechanism by which localized ceramide-driven membrane remodeling is propagated across the cell to coordinate adaptation and preserve homeostasis under inflammatory stress.

T15

The bond that breaks the heart - and the liver, and the kidney, and the...

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Overnutrition, inflammation, and genetic aberrations promote the accumulation of sphingolipids such as ceramides, which alter metabolic programs and induce apoptosis in a wide range of cell types. In humans, serum ceramides are biomarkers of diabetes and major adverse cardiac events, and clinics have started measuring circulating ceramides as markers of disease risk. In rodents, inhibiting ceramide biosynthesis ameliorates diabetes, steatohepatitis, kidney injury, and heart failure. The author will discuss the evolution of this pathway as a signal of lipid excess and the therapeutic potential of a new approach, involving the elimination of a double bond, that inactivates ceramides and ameliorates cardiometabolic pathologies.

The author is a co-founder and shareholder of Centaurus Therapeutics, inc.

Identifying and validating ceramide transfer protein binding partners

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Sphingolipids (SLs) are crucial membrane components with key roles in signal transduction and cell metabolism. A central step in SL metabolism is the production of ceramides (Cer), which are abundant in cell membranes and play crucial roles in cellular functions such as stress response, senescence, apoptosis, cell cycle, differentiation, and neuronal maturation. The ceramide transfer protein (CERT) transports Cer from the endoplasmic reticulum to the Golgi apparatus for further modification into more complex SLs. CERT has two isoforms, CERTL and CERT, which differ slightly in their middle region and are both composed of a pleckstrin homology domain, a middle region, and a StAR-related lipid transfer domain. CERT is expressed ubiquitously but is particularly important in the central nervous system, where it is associated with embryogenesis and brain development, and can also bind C1q to activate the complement cascade, highlighting a role in immune responses. Despite its significance, CERT's molecular mechanisms and broader interactome remain poorly understood. To identify CERT interactors, we employed a yeast two hybrid screen analysing each region of the protein individually. Candidate interactions are being validated through complementary approaches, including BioID and NanoBRET. Using these assays, we confirmed the known interactor VAP-A, supporting the validity of our screening and validation pipeline. We also identified additional candidate interactors spanning functions such as mitochondrial activity, apoptosis, cell growth, vesicle transport, and protein degradation, including ORMDL1, a regulator of serine palmitoyltransferase and de novo ceramide biosynthesis, and RTN1, an ER-shaping protein implicated in apoptosis and neuronal differentiation. Validation of these candidates is ongoing. These findings lay the groundwork for future research to clarify CERT's molecular mechanisms and interactome, and its potential as a therapeutic target in diseases related to ceramide metabolism.



Disease-associated SPT variants drive distinct sphingolipid remodeling states

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Sphingolipids are essential structural and signaling components of mammalian cells and are particularly important for neuronal membrane organization, signaling, and survival. Serine palmitoyltransferase (SPT), a multi-subunit enzyme complex, catalyzes the first and rate-limiting step of de novo sphingolipid synthesis by generating long-chain bases from palmitoyl-CoA and L-serine. SPT activity is controlled by ceramide-mediated feedback inhibition, but the enzyme can also use alternative amino acid substrates, such as L-alanine, leading to the formation of non-canonical 1-deoxysphingolipids (1-deoxySL). Pathogenic variants in the SPT subunits SPTLC1 and SPTLC2 are associated with distinct neurological disorders, including hereditary sensory and autonomic neuropathy type 1, amyotrophic lateral sclerosis, and mixed sensory-motor neuropathies. However, a systematic comparison of the metabolic consequences of these disease-associated variants has been lacking.

Using stable isotope tracing and LC-MS/MS-based lipidomics, we compared representative disease-associated variants in SPTLC1 and SPTLC2. ALS-associated variants showed increased canonical sphingolipid synthesis and impaired ceramide-mediated feedback regulation, resulting in accumulation of ceramides and sphingomyelins. In contrast, HSAN1-associated variants altered SPT substrate selectivity, promoting 1-deoxySL production while reducing canonical sphingolipid synthesis. Variants associated with mixed sensory-motor disease exhibited a third metabolic profile, with increased levels of both canonical sphingolipids and 1-deoxysphingolipids. Together, lipidomic profiling separated ALS, HSAN1, and mixed variants into three distinct sphingolipid remodeling states. These findings show that disease-associated SPT variants drive phenotype-specific changes in sphingolipid metabolism and identify metabolic signatures that may explain the divergent clinical presentations of SPT-associated neuropathies. They further suggest that therapeutic strategies should be adapted to the underlying metabolic state rather than the affected gene alone.



The potential role of nuclear sphingomyelin in non-canonical exosome biogenesis

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Sphingomyelin (SM) is the most abundant sphingolipid in the nucleus and, together with cholesterol, contributes to the formation of specialized lipid microdomains in the nuclear membrane. These domains have been proposed as regulatory platforms involved in chromatin organization and transcriptional control. We previously demonstrated that SM promotes neuronal differentiation and that SM-enriched nuclear lipid microdomains (NLMs) protect specific non-coding RNAs, including miRNAs, involved in the regulation of genes controlling neurodifferentiation and sphingolipid metabolism. Based on these findings, we investigated whether SM-enriched NLMs may also be involved in a non-canonical pathway of exosome biogenesis, potentially providing a mechanism for the propagation of SM-induced differentiation signals to neighboring cells and representing a novel interface between nuclear regulation and intercellular communication. Using embryonic hippocampal cells (HN9.10e cell line), we identified several features supporting a possible relationship between NLMs and exosomes. Exosomes released by HN9.10e cells showed a lipid composition enriched in SM, ceramide and phosphatidylcholine, closely resembling that of NLMs. Moreover, comparison of miRNA profiles revealed a high degree of similarity between the two compartments: 273 miRNAs were common to NLMs and exosomes, 30 of which were significantly enriched. Notably, the exosomal marker CD63 was detected within the nucleus, while nuclear material was identified inside exosomes. Finally, using a synthetic membrane system, we demonstrated a physical association between specific miRNAs and SM, providing mechanistic support for the possibility that SM-associated miRNAs may be incorporated into extracellular vesicles during their biogenesis. Together, these findings support the hypothesis that SM-enriched NLMs are involved in neurodifferentiation and may represent a previously unrecognized site of non-canonical exosome biogenesis, linking nuclear lipid microdomains to extracellular vesicle production and the propagation of differentiation signals between cells. Financial support by Levante SRL and Dia.Metra SRL

Total sphingomyelin synthesis deficiency promotes lipodystrophy and prevents adipogenesis

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Lipodystrophy is characterized by a loss of adipose tissue, severe insulin resistance, fatty liver, and dyslipidemia. We and others found that blocking the first and key enzyme, serine palmitoyltransferase (SPT), in adipose tissue causes lipodystrophy. Sphingomyelin synthase (SMS) acts downstream of SPT activity and could be involved in the process of lipodystrophy. There are two isoforms, SMS1 and SMS2, which utilize ceramide to produce SM, thus influencing both ceramide and SM. In the current study, we utilized inducible *Sms1* (*iSms1*) knockout (KO) mice and *iSms1* KO/germline *Sms2* KO mice. As expected, we found SM reduction and glucosylceramide accumulation in adipose tissues of these mice. To our surprise, we did not find significant changes on ceramide, which could induce apoptosis. The *iSms1* KO mice have no lipodystrophy under chow diet and this could be due to the existence of SMS2. Indeed, the lipodystrophy was observed in *iSms1* KO/*Sms2* KO mice. Further, we found that *iSms1* KO/*Sms2* KO adipocytes underwent severe apoptosis and recruited dramatic numbers of macrophages for phagocytosis. We next supplemented *iSms1* KO/*Sms2* KO mice with SM and found the treatment can significantly reverse the double deficiency-mediated apoptosis. To investigate the mechanism, we performed immunoblots and found that the double deficiency can significantly induce caveolin-1 and PTEN (phosphatase and Tensin homolog), thus, inhibiting the level of activated-AKT, a well-known anti-apoptotic factor. Importantly, supplementing SM can reverse these observed changes. To study the effect of SMS deficiency on adipogenesis, we utilized stromal vascular fraction (SVF) from mouse adipose tissues and found that lacking SMS activity can significantly blunt adipocyte maturation through induction of membrane-bound cadherin, a negative regulator of adipogenesis. In conclusion, total SMS activity-mediated SM but not ceramide changes in adipose tissues contribute to adipocyte integrity and adipogenesis. Fine-tuning SMS activity could provide a new approach for the treatment of metabolic diseases.

Targeting sphingolipid metabolism regulates tumor growth, metastasis, and anti-cancer therapyBesim Ogretmen*Medical University of South Carolina and Hollings Cancer Center, Charleston, SC, USA*

Bioactive ceramide induces cell death in part by promoting mitophagy. C18-ceramide levels are commonly reduced in head and neck squamous cell carcinoma (HNSCC), which correlates with poor prognosis, suggesting the potential of harnessing ceramide for cancer treatment. We evaluated the ability of the ceramide analog LCL768 to induce mitophagy and metabolic stress in HNSCC. In addition, LCL768-driven mitophagy altered mitochondrial metabolism, resulting in fumarate depletion and leading to tumor suppression *in vivo*. Exogenous fumarate supplementation prevented LCL768-mediated mitophagy, mitochondrial trafficking of CerS1, ER-mitochondrial tethering, and tumor suppression in mice.

However, a mechanistic understanding of aging-mediated changes in mitochondrial bioenergetics and lipid metabolism that affect T cell function needs to be defined. A bioactive sphingolipid ceramide, induced by aging stress, mediates mitophagy and cell death; however, the aging-related roles of ceramide metabolism in regulating T cell function remain unknown. We showed that activated T cells isolated from aging mice exhibit elevated accumulation of C14-/C16-ceramide in mitochondria, generated by ceramide synthase 6, leading to mitophagy/mitochondrial dysfunction. Mechanistically, aging-dependent mitochondrial ceramide inhibited protein kinase A, leading to mitophagy in activated T cells. This aging/ceramide-dependent mitophagy attenuated the anti-tumor functions of T cells *in vitro* and *in vivo*. Also, inhibition of ceramide metabolism or PKA activation by genetic and pharmacologic means prevented mitophagy and restored the central memory phenotype in aging T cells.

Alzheimer's disease (AD) patients have a decreased incidence of cancer. A cross-sectional analysis of a nationwide sample of adults found 21-fold higher odds of cancer diagnosis in non-AD compared to AD patients. We demonstrated that mitochondrial localization of AD-associated amyloid- β precursor protein (APP) and its cleavage product, amyloid- β 40, but not mutant APP lacking a mitochondrial localization signal, inhibits lipid stress-mediated hyperactive mitophagy in aging T-cells, thereby improving their anti-tumor functions. The growth of melanoma xenograft or carcinogen-induced oral cancer models was highly reduced in AD mice. Additionally, adoptive cell transfer (ACT)-based immunotherapy using aging T cells isolated from AD mice suppressed tumor growth. The metabolic signature of stress-dependent mitophagy in T cells showed fumarate depletion, which was linked to decreased succination of Parkin and enhanced mitochondrial damage. Mechanistically, APP interaction with the TOMM complex at the outer mitochondrial membrane attenuated the trafficking of ceramide synthase CerS6 to mitochondria in aging AD T-cells, preventing ceramide-dependent mitophagy. Moreover, T cells isolated from aging donors showed elevated mitophagy upon fumarate depletion, a phenotype restored in T cells isolated from age-matched AD patients.



In addition, crosstalk between metabolic and signaling events that induce tumor metastasis remains elusive. We recently determined that oncogenic sphingosine 1-phosphate (S1P) metabolism induces intracellular C3 complement activation, thereby enhancing migration/metastasis. We demonstrate that increased S1P metabolism activates C3-complement processing through S1P receptor 1 (S1PR1). S1P/S1PR1-activated intracellular C3b- α_2 is associated with PPIL1 through glutamic acid 156 (E156) and aspartic acid 111 (D111) residues, resulting in NLRP3/inflammasome induction. Inactivation mutations of S1PR1 to prevent S1P signaling or mutations of C3b- α_2 to prevent its association with PPIL1 attenuate inflammasome activation and reduce lung colonization/metastasis in mice. Also, activation of the S1PR1/C3/PPIL1/NLRP3 axis is highly associated with human metastatic melanoma tissues and patient-derived xenografts (PDXs). Moreover, targeting S1PR1/C3/PPIL1/NLRP3 signaling using molecular, genetic, and pharmacologic tools prevents lung colonization/metastasis of various murine cancer cell lines using WT and C3a-receptor1 knockout (C3aR1^{-/-}) mice. These data provide strategies for treating high-grade/metastatic tumors by targeting the S1PR1/C3/inflammasome axis. Moreover, we provide new data about the possible roles of the S1P transporter, Spns2, in inducing cancer metastasis and response to immunotherapy.

For our translational studies, targeting SphK2 and sphingolipid metabolism using Opananib in a phase II clinical trial for the treatment of patients with advanced prostate cancer has been completed, and the data have been recently published. To this end, reprogramming T cells to modulate their metabolic states, including regulation of lipid metabolism, seems to be an effective way to improve CAR-T-based cellular immunotherapy for the treatment of patients with advanced cancers.



Multiple mechanisms converge on deregulation of sphingolipid and glycosphingolipid metabolism in colorectal carcinoma

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Colorectal cancer (CRC) development is associated with profound alterations in lipid metabolism, particularly sphingolipids (SLs) and glycosphingolipids (GSLs), which regulate key cellular processes involved in tumor progression. Most previous studies have focused on the sphingosine-1-phosphate/ceramide ratio or a limited set of GSLs. Here, we provide a broader and more detailed characterization of SL/GSL metabolic changes in CRC tumor tissues. We observed increased levels of sphingosine, sphingosine-1-phosphate, and ceramide-1-phosphate in CRC tissues, accompanied by upregulation of key SL metabolism genes (CERS2, CERS6, ASAH1, SPHK1). This was paralleled by enhanced expression of fatty acid synthesis genes (FASN, SCD, ACSL1/4), suggesting increased availability of fatty acid-CoA substrates for SL biosynthesis. Additionally, CRC tissues exhibited elevated levels of glucosylceramides, lactosylceramides, and more complex GSLs (GM3, GM2, GM1a, GD3, GA2, Gb3), indicating widespread dysregulation of GSL metabolism. Notably, these increases were largely independent of changes in GSL synthase expression (except B4GALT5/6) and may instead reflect enhanced availability of UDP-glucose and UDP-galactose, co-substrates of glucosylceramide and lactosylceramide synthesis. This is supported by increased expression of GLUT1 and SLC35A2, as well as upregulation of Golgi-associated regulators of GSL synthesis (GOLPH3, GRASP55, AUTS2). Overall, our findings suggest that SL/GSL alterations in CRC might arise from multi-level metabolic reprogramming involving substrate availability, lipid transport, and Golgi function, rather than isolated enzyme changes. These dysregulated pathways highlight potential diagnostic biomarkers and therapeutic targets in CRC.



Targeting ELOVL1 impacts sphingolipid metabolism, reduces the aggressiveness of Triple-negative breast cancer and enhances the efficacy of immunotherapy

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Introduction: Triple-negative breast cancer (TNBC) is characterized by the absence of estrogen and progesterone receptors, as well as HER2, leaving only a few targeted therapeutic options. TNBC is highly aggressive and invasive, with over 35% of patients do not respond to standard chemo-immunotherapy (anti-PD-1 plus chemotherapy) due to an immunosuppressive tumor microenvironment (TME). This highlights the need for new therapeutic targets.

Results: Using high-resolution mass spectrometry on patient cohorts, our team demonstrated that TNBC tumors exhibit a sphingolipid metabolism reprogramming, favoring the production of C24:0 very long-chain sphingolipids (C24:0 VLC SL) compared to other breast cancer (BC) subtypes and healthy tissue. This C24:0 VLC SL signature positively correlates with an immunosuppressive signature in the TME, as revealed by flow cytometry and transcriptomic analyses. This immunosuppressive signature is characterized by tumor infiltration by Tregs and macrophages, as well as expression of immune checkpoints such as PD-1 and TIGIT. This C24:0 VLC SL signature could be explained by the higher expression of ELOVL1 in TNBC tumors compared to the other BC subtypes, as well as in the aggressive and immunoresistant Basal-Like Immunosuppressed TNBC subtype, as revealed by *in silico* analyses. Moreover, high ELOVL1 expression correlates with poor overall and disease-free survival. We demonstrated that, epigenetic (shRNA) and pharmacological (*ELOVL1-IN-1*) inhibition of ELOVL1, reduced C24:0 VLC SL, cell proliferation, motility and velocity in TNBC cell lines *in vitro*. *In vivo*, downregulation of ELOVL1 in TNBC tumor cells with shRNA significantly impaired tumor growth. Moreover, systemic pharmacological inhibition of ELOVL1 improved anti-PD-1 therapy efficacy by enhancing the recruitment of favorable tumor-infiltrating lymphocytes into the TME.

Conclusion: Targeting ELOVL1 thus represents a promising strategy for overcoming immuno-resistance and improving survival in patients with TNBC.

Sphingosine-1-phosphate orchestrates inflammation and fibrosis in benign uterine disorders

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Introduction: Benign uterine disorders, including endometriosis, adenomyosis and uterine fibroids, are among the most prevalent gynecological diseases affecting women of reproductive age and represent a major cause of pelvic pain, abnormal uterine bleeding, infertility and adverse pregnancy outcomes. Despite their high prevalence and clinical impact, their pathogenesis remains incompletely understood, and current treatments mainly rely on surgery and hormonal therapies, both limited by symptom recurrence and side effects. Although clinically distinct, these disorders share common pathogenic mechanisms, including chronic inflammation, fibrosis and aberrant tissue remodeling. Increasing evidence identifies the pleiotropic bioactive sphingolipid sphingosine-1-phosphate (S1P) as a key regulator of these processes.

Results: Our studies have demonstrated that the S1P signaling network is profoundly dysregulated across benign uterine disorders. In uterine fibroids, S1P promotes profibrotic signaling through interaction with activin A. In adenomyosis, remodeling of S1P metabolism and receptor expression is associated with the fibrotic phenotype. In endometriosis, S1P orchestrates inflammation, oxidative stress, epithelial-mesenchymal transition and fibrosis through receptor-specific signaling pathways. More recently, we identified S1P as a central mediator of immune-stromal communication, sustaining chronic inflammation and self-amplifying fibrotic circuits.

Conclusions: Collectively, our findings identify S1P signaling as a shared molecular hub linking inflammation, fibrosis and aberrant tissue remodeling across benign uterine disorders. These studies provide new mechanistic insights into disease pathophysiology and support the development of innovative biomarkers as well as non-hormonal therapeutic strategies targeting the S1P axis.



Resolving the expression–function paradox of sphingosine-1-phosphate lyase reveals a key regulator of metastasis in head and neck squamous cell carcinoma

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Introduction: Metastasis is the principal cause of mortality in head and neck squamous cell carcinoma (HNSCC), yet robust molecular predictors remain undefined. The bioactive lipid sphingosine-1-phosphate (S1P) drives tumor cell migration and invasion, positioning its degradation by sphingosine-1-phosphate lyase (SGPL1) as a central regulatory step. However, conflicting evidence regarding SGPL1 expression and clinical outcome indicates a fundamental gap in current biomarker concepts.

Results: We identify and resolve a critical expression–function paradox of SGPL1 in HNSCC. While transcriptomic datasets suggest that high SGPL1 expression correlates with improved metastasis-free survival, protein-level analyses fail to reproduce this association. We demonstrate that this discrepancy arises from isoform-specific differences and incomplete antibody detection, rendering expression-based assessments insufficient. Using a fluorescence-based activity sensor, we establish SGPL1 functionality as the decisive parameter governing tumor cell behavior. Reduced SGPL1 activity is associated with increased motility, altered adhesion dynamics, and enhanced clonogenicity. Functional restoration of SGPL1 reverses this phenotype and suppresses invasive capacity. Unbiased metabolomic profiling (LC-MS, GC-MS) further links SGPL1 activity to distinct sphingolipid signatures, establishing a direct functional link between SGPL1 activity and metastatic behavior.

Conclusions: Our findings redefine SGPL1 from a descriptive marker to a functional regulator of metastasis in HNSCC. Critically, they demonstrate that enzymatic activity, rather than expression alone, determines biological outcome. This work establishes SGPL1 functionality as a mechanistically grounded biomarker and defines sphingolipid metabolism as a targetable axis for anti-metastatic intervention.



S1P-induced $\beta 8$ integrin: a driver of primary melanoma progression?

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Introduction: Melanoma is the deadliest form of skin cancer due to its high capacity to metastasize. Despite therapeutic advances, the prognosis of patients with metastatic disease who do not respond to treatment remains uncertain. A major challenge is the identification of early biomarkers capable of predicting metastatic risk and guiding clinical decisions. The transition from primary to metastatic melanoma involves major phenotypic changes, including alterations in sphingolipid metabolism that lead to increased production of sphingosine-1-phosphate (S1P), a bioactive lipid that promotes tumor progression.

Results: We identified a positive association between a high intratumoral S1P production score and elevated expression of $\beta 8$ integrin (ITGB8), a key activator of latent transforming growth factor- β (LAP-TGF- β). Mechanistically, we demonstrated that S1P induces ITGB8 expression through S1PR2-dependent activation of the transcription factor c-JUN in multiple melanoma cell lines. In murine melanoma models, we previously showed that silencing sphingosine kinase 1 reduced TGF- β expression, fibroblast activation, and the intratumoral Treg/CD8⁺ T-cell ratio, highlighting the role of S1P signaling in shaping a pro-tumoral microenvironment.

We are currently investigating whether S1P-induced $\beta 8$ integrin promotes TGF- β activation and assessing the consequences of this pathway on fibroblast activation and immune regulation in vitro and in vivo.

Conclusion: Our findings support a novel role for the S1P-ITGB8 axis in melanoma progression. Elucidating how this pathway regulates TGF- β activation may reveal new biomarkers of metastatic risk and identify actionable therapeutic targets to limit tumor progression and immunosuppression.



Sphingosine-1-phosphate-induced relaxation of human coronary arteries and its impairment in ischemic heart disease

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Introduction: Sphingosine-1-phosphate (S1P) has been implicated in multiple cardiovascular functions; however, its vasoactive effects in human coronary arteries remain poorly understood. We aimed to investigate the role of S1P in human coronary vasomotor regulation and its alteration in ischemic heart disease (IHD).

Segments of the left anterior descending coronary artery were isolated from explanted hearts of patients undergoing heart transplantation due to or without IHD. S1P- and bradykinin (BK)-induced changes in vascular tone were assessed with wire myography after precontraction. In some experiments pharmacological inhibition of S1PR1, S1PR3, nitric oxide synthase (NOS), or endothelial denudation was applied. Immunohistochemistry was performed on coronary sections using antibodies against S1PR1, S1PR3, and the endothelial marker CD31 to determine receptor localization. **Results:** Coronary arteries of non-IHD patients showed marked vasorelaxation in response to S1P, which was similar to the effect of the endothelium-dependent vasodilator BK. Immunostaining revealed the expression of S1PR1 and S1PR3 predominantly in the endothelium. S1P-induced relaxation was abolished by endothelial removal, NOS inhibition, and selective S1PR1 blockade, while S1PR3 inhibition had no significant effect, identifying endothelial S1PR1-mediated NO release as the main signaling pathway. Interestingly, S1P failed to induce vasorelaxation in arteries from IHD patients, whereas the effect of BK was not altered, suggesting a selective impairment of S1P signaling rather than generalized endothelial dysfunction. **Conclusions:** These findings provide the first direct evidence that S1P relaxes human coronary arteries via an endothelial S1PR1–NOS-dependent mechanism, which is significantly impaired in IHD and may contribute to coronary vascular dysfunction.

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The adaptive role of sphingosine 1-phosphate in red blood cells to hypoxic conditions in respiratory diseases

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Introduction: Respiratory diseases contribute to more than 8 million deaths annually worldwide. Hypoxia is a common feature of severe respiratory diseases. The major cause is insufficient oxygenation of red blood cells (RBC) due to damaged lungs. RBC are characterized by comparably high micromolar concentrations of sphingosine 1-phosphate (S1P), which can bind to and stabilize the tense (T-)state of hemoglobin. As a result, the oxygen-hemoglobin dissociation curve is shifted to the right. This right shift creates a lower oxygen affinity of hemoglobin. The functional consequence is improved oxygen supply in tissues and aggravated oxygen uptake in the lung. In order to test the relevance of this mechanism in respiratory diseases, we investigated S1P-driven adaptive responses in RBC derived from acute pneumonia, chronic obstructive pulmonary disease (COPD) and long COVID patients.

Results: While long COVID patients showed no obvious alterations in the production of 2,3-bisphosphoglycerate (2,3-BPG), pneumonia patients were characterized by increased 2,3-BPG concentrations, which correlated with increased sphingosine kinase activity. 2,3-BPG stabilizes the T-state of hemoglobin and induces a right-shift of the oxygen-hemoglobin dissociation curve. As a result, oxygen uptake of hemoglobin was dampened. While total S1P levels in RBC were not significantly altered, pneumonia and long COVID patients demonstrated lower expression of the S1P degrading enzyme S1P-lyase and decreased ceramide levels in RBC, suggesting a shift towards S1P production. In addition, flow induced dispersion analysis (FIDA) indicated a decreased hydrodynamic radius of hemoglobin in the presence of S1P and 2,3-BPG, which pointed to the stabilization of the T-state of hemoglobin.

Conclusion: Respiratory diseases are characterized by adaptive responses of S1P signaling in RBC which lead to altered oxygen supply under hypoxic conditions.



T28

Sphingosine 1-phosphate bound to HDL promotes of cerebrovascular health and cognitive functions

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Sphingosine 1-phosphate (S1P), a circulating lipid that is bound to HDL via Apolipoprotein M (ApoM) signals via vascular endothelial cell (EC) G protein-coupled S1P receptors. This signaling axis promotes systemic vascular barrier function, blood flow and suppresses EC injury and inflammation. The central nervous system (CNS) endothelium is endowed with specialized barrier (AKA blood brain barrier; BBB) and unique transcriptional program which allows optimal support of the brain and spinal cord. We show that during vertebrate aging, reduced plasma ApoM, decreased signaling of the CNS endothelium contributes to BBB defects. We also provide evidence to suggest that this pathway is therapeutically tractable with engineered S1P chaperones derived from ApoM. Therapeutic administration of stable, engineered ApoM protein leads to correction of CNS endothelial defects, and improved spatial memory in aged mice. Axonal structure is improved in the hippocampal neurons after this therapeutic rescue. Transcriptomic profiling studies suggest improved lysosomal function in hippocampal neurons.

We propose that rejuvenation of the aged CNS endothelium improves neuronal function and cognition.

Molecular pharmacology of blocking sphingosine-1-phosphate transport

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Sphingosine 1-phosphate (S1P) is a bioactive lipid that regulates the growth, survival, and migration of several cell types. Targeting the S1P pathway has led to the development of S1P1 receptor modulators (SRMs). S1P that binds to cell-surface lymphocyte S1P receptors is supplied by the transporters Mfsd2b and Spns2. Mice lacking spinster homolog 2 (Spns2) are lymphopenic and exhibit low lymph S1P concentrations. Here, we report the development of S1P transport blockers (STBs) and examine whether this genetic phenotype can be recapitulated using small molecules.

Profiling of our lead STB, SLF80821178 ($IC_{50} = 50$ nM), showed efficacy in a multiple sclerosis model; however, unlike the SRM fingolimod, it neither decreased heart rate nor compromised lung endothelial barrier function. Notably, while Spns2-null mice display a sensorineural hearing defect, mice chronically treated with SLF80821178 maintained normal hearing acuity. STBs such as SLF80821178 produce a dose-dependent decrease in peripheral blood lymphocyte counts; however, in contrast to findings in Spns2-deficient mice, lymph S1P concentrations were not significantly altered at doses producing maximal lymphopenia. Finally, cryo-EM characterization of SLF80821178 bound to Spns2 revealed binding within the S1P channel, with key amino acid interactions key to S1P transport blockade.



Photoactivatable sphingosine reveals distinct target profiles in SphK1/2-overexpressing cells and uncovers HN1 and HN1L as regulators of sphingosine-induced Ca^{2+} signaling

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Introduction: Sphingosine-1-phosphate (S1P) is a well-established extracellular signaling molecule, while intracellular targets of S1P and its precursor sphingosine remain poorly characterized. Photoactivatable and clickable (pac-) sphingosine has been developed for identification of sphingolipid-binding proteins. Here, we optimized the conversion of pac-sphingosine to pac-S1P by overexpressing sphingosine kinase (SphK)-1 or -2 in HEK-293 cells and analyzed the target profiles by proteomics.

Results: HEK/SphK1 and HEK/SphK2 cells were incubated with 10 μM pac-sphingosine for 10 min in the presence of a ceramide synthase inhibitor. Thereby, pac-S1P formation was increased by ~8-fold compared with vector-transfected cells. Proteomic analyses of UV-irradiated pac-sphingosine-treated cells identified 95 specifically enriched proteins (fold change >2) in HEK/SphK1 cells and 156 proteins in HEK/SphK2 cells. Only 18 proteins were found in both datasets, revealing distinct signaling networks of SphK1 and SphK2. To assess the relevance of these findings in a physiological cellular context, parallel experiments were performed in untransfected human umbilical vein endothelial cells (HUVECs), with 351 enriched proteins. Comparison with HEK/SphK1 and HEK/SphK2 showed only limited overlap (16 and 5 proteins, respectively). Among the identified candidates, HN1L, a known mediator of NAADP-induced Ca^{2+} release, was enriched ~3-fold in HEK/SphK2 cells. Moreover, the related protein HN1 was enriched ~6-fold in HEK/SphK1 cells and ~15-fold in HUVECs. Functional studies demonstrated that overexpression of HN1 or HN1L significantly enhanced sphingosine-induced $[\text{Ca}^{2+}]_i$ increases in HEK-293 cells.

Conclusions: Our results identify HN1 and HN1L as novel regulators of sphingosine-dependent Ca^{2+} signaling and provide new insights into the cellular interactomes of sphingosine and S1P.



Beyond the brain: sphingosine 1-phosphate dysregulation and skeletal muscle impairment in Huntington's disease

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Introduction: Huntington's disease (HD) is increasingly recognized as a multisystem disorder in which skeletal muscle impairment substantially contributes to disease progression and reduced quality of life. Although alterations of sphingosine 1-phosphate (S1P) metabolism have been extensively documented in the HD brain, whether dysregulation of this signaling pathway also contributes to HD skeletal muscle dysfunction remains unknown.

Results: Here, we demonstrate that skeletal muscle from R6/2 and zQ175 HD mouse models exhibits profound alterations in S1P metabolism and signaling, including reduced expression of ceramide synthase 1 (CerS1), sphingosine kinase 1 (SK1), spinster homolog 2 (Spns2) and the S1P receptor S1P₂. These molecular alterations were associated with impaired expression of the myogenic differentiation markers, such as, myogenin reduction, abnormal caveolin-3 accumulation, with altered autophagy-related signaling, and with dysregulated AKT, JNK, ERK1/2 and p38 MAPK activation, supporting the existence of defective muscle homeostasis in HD. Pharmacological modulation of S1P signaling partially or completely reversed these abnormalities. While both the SK1 activator K6PC-5 and the S1P lyase inhibitor 2-acetyl-4(5)-tetrahydroxybutyl imidazole (THI) restored myogenin expression, only K6PC-5 normalized S1P₂, caveolin-3, LC3-II levels and stress-related signaling pathway activation, indicating that direct SK1 activation provides broader biological benefits than inhibition of S1P degradation alone.

Conclusions: These findings identify defective S1P signaling as a previously unrecognized mechanism contributing to skeletal muscle pathology in HD and establish SK1 activation as a promising strategy to restore muscle homeostasis. Together with the previously demonstrated neuroprotective and peripheral effects of K6PC-5, our results further support pharmacological activation of SK1 as a systemic therapeutic approach capable of targeting both central and peripheral manifestations of Huntington's disease.



Modelling genetic defects in sphingolipid biosynthesis using CRISPR-Cas9-edited HEK-293T cells

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Sphingolipid metabolism is essential for membrane integrity, signaling, and neurodevelopment. Inherited defects in this pathway cause severe neurological disorders. HEK-293T cells engineered by CRISPR-Cas9 editing represent a model to dissect the molecular consequences of enzymatic blocks in ceramide and glycosphingolipid synthesis. In this framework, we engineered HEK-293T cells to introduce patient-derived mutations identified in *DEGS1*, *B4GALT5*, and *ST3GAL3*, thereby generating faithful disease-relevant models of biosynthetic sphingolipid disorders. CRISPR-Cas9 disruption of *DEGS1* is expected to impair the conversion of dihydroceramide to ceramide, leading to substrate accumulation and altered ceramide pool. This defect is predicted to affect downstream sphingolipid homeostasis and membrane-associated signaling. Loss of *B4GALT5* interrupts galactose transfer to glucosylceramide-derived intermediates, limiting lactosylceramide formation and the synthesis of more complex glycosphingolipids. Accordingly, this block should produce accumulation of upstream species and depletion of downstream neutral glycosphingolipids. Deficiency of *ST3GAL3* compromises sialylation of different glycosphingolipids acceptors, reducing ganglioside biosynthesis (e.g. sLC4 and GM1b) and reshaping the sialylated sphingolipid landscape. Together, these models provide a controlled system to define enzyme-specific lipid signatures and to distinguish primary metabolic effects from compensatory remodeling. The HEK-293T CRISPR-Cas9 platform is a powerful tool to model biosynthetic sphingolipid disorders with high mechanistic precision. Parallel interrogation of *DEGS1*, *B4GALT5*, and *ST3GAL3* deficiency allows direct comparison of defects affecting ceramide formation, oligosaccharide chain elongation, and terminal sialylation. This approach is particularly valuable for defining the metabolic alterations underlying inherited sphingolipid diseases and for establishing the biochemical basis of their pathogenic process.



Probing the ganglioside interactome using synthetic bifunctional gangliosides

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Gangliosides, sialic acid bearing glycosphingolipids, are expressed on the outer leaflet of plasma membranes of all vertebrate cells. They function in regulatory pathways involved in cell-cell adhesion, signal transduction, and ion transport. Ganglioside expression and dysregulation are linked to human diseases including neurodegeneration, diabetes, and cancer.

Physiological functions of gangliosides often result from their binding to complementary binding proteins in one of two modes – *cis* and *trans*. In *cis*, gangliosides bind to transmembrane proteins in the same membrane leaflet to regulate protein signaling. In *trans*, they bind to proteins on other cells or in the extracellular milieu. The goal of this project was to synthesize chemical probes to identify the entire “ganglioside interactome” – both *cis* and *trans*.

To capture both *cis* and *trans* interactions, we chemically installed bifunctional probes in the ceramide or on the glycan of selected gangliosides. Probes carried a diazirine for photoaffinity covalent capture of adjacent proteins, and an alkyne for click chemistry. An 18-atom linear probe was installed in place of the fatty acid amide of ceramide to mimic its structure. A 7-atom probe was installed on the ganglioside sialic acid or galactose to minimally disrupt glycan structures. Major human brain and peripheral tissue gangliosides were chosen as parent molecules.

The ganglioside probes were complexed with methyl- β -cyclodextrin to deliver them to the outer leaflet of the plasma membrane of live human cells in culture. Photoactivation, cell solubilization, and click-chemistry biotinylation allowed us to capture ganglioside-associated proteins, which were identified by proteomic mass spectrometry. Ceramide-based probes selectively captured integral plasma membrane proteins, whereas glycan-based probes captured other cell surface proteins. Hundreds of proteins were identified as high-probability ganglioside binding proteins, including ion channels, receptor tyrosine kinases, Wnt receptors, and cell adhesion molecules (integrins, cadherins, laminins). These included proteins previously known to be regulated by gangliosides and others unanticipated by prior data.



Glycosphingolipid engineering in pursuit of ganglioside-protein interactions

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Gangliosides interact with a repertoire of proteins, recruiting ligands within the same membrane leaflet or across opposing membranes, positioning them as key molecular players in immunity, signal transduction and neurotransmission. Being highly enriched in mammalian brain, understanding their neurochemical roles has become a central pursuit in glycobiology. To contribute to this endeavor, our group employs several approaches aimed at characterizing ganglioside-protein interactions and elucidating the functional roles gangliosides exert on specific neuronal proteins.

Our efforts build on data obtained from transgenic mouse models lacking GM2/GD2 synthase or GD3 synthase. However, since mouse models do not represent an entirely accurate phenocopy of the human diseases arising from the same mutations, we are currently focusing our efforts on cellular models by implementing both human and murine neuroblastoma cell lines, which allows for the comparison of key molecular differences in ganglioside effects between them, and, potentially identifies results that can be translated to patients. We modified the cell lines using CRISPR/Cas9 technology to knock out *ST3GAL5/St3gal5* coding for GM3 synthase and *ST8SIA1/St8sia1* coding for GD3 synthase. These functional knock-outs are used to investigate the expression and function of selected proteins, such as sodium/potassium ATPase, but also to determine specific ganglioside interactors by proximity ligation assays. Furthermore, to complement these findings, we are developing a copper-free click chemistry-based system of biorthogonal ganglioside synthesis, allowing real-time monitoring of ganglioside membrane incorporation, while minimizing the interference of the fluorophore with native ganglioside-protein interactions present in fluorescent cell imaging. Our ultimate goal is to obtain high-resolution *in vitro* data on the physiological interactions between gangliosides and their protein partners which could serve as a strong foundation for developing new strategies for managing disorders linked to ganglioside metabolism.



Multimodal MRI reveals structural and microstructural brain alterations in GD3 synthase-deficient mice

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Gangliosides, sialylated membrane glycosphingolipids, are expressed with remarkable abundance and diversity in the mammalian central nervous system (CNS). Many of the cellular and physiological roles of gangliosides and their involvement in neurodevelopment and neurodegeneration have been revealed by studying phenotypes of mouse models with genetically modified ganglioside synthesis. Since different classes of lipids show distinct magnetic resonance (MR) signals, brain imaging techniques may provide significant clues on how glycolipidomic signatures of different cell types and brain areas relate to brain structure and function. Here, we investigated the association of specific molecular composition of membranes in brain of GD3 synthase deficient mice (*St8sia1 null*), lacking b- and c-series and overexpressing a-series of gangliosides, with changes in brain morphology, by using *ex vivo* magnetic resonance imaging. Regional brain volume, diffusion tensor imaging (DTI) metrics, and T1 signal intensity were analyzed in 10 *St8sia1 null* and 9 wild-type male mice, aged 5-6 months. The MRI alterations in *St8sia1 null* vs. wild-type brains were most prominent in brain regions responsible for sensory processing, motor control, learning and memory. Additionally, in order to relate distinct *St8sia1 null* MRI phenotypes to gene expression profiles in different cell types, we performed spatial transcriptomic and cell-type enrichment analysis by compiling our and the Allen Brain Atlas data, and identified a strong association with cortical and cerebellar astrocytes, Bergmann glia and oligodendrocytes. The findings identify the structural correlates of major neurobehavioral phenotypic features of *St8sia1 null* mice and bring a novel perspective on gangliosides as essential modulators of brain microarchitecture, structure and function.



Role of gangliosides in extracellular vesicle secretion and fate: implications for misfolded protein diseases

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Gangliosides are glycosphingolipids highly enriched in the brain. They play important modulatory functions in cell signaling and cell to cell communication. The importance of gangliosides in the brain is underscored by the fact that defects in the biosynthesis of gangliosides lead to early onset neurodegenerative diseases. Changes in ganglioside levels occur in Parkinson's disease (PD), Huntington's disease (HD) and Alzheimer's disease (AD), but their relevance to disease pathogenesis and progression remains unclear.

Restoration of ganglioside levels in cell and animal models of HD through administration of GM1, a major brain ganglioside, slows down neurodegeneration and corrects all disease symptoms. I will discuss a novel role of gangliosides in the biogenesis of extracellular vesicles (EVs) and in the secretion and clearance of pathogenic misfolded proteins via EVs, which might contribute to the neuroprotective effects of GM1 and other gangliosides. Our data indicate that complex gangliosides, exogenously administered or endogenously synthesized, increase the production of EVs carrying misfolded proteins and promote their uptake and clearance by microglia, thus attenuating proteotoxic stress in vulnerable neurons.

The endogenous sphingolipid sulfatide affects inflammation via multiple receptors – TLR4/MD-2, TLR2 and the Inflammasome

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Introduction: Pattern Recognition Receptors (PRRs) sense pathogen-associated molecular pattern (PAMP) molecules and initiate inflammation. Lipopolysaccharide (LPS), derived from Gram-negative bacteria, is sensed by the cell-surface receptor toll-like receptor 4 (TLR4) and the cytosolic receptor caspase-11/4/5, also known as the non-canonical inflammasome. Lipopeptides, derived from Gram-positive bacteria, are sensed by TLR2. These receptors are also thought to recognize endogenous damage-associated molecular pattern (DAMP) molecules released from stressed or dying cells, leading to autoinflammation and autoimmunity. Yet, the identity of such endogenous ligands is still poorly understood. We reported that the endogenous lipid C16-sulfatide (3-O-sulfogalactosylceramide) mimics LPS by binding to TLR4/MD-2, leading to its inhibition in human macrophages (Su *et al.* PNAS 2021). In the current research, we examined the activity of sulfatides towards TLR2 and towards the non-canonical inflammasome.

Results: Extracellular sulfatides competitively inhibit the activity of TLR4/MD-2 and TLR2 agonists in human macrophages. C16-sulfatide is an order of magnitude more potent towards TLR2 relative to TLR4. Structural studies indicate that sulfatides act as antagonists of these two receptors by mimicking their PAMPs. While TLR4/MD-2 senses only short fatty acid (FA) chain sulfatides, TLR2 is sensitive to a wide range of native sulfatide variants, and in particular to long FA chain sulfatides.

Native extracellular sulfatides, in particular with long FA chain, also inhibit inflammasome triggering by cytosolic LPS, in a non-competitive manner. In contrast, cell-delivered sulfatides, in particular with short FA chain, activate the canonical inflammasome via caspase-11, as well as independently.

Conclusions: Sulfatides, native glycosphingolipids in mammals, modulate inflammation in a context-dependent manner. Extracellular sulfatides inhibit TLR4, TLR2 and the non-canonical inflammasome, while intracellular sulfatides trigger the inflammasome.



The sphingolipid-metabolizing enzyme β -galactosylceramidase promotes melanoma growth by activation of a tumor cell pro-inflammatory signaling

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Metastatic melanoma is the most lethal form of skin cancer. Despite recent advances, the biological mechanisms driving melanoma progression remain unclear. Sphingolipids are key regulators of cancer-related processes and play pivotal roles in tumor progression. In this context, expression of the enzyme β -galactosylceramidase (GALC) progressively increases from benign nevi to metastatic melanoma, suggesting a pro-oncogenic role. Functional studies have shown that GALC promotes melanoma cell proliferation, migration, tumorigenic and metastatic potential. However, the molecular mechanisms underlying its pro-tumorigenic activity remain unclear. Here, we performed an untargeted lipidomic analysis of GALC-overexpressing (upGALC) melanoma cells, that revealed extensive lipid remodeling, characterized by increased levels of all sphingolipid classes and only minor changes in free cholesterol content. These alterations modified the Cho/PL, Cho/SM, and Cho/saturated SM ratios, increased unsaturated PE levels, and altered plasma membrane and lipid raft dynamics, as confirmed by FRAP analysis and PDA staining. These changes were associated with enhanced intracellular signaling with activation of the PLA2/COX2/NF- κ B axis. Furthermore, gene expression profiling revealed significant enrichment of genes involved in inflammatory response, cytokine–receptor interaction, and NF- κ B signaling pathways. The enhanced pro-inflammatory phenotype of upGALC cells was confirmed *in vitro* and in mouse and zebrafish xenograft models. Finally, pharmacological inhibition of the GALC-induced inflammatory phenotype using NSAIDs, both *in vitro* and *in vivo*, demonstrated that inflammation is a key mediator of the pro-tumorigenic activity of GALC in melanoma cells. Overall, our findings indicate that GALC promotes plasma membrane remodeling, leading to the establishment of a pro-inflammatory phenotype that supports melanoma progression. These findings confirm a non-redundant role of GALC in melanoma progression and suggest that targeting GALC-dependent inflammatory signaling may represent a promising therapeutic strategy for melanoma.



The glycolipid transfer protein GLTP as a modulator of COPII vesicle export from the ER and of cellular GSL homeostasis

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The glycolipid transfer protein (GLTP) is linked to a multitude of cellular processes aside from its best-known function as a lipid transport protein. Based on previous research and hypotheses, it is proposed that it may also act as a sensor and regulator of glycolipid homeostasis in the cell. Furthermore, through its previously determined interaction with the ER membrane protein VAP-A (vesicle-associated membrane protein-associated protein A), GLTP may also be involved in facilitating vesicular transport in the cell. Here, we propose a role of GLTP in regulating the transfer of ceramide from the ER to the Golgi apparatus. We propose that the effect of GLTP expression levels on the glycolipid homeostasis is related to the observed effect of GLTP on vesicle trafficking; ceramide for GSL synthesis is likely primarily transferred from the ER to the Golgi through vesicular transport, and the observed effects of GLTP fit this hypothesis. The restriction of GLTP/VAP-interaction by GLTP-GSL binding, through a conformational change in the FFAT-like motif of GLTP occurring as a result of GSL binding, likely functions as the regulatory mechanism. *In vivo*, the regulatory mechanism functions through a feedback loop, where increased cellular levels of GSLs would lead to lower GLTP/VAP-interaction, decreasing ceramide transfer to the *cis*-Golgi and decreasing GSL synthesis, and vice versa.



Selective impact of glycosphingolipids on melanoma progression and response to immune checkpoint inhibitors

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Cutaneous melanoma is the deadliest form of skin cancer. Various mutations are involved in melanoma development, including oncogenic mutations in the *BRAF* and *NRAS* genes. The prognosis for patients with advanced melanoma has improved with the use of immune checkpoint inhibitors (ICI), such as anti-CTLA-4 and anti-PD-1. However, more than 50% of patients do not respond to treatment or experience a relapse. The resistance mechanisms are partially characterized and include alterations in the sphingolipid metabolism in tumors. Interestingly, my team identified a significant enrichment of glycosphingolipids in melanoma cells, as well as in the plasma of advanced melanoma patients who did not respond to ICI treatment. Glucosylceramide synthase (GCS, encoded by the *Ugcg* gene), which synthesizes the precursor of most glycosphingolipids, has been shown to promote tumor progression and resistance to ICI therapy in various solid cancer models. We then aimed to evaluate the impact of targeting GCS by either knocking out the *Ugcg* gene in cells, or by inhibiting GCS activity pharmacologically, using different mouse melanoma models. Our results showed that targeting GCS reduced glycosphingolipid production, while increasing intracellular levels of ceramide and sphingomyelin. Targeting GCS did not affect cell proliferation *in vitro*, except for the 1014 *Nras*-mutated melanoma cell line, which exhibited reduced proliferation under serum-starvation conditions. In mice, GCS inhibition had no significant impact on three B16 melanoma models regardless of their sensitivity to ICI. However, it did sensitize the *Nras*-mutated and poorly immunogenic 1014 melanoma model to ICI, a phenomenon associated with enhanced T cell-dependent immune responses. Overall, our results suggest that targeting GCS has a limited impact on melanoma growth and response to ICI in B16 (*Nras* WT) models. However, it may partially overcome resistance to ICI in an *Nras*-mutated melanoma model.

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From enzyme dysfunction to altered sialylation: defining the biochemical signature of ST3GAL3-CDG

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ST3GAL3 is a sialyltransferase mediating transfer of sialic acid to terminal galactose residues in glycoproteins and glycosphingolipids. Biallelic loss-of-function variants in ST3GAL3 have been associated with a rare neurodevelopmental disorder mainly characterized by epileptic encephalopathy, developmental delay and intellectual disability, and proposed as a novel congenital disorder of glycosylation (CDG). Although implicated in the biosynthesis of sialylated glycoconjugates, ST3GAL3 physiological substrates and disease pathogenesis mechanisms remain incompletely defined. Biochemical characterization of pathogenic ST3GAL3 variants was performed in HEK-293-T cells following transient overexpression, with enzymatic activity quantified by LC-MS/MS using both artificial and physiological substrates. All variants showed complete loss of catalytic activity, except p.A13D retaining residual function, confirming loss of enzyme activity as the primary pathogenic mechanism. To assess disease-associated alterations in patient-derived material, quantitative glycosphingolipid profiling was performed on plasma and fibroblasts from individuals carrying pathogenic ST3GAL3 variants. Plasma showed only subtle changes, whereas fibroblasts exhibited reduced sialyl-lactotetraosylceramide (sLc4) with concomitant accumulation of its precursor lactotetraosylceramide (Lc4), consistent with impaired sialylation of lacto-series glycosphingolipids. Additionally, an ion mobility-enabled LC-MS workflow is being implemented to improve separation of isomeric glycosphingolipid species and refine characterization of putative ST3GAL3-dependent products. In parallel, preliminary glycoproteomic analyses on plasma suggest possible alterations in additional glycoconjugates, warranting further investigation into the broader impact of ST3GAL3 deficiency on cellular sialylation. These findings support ST3GAL3 deficiency as a putative CDG associated with defective sialylation. Patient-derived fibroblasts represent a sensitive matrix for biochemical characterization, while plasma profiling may support future biomarker development and diagnostic screening.



The crucial role of sphingolipid glycan code: the GM1 oligosaccharide

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Gangliosides are essential components of neuronal membranes, where their oligosaccharide moieties regulate membrane signaling and protein interactions. Among them, GM1 plays a pivotal role in maintaining neuronal function. During aging, GM1 levels decline, disrupting key signaling pathways and contributing to the onset and progression of neurodegenerative conditions. Although GM1 replacement therapy has shown therapeutic potential, the amphiphilic nature limits its clinical application. Recently we have identified the GM1 oligosaccharide (GM1-OS) as the key bioactive component responsible for GM1's neuroprotective effects. Unlike the parental ganglioside, GM1-OS is water-soluble and efficiently penetrates the central nervous system. Through multidisciplinary research, we demonstrated that GM1-OS can restore multiple neuronal functions by targeting key pathological mechanisms such as inflammation, mitochondrial dysfunction, excitotoxicity, and protein aggregation—hallmarks of neurological diseases like Parkinson's and Amyotrophic Lateral Sclerosis.

By retaining the therapeutic benefits of GM1 without its delivery limitations, GM1-OS emerges as a strong candidate for disease-modifying therapy in neurodegenerative disorders. Its multifunctional action positions it as a promising agent for future clinical development.



Ion mobility mass spectrometry: a new dimension in brain ganglioside research

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Introduction: Ion mobility mass spectrometry (IMS-MS) extends conventional MS by separating ions according to their size, shape, and charge. This additional separation enables the characterization of complex and isomeric lipids that are difficult to resolve by mass alone. By combining high sensitivity with enhanced structural resolution, IMS-MS provides a powerful approach for mapping ganglioside diversity and revealing molecular changes associated with brain diseases.

Results: In the past few years, our group made several pioneering contributions to the application of IMS-MS and tandem MS to the analysis of brain gangliosides. We developed workflows capable of separating and characterizing isomeric complex gangliosides directly from brain extracts. A major achievement is the differentiation of gangliosides sharing the same nominal mass but differing in glycan branching, sialylation pattern, or ceramide composition. Using collision cross section measurements together with high-resolution MS/MS, we succeeded in establishing structural fingerprints for several brain ganglioside classes. In disease-related studies, we identified specific alterations in ganglioside profiles associated with the pathological brain condition, including changes in the expression of species, their glycan and ceramide composition and unsaturation. Our IMS-MS approaches allowed the detection of low-abundance structures that were not resolved by conventional MS. We also demonstrated that IMS-MS improves the identification of biomarkers by reducing spectral overlap in complex mixtures.

Conclusions: The results revealed that subtle structural changes in brain gangliosides can be detected in both physiological and pathological conditions, establishing IMS-MS as a powerful tool in neuro-glycolipidomics. IMS-MS also facilitated the discovery of numerous previously unreported species and candidate biomarkers associated with cancer and neurological disorders.



Sphingolipid imbalance as a novel therapeutic target in Rett syndrome: a drug repositioning approach

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Rett syndrome (RTT, OMIM 312750) is a rare neurodevelopmental disorder primarily affecting girls, characterized by motor and cognitive impairment, caused by mutations in the X-linked *MECP2* gene, which encodes an epigenetic factor crucial for brain function. RTT pathogenesis remains poorly understood, and no definitive cure is currently available. Our previous work, performed in the brain of *Mecp2*^{-/-} male and *Mecp2*^{+/-} female mice, in murine neurons carrying the common RTT hotspot mutation MeCP2^{R306C}, and in patient-specific MeCP2^{R255X} and MeCP2^{G252Rfs7ter} iPSC-derived neurons, revealed altered levels of several sphingolipids (SLs) and aberrant expression of multiple genes involved in SL metabolism (sphingo-genes). These data uncover a previously unrecognized role of MeCP2 in SL homeostasis, support the involvement of altered SL metabolism in RTT, and provide a rationale for exploring SL-targeted therapeutic strategies to improve disease outcomes. Treatment of *Mecp2*^{-/-} mice with Ozanimod (RPC-1063), a next generation sphingosine-1-phosphate receptor modulator able to cross the blood-brain barrier and approved for the treatment of other neurological disorders, prolonged the lifespan, reduced RTT phenotypic score, and prevented body weight loss. Furthermore, Ozanimod restored the expression of the neurotrophic factors BDNF and IGF-1, positively affected ERK1/2 signaling, and normalized the expression of multiple sphingo-genes in the *Mecp2*^{-/-} brain. Moreover, we confirmed its beneficial effects on altered neuronal morphology and sphingo-gene dysregulation in patient-specific iPSC-derived neurons. The effect of Ozanimod on aberrant SL levels in the different RTT models is under investigation. We are also assessing SL metabolism dysregulation and the efficacy of Ozanimod treatment in *Mecp2*^{R306C} mice, an *in vivo* model genetically closer to RTT patients.

Collectively, our data support the potential of Ozanimod as a novel therapeutic strategy to restore SL homeostasis and improve RTT-related phenotypes.



Sphingolipid control of brain serotonergic signaling and its consequences for behavior and mental disorders

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Sphingolipids shape structure and function of neuronal synapses in a dynamic way and may, with this function, be at a nodal point for behavioral control and plasticity. Disruption of the sphingolipid system may, therefore, translate into aberrant neurotransmitter signaling and mental disorders. In a second step, humans frequently use alcohol to self-manage disadvantageous mental states. A crucial system for control of virtually all behavior is the serotonergic (5-HT) system. We have investigated the role of neutral sphingomyelinase (NSM) in serotonergic neurons of the brain in emotional behavior and alcohol consumption in a sex-dependent way. In order to address this question, we created mice with an inducible NSM deletion in serotonergic neurons at adult age (NSM^{flox/flox} Tph2-iCreER). Behavioral characterization showed a sex-specific role of NSM in serotonergic neurons. Female NSM^{flox/flox} Tph2-iCreER mice showed significant reduction in depression-related behaviors, that was accompanied by a strong increase in 5-HT tissue level. This effect dissociated from cognitive performance, in that recognition- and short-term memory was not changed. Male NSM^{flox/flox} Tph2-iCreER mice, in contrast, did not show changes in the emotional phenotype, but improved recognition- and short-term memory. This was not accompanied by altered 5-HT tissue levels. The positive emotional phenotype in females resulted in a significantly lower alcohol consumption in single housing- as well as social housing free choice tests, which was not observed in males. Altogether, these findings show a largely sex-specific role of the NSM in one of the most important neuromodulatory systems of the brain, controlling emotional behavior and subsequent alcohol consumption. This may further suggest to target NSM function in a pharmacotherapy of depression and/or alcohol use disorder, in a personalized, sex-specific way.



Role of sphingolipids in fractalkine mediated myelin repair

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Remyelination is a complex event that requires the orchestrated intervention of various cell types present in the lesion niche. Recruitment of oligodendrocyte precursor cells (OPC) to the injured site, while crucial for remyelination, sometimes fails, thus facilitating this process and stimulating OPC maturation into myelinating oligodendrocytes (OL) could prevent disease progression. Fractalkine (FKN) is able to promote remyelination acting both on OPC and microglia, enhancing the proliferation and the differentiation of the former.

Analysis of the sphingolipid content of OPC, differentiating OL and BV2 microglial cells treated with FKN revealed that while the treatment does not affect the phospholipid content, sphingolipid composition is altered. In particular, total levels of ceramide, a sphingolipid that represents not only an important pro-apoptotic signal, but also a signal for the rearrangement of sphingolipid- and cholesterol-rich signaling platforms, is significantly reduced in all cells treated with FKN. Moreover, treated BV2 cells also show reduced cholesterol levels, however cholesterol content in OPC and OL remains unchanged.

Protein levels of integrin receptors and kinases of the Src family in OPC and BV2 cells treated with FKN have also been analyzed. In BV2 cells, the treatment leads to a decrease in the levels of integrin receptor subunits αV and $\beta 3$, while no difference is observed for Src nor Lyn. On the other hand, FKN in OPC induces an increase in integrin αV and $\beta 3$. Moreover, while total levels of Lyn and Src kinases are reduced, there is an increase in their activation.

Altogether, these results support the notion that FKN signaling might be mediated by alterations of lipid-dependent membrane organization and/or signaling in different glial cells present in the lesion niche.



The two faces of glucosylceramide catabolism in neuronal life

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Glucosylceramide (GlcCer) is increasingly recognized as a central hub of glycosphingolipid metabolism and an important regulator of plasma membrane organization and function. In mammalian cells, GlcCer metabolism is tightly controlled by glycosyltransferases, trafficking proteins, and β -glucosylceramidases. Three β -glucosylceramidases have been identified to date: the lysosomal β -glucocerebrosidase (GCase/GBA1), the cytosolic β -glucosidase (GBA3), whose physiological role in GlcCer metabolism remains controversial, and the non-lysosomal β -glucosylceramidase (NLGase/GBA2), a plasma membrane-associated enzyme. The biological functions of these enzymes critically depend on their subcellular localization, expression, and their ability to generate ceramide within specific cellular compartments. We demonstrated that the aberrant localization of GCase and NLGase at the plasma membrane results in the generation of ectopic ceramide, leading to impaired cell proliferation in fibroblasts. In neurons, however, plasma membrane-associated ceramide production plays a dual role. During neuronal differentiation, the activity of NLGase progressively increase, leading to local ceramide generation. Remarkably, unlike other cell types, NLGase becomes the predominant β -glucosylceramidase activity in fully differentiated neurons and adult brain. Moreover, NLGase overexpression promotes neuronal differentiation, supporting a key role for this enzyme in neuronal maturation. Conversely, in senescent neurons, a marked increase in plasma membrane-associated GCase activity is observed, suggesting that alterations in the spatial regulation of ceramide production shift its biological function from a pro-differentiation signal to a pro-senescent one. Interestingly, GCase exerts a dual effect on neuronal physiology. Its aberrant accumulation at the plasma membrane promotes neuronal senescence through excessive local ceramide production. Conversely, GCase loss of function also leads to neuronal dysfunction, although through a distinct mechanism. GCase deficiency is now recognized as one of the major genetic risk factors for Parkinson's disease and other neurodegenerative disorders. Beyond impairing lysosomal function, GCase deficiency induces profound alterations in plasma membrane lipid composition and organization as a consequence of aberrant lysosomal exocytosis. These membrane changes compromise membrane-associated signaling and ultimately promote neuronal dysfunction. Overall, our findings indicate that the biological outcome of GlcCer catabolism is determined not only by enzyme activity but also by its spatial regulation. Properly controlled plasma membrane-associated β -glucosylceramidase activity supports neuronal differentiation, whereas its dysregulation—either through aberrant plasma membrane localization or GCase loss of function—drives neuronal senescence and neurodegeneration.

Glutamate release by regulated exocytosis in human glioblastomas

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Introduction: Glioblastomas (GBMs) are the most common primary malignant brain tumors in adults. They are one of the deadliest cancers, having a median survival of 14 months despite of standard of care (surgery, radiotherapy, and chemotherapy with temozolomide). GBMs are characterized by extensive dispersal throughout the brain, indicative of their highly invasive nature. Identifying novel treatments that stop/attenuate the progression of GBMs will be life altering. We propose a novel hypothesis that human GBM invasiveness is promoted by regulated exocytotic secretion of glutamate (Glu), a transmitter known to stimulate GBM cell motility and invasion. GBM invasiveness can be stimulated by bradykinin (BK), a signaling molecule generated at the interface between the brain parenchyma and vasculature. BK activates B2 receptors (B2Rs). BK-evoked exocytosis was blocked in B2R high-expressing PDX GBM cells by icatibant and via transcriptional silencing of VAMP2. The effects were studied in both *ex vivo* and *in vivo* settings: A) Real-time visualization of human PDX GBM cell invasion into the normal brain parenchyma was examined in mouse acute slice preparations using laser scanning confocal microscopy. B) In mice bearing implanted PDXs, we will assess animal survival, tumor volume and GBM cells invasiveness.

Results: Our data demonstrate that B2Rs are abundantly expressed on a subset of human GBMs. Our data further reveal that B2R activation in GBM cells causes exocytotic/vesicular glutamate release from human GBM patient-derived xenolines/xenografts (PDX), the best pre-clinical model of GBM. Blocking this pathway increases survival time and decreases the GBM tumor progression *in vivo*. We posit that BK-evoked vesicular release of Glu could be a common mechanism of Glu release from GBMs and a therapeutic target to reduce invasiveness.

Conclusions: The data generated is highly relevant for the development of adjuvant treatments for people suffering from glioblastoma.



Role of hypothalamic de novo ceramides synthesis in resistin-induced hypothalamic inflammation and insulin resistance: impact on glucose homeostasis

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In the context of obesity, ectopic accumulation of lipids, particularly ceramides in the hypothalamus leads to lipotoxicity, a process that greatly contributes to the development of hypothalamic insulin resistance as well as the impairment of glucose homeostasis. Besides lipids, the hypothalamus is also subjected to changes in circulating factors originated from adipose tissue. Among these factors, the adipokine resistin is a key factor linking obesity with insulin resistance and type 2 diabetes. We have reported that resistin, through hypothalamic toll-like receptor 4 (TLR4), induces insulin resistance and promotes hypothalamic inflammation. The present study aimed to investigate the potential link between the de novo ceramide synthesis and resistin's effect in the hypothalamus.

We showed that resistin impaired insulin signaling in hypothalamic neuronal mHypoA cells and increased mRNA expression of inflammatory cytokine in mHypoA and microglial SIMA9 cells. Myriocin and TAK-242 restored insulin signaling in resistin-treated mHypoA cells and blunted the inflammatory response both in mHypoA and SIMA9 cells. Resistin promotes the enrichment of various ceramide species in mHypoA and SIMA9 cells in a myriocin-dependent manner. Chronic resistin ICV infusion reactive microgliosis as an inflammatory response in the hypothalamus. Interestingly, genetic blockade of hypothalamic de novo ceramide synthesis, through ICV injection of Ad-shSPT1, counteract resistin-induced hypothalamic microgliosis. Moreover, AdshSPT1 prevented central resistin-induced glucose intolerance. In liver, the blockade of hypothalamic de novo ceramide synthesis prevents central resistin-induced hepatic gene expression of the key gluconeogenic enzymes.

Taken together, hypothalamic de novo ceramide synthesis is a new regulatory pathway involved in central resistin-induced hypothalamic inflammation and glucose homeostasis dysregulation.



Glycosphingolipid dysregulation in Parkinson's disease

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GBA1 encodes glucocerebrosidase, a lysosomal enzyme responsible for the last step in glycosphingolipid (GSL) catabolism by hydrolyzing glucosylceramide into ceramide. Biallelic *GBA1* mutations cause Gaucher disease, while heterozygous variants represent the strongest known genetic risk factor for Parkinson's disease (PD). Although alterations in specific GSLs have been reported in *GBA*-associated PD (*GBA*-PD), the overall glycosphingolipid profile has not been fully defined, and it remains unclear how *GBA* mutations contribute to PD pathogenesis. To better investigate the role of *GBA* and glycosphingolipids in PD, we combined two highly sensitive approaches. Specifically, we quantified plasma glycosphingolipids using an HPLC-based method in 148 individuals (49 healthy controls, 51 idiopathic PD [iPD], and 48 *GBA*-PD) and performed RNA sequencing on postmortem PD brain region to investigate dysregulated genes and pathways linked to GSL metabolism.

Total glycosphingolipid levels were significantly increased in *GBA*-PD compared with healthy controls, whereas they are reduced in iPD. Sex-stratified analysis revealed that the increase in *GBA*-PD was significant only in females, while the decrease in iPD was significant only in males. Moreover, among *GBA1* variants, N370S seems to be the main driver of glycosphingolipid increase, with the strongest effect observed in females. Consistently, transcriptomic analysis identified dysregulation of multiple genes involved in glycosphingolipid pathways in PD brain tissue. Overall, our findings suggest that *GBA*-PD is characterized by a distinct, sex- and genotype-dependent glycosphingolipid signature. In parallel, the transcriptional dysregulation of genes involved in glycosphingolipid metabolism in PD brain tissue supports a broader role for altered GSL homeostasis in PD pathogenesis. Together, these findings highlight glycosphingolipid metabolism as a promising source of biomarkers and therapeutic targets.



Targeting sphingolipid metabolism rescues cognitive deficits and hippocampal integrity in Huntington's disease mouse models

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Introduction: Huntington's disease (HD) is a fatal neurodegenerative disorder characterized by progressive cognitive decline, for which no disease-modifying therapy is currently available. Altered sphingolipid metabolism is an early and consistent feature of HD and represents a promising therapeutic target. In this study, we investigated whether pharmacological inhibition of sphingosine-1-phosphate lyase (SGPL1) with THI could ameliorate cognitive dysfunction in two genetically distinct HD mouse models (R6/2 and zQ175). Mice received chronic THI treatment (0.1 mg/kg, i.p.), followed by behavioral, lipidomic, molecular, ultrastructural, electrophysiological, and histological analyses of the hippocampus.

Results: THI significantly rescued recognition memory deficits in both HD models. Mechanistically, THI restored sphingosine-1-phosphate (S1P) homeostasis, normalized glycosphingolipid metabolism, improved myelin integrity, preserved synaptic structure and neuronal excitability, enhanced endolysosomal-autophagic activity, reduced mutant huntingtin aggregation, and increased BDNF expression. The therapeutic effects were consistently reproduced in the heterozygous zQ175 model, supporting the robustness of the findings.

Conclusion: These findings identify SGPL1 inhibition as a promising therapeutic strategy for HD and establish sphingolipid metabolism as a central regulator of hippocampal homeostasis. Targeting this pathway simultaneously ameliorates multiple pathological processes implicated in HD, providing a mechanistic framework for the development of lipid based therapeutic approaches.

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Sphingolipid dysregulation across neurodegenerative disease: genotype-dependent lipid alterations in Alzheimer's disease mice and GBA-associated Parkinson's disease

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Sphingolipid metabolism is increasingly recognised as a genetically modulated feature of neurodegenerative disease. In Alzheimer's disease (AD), the APOE ε4 allele is the strongest genetic risk factor and is linked to altered lipid metabolism early in disease, while in Parkinson's disease (PD), mutations in GBA1, encoding the glycosphingolipid (GSL)-degrading enzyme glucocerebrosidase, represent the most significant genetic risk factor. We addressed sphingolipid metabolism in two research lines: an APOE genotype-based mouse model of AD, and human and cross-species models of GBA-associated PD.

Plasma and brain sphingolipid profiles (110 species) were analysed in APOE3/4 and E3/4FAD mice at 2-, 4- and 6-months using LC-MS/MS. Early plasma sphingolipid changes were driven primarily by APOE genotype rather than AD pathology, with APOE4 carriers showing increased sphingomyelins and reduced ceramides regardless of AD status. APOE- and AD-related brain differences were mainly detectable in cortical sphingolipids. We are now extending this to the full brain and plasma lipidome (>2000 lipids, 15 classes), with preliminary results showing genotype- and age-dependent phosphatidylcholine and phosphatidylethanolamine alterations detectable in plasma from 2 months. In parallel, using a refined HPLC-based GSL quantification method, GSL metabolism was found to be consistently elevated in plasma of GBA mutation carriers with sex-specific differences, region-specific changes in the PD brain, and altered in GBA-PD-mutant *Drosophila* and *C. elegans* models.

These findings show sphingolipid alterations are genotype- and region-dependent, with sex-specific effects seen in GBA-PD and reported in human AD lipid studies. This supports sphingolipid metabolism as a shared, genotype-linked feature of neurodegeneration across AD and PD, and suggests sphingolipid-modulating therapies may hold cross-disease relevance.



Lysosomal dysfunction in GCase-deficient dopaminergic neurons induces plasma membrane alterations and metabolic rewiring

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Lysosomal function is essential for cellular homeostasis, coordinating macromolecule degradation and recycling, mitochondrial quality control, and metabolic adaptation. In neurons, lysosomal dysfunction is increasingly recognized as a key driver of ageing and neurodegeneration. Deficiency of β -glucocerebrosidase (GCase), the lysosomal enzyme responsible for glucosylceramide (GlcCer) degradation, impairs lysosomal function and is associated with neurodegenerative disorders. However, the mechanisms linking lysosomal dysfunction to neuronal degeneration remain incompletely understood. To investigate these mechanisms, we used human iPSC-derived dopaminergic neurons chronically treated with conduritol B epoxide (CBE), a selective GCase inhibitor. CBE-treated neurons exhibited neurodegenerative features accompanied by GlcCer accumulation within lysosomes and at the plasma membrane, driven by aberrant activation of lysosomal exocytosis. At the plasma membrane, GlcCer preferentially accumulated in lipid rafts, altering both their sphingolipid composition and associated protein landscape. Given the central role of lysosomes in nutrient recycling, we next examined the metabolic consequences of chronic lysosomal dysfunction. CBE-treated neurons underwent profound metabolic rewiring characterized by reduced glucose uptake and utilization, alongside increased branched-chain amino acid (BCAA) catabolism to sustain energy production. In parallel, these neurons became increasingly dependent on extracellular amino acids to support anabolic pathways when lysosomal nutrient recycling is impaired. Together, our findings show that chronic lysosomal dysfunction extends far beyond defective substrate degradation, triggering plasma membrane remodeling and a profound metabolic adaptation that increases neuronal dependence on extracellular nutrients. These adaptations are likely to contribute to neuronal vulnerability in GCase-associated neurodegeneration and identify metabolic rewiring as a potential pathogenic mechanism downstream of lysosomal dysfunction.



Glycosphingolipid mapping reveals a unique ganglioside signature of murine cortical mitochondria

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Gangliosides, sialic acid-bearing complex glycosphingolipids, are primarily considered residents of the plasma membrane, where they exert their most prominent effects. However, studies dealing with their intracellular roles provide evidence that gangliosides are implicated in physiological processes taking place in different cell compartments as well. Furthermore, disorders such as infantile-onset symptomatic epilepsy syndrome caused by the deficiency of GM3 synthase show characteristics of mitochondrial disease, suggesting that gangliosides participate in mitochondrial molecular architecture. The aim of our study was to analyze the mitochondrial gangliosidome of murine cortex by employing several complementary approaches. We firstly isolated the mitochondria using antibody-labeled magnetic beads, yielding intact and viable mitochondria of high purity. We assessed the purity via Western blotting for mitochondrial and other cellular membrane markers, while the transmission electron microscopy (TEM) provided insight into mitochondrial morphology through morphometric analysis. Immunofluorescent imaging was additionally performed to determine the viability of mitochondria with a mitochondria-specific dye labeling live mitochondria, but also to determine the presence of gangliosides in mitochondrial membranes by using specific anti-ganglioside antibodies. Lastly, we performed structural characterization of gangliosides extracted from isolated mitochondria by ion mobility and collision-induced dissociation mass spectrometry (IMS MS and CID MS/MS). Both MS approaches and immunofluorescent imaging confirmed the presence of gangliosides within the mitochondrial membrane, particularly GD3, GM1 and GD1a. The high-resolution MS comparison of cell membrane to mitochondrial gangliosidome showed a unique ganglioside pattern in mitochondria, enriched in simpler ganglioside species. These findings establish mitochondria as a distinct ganglioside-containing compartment, opening new avenues for comprehensive understanding of glycosphingolipid roles in diverse cellular processes.



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ABSTRACTS OF POSTERS

Chronic psychotropic drug exposure and lipid remodeling in metabolic tissues of middle-aged female rats

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Psychotropic drugs can interfere with lipid homeostasis and contribute to peripheral and central metabolic adverse effects. Aripiprazole and trazodone inhibit 7dehydrocholesterol reductase and may therefore disturb sterol availability, membrane organization and the composition of cholesterol-dependent lipid rafts. Because these microdomains are enriched in sphingolipids, gangliosides, and membrane proteins, drug induced sterol imbalance may be accompanied by broader tissue-specific lipid remodeling. This ongoing study investigates the individual and combined effects of aripiprazole and trazodone in female Sprague-Dawley rats, incorporating sex as a biological variable. The animals were acquired as retired breeders at 8–9 months of age, and treatment began when they were approximately 12 months old, corresponding to the reproductive-senescent transition. Rats were assigned to four groups: a control group receiving the vehicle (5% dimethyl sulfoxide in saline), an aripiprazole group receiving 2.5 mg/kg, a trazodone group receiving 5 mg/kg, and a combined-treatment group receiving aripiprazole and trazodone at the same respective doses. Following sacrifice, matrix assisted laser desorption/ionization time-of-flight imaging mass spectrometry (MALDITOF IMS) will be used for untargeted lipid profiling of key metabolic tissues, primarily liver and kidney, with selected brain regions considered as an additional target of central metabolic effects. The analysis will assess drug-associated changes in the relative abundance and tissue distribution of lipid species, including phospholipids, sphingolipids and sterol-related signals. We hypothesize that combined aripiprazole and trazodone treatment will induce the most pronounced lipidomic alterations, reflecting synergistic disruption of sterol metabolism and cholesterol-dependent membrane microdomains. This approach may reveal tissue-specific lipid signatures linking psychotropic polypharmacy with metabolic complications in aging females.



Complex HPLC/MS methodologies for the broad profiling of sphingolipids and glycosphingolipids

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Resolving the structural complexity of the sphingolipidome remains a significant challenge in biomedical research, where trace abundances, matrix induced ion suppression, and critical isomeric species such as glucosylceramide vs. galactosylceramide frequently lead to analytical misidentification. To address these bottlenecks, a comprehensive, high-throughput HPLC/MS platform was optimized for wide-scale, high-confidence profiling of diverse (glyco)sphingolipid classes across various biological matrices, including cell lines, solid tissues, and biofluids. The established workflows integrate standardized sample handling, robust extraction protocols, multi-column chromatographic separation, and highly sensitive tandem mass spectrometry detection in targeted multiple reaction monitoring mode. This methodology allows for the simultaneous screening and absolute quantification of a vast range of lipid classes, spanning from simple sphingoid bases and standard ceramides to complex glycosphingolipids, globosides, and complex sialylated gangliosides. The validation of these workflows demonstrates high reproducibility and precision, making the platform ideal for mapping precise metabolic shifts in translational oncology and biomarker discovery. These fully established analytical capabilities and lipidomic workflows are presented as a robust screening platform available for collaborative research and multi-center scientific projects.

Sphingolipid remodeling induced by α -synuclein in human epidermal equivalents: exploring novel peripheral biomarkers for Parkinson's disease

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Introduction: The pathological accumulation of α -synuclein in the brain is a hallmark of Parkinson's disease (PD). Aggregated α -synuclein is detected not only in the brain but also in the cerebrospinal fluid, blood, and peripheral tissues, including the skin. Due to the interplay between central and peripheral inflammation, PD is associated with inflammatory skin disorders characterized by impaired epidermal barrier function. These observations suggest that the skin may reflect brain α -synuclein biosignatures. To identify candidate cutaneous biomarkers for the early PD diagnosis, we exposed both 2D and 3D human epidermal equivalents, which mimic the multilayered, terminally differentiated human epidermis *in vitro*, to α -synuclein.

Results: Gene expression profile was assessed by Real-time RT-PCR and CARD arrays to analyze markers of keratinocyte activation (K6, K17) and inflammation/stress response (IL1 α , IL-1 β , IL-6, IL-8, TNF- α , catalase, COX-2), together with markers of barrier function and enzymes involved in ceramide, fatty acid, and sphingomyelins metabolism. Liquid chromatography-mass spectrometry was performed to characterize the profile of lipids involved in energy metabolism and barrier function, including fatty acids, triglycerides, sphingomyelins, cholesterol, and ceramides. The results indicated that α -synuclein triggers an inflammatory response and alters lipid metabolism. Specifically, a trend toward increased levels of bioactive lipid precursor was observed in the 2D models. Furthermore, the 3D models, upon exposure to α -synuclein, exhibited an increase in lipids associated with epidermal barrier function, including short-chain ceramides and glycosphingolipids. **Conclusions:** The α -synuclein-induced protein and lipid alterations in the *in vitro* epidermal model underscore the role of the skin as a reporter of central neuroinflammatory and neurodegenerative processes.

Quantitative analysis of skin sphingolipids in childhood cancer survivors

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Childhood cancer survivors are at increased risk of developing a broad spectrum of late adverse effects that may involve virtually any organ system. Their individual risk is influenced by multiple factors, making accurate prediction challenging. Premature aging is considered one of the key mechanisms underlying the development of these late effects. While biomarkers of biological aging, including p16^{INK4a} expression and skin aging parameters, have been associated with longevity and age-related changes in the general population, premature skin aging in childhood cancer survivors remains insufficiently investigated.

We hypothesize that skin alterations may represent a non-invasive biomarker for identifying survivors at increased risk of late adverse effects. To test this hypothesis, we evaluated skin barrier function in childhood cancer survivors treated with chemotherapy, radiotherapy, combined chemo-radiotherapy, or surgery by measuring transepidermal water loss (TEWL). A trend toward higher TEWL values compared with healthy controls suggested impaired skin barrier integrity in cancer survivors. In parallel, targeted LC–MS analysis of the upper stratum corneum, using synthesized ceramide internal standards including the skin-specific acylceramide EOS, was performed to quantify skin ceramides. Ceramides are essential components of the epidermal permeability barrier and are closely linked to cancer, cancer therapy, cellular senescence, and aging-related processes. Preliminary results revealed significant differences in selected ceramide classes between childhood cancer survivors and healthy controls. Further analyses in a larger cohort are underway to validate these findings.

Our long-term goal is to establish a novel, non-invasive diagnostic approach based on skin biomarkers for the prediction of late adverse effects in childhood cancer survivors.

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Improved synthesis and quantification of ultralong ω -O-acylceramides for targeted delivery in a psoriasis mouse model

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Ultralong ω -O-acylceramides (acylCer) are essential components of the human skin barrier located in the outermost layer of the skin, the stratum corneum. They are present either as free lipids within the extracellular lipid matrix, together with other ceramide species, free fatty acids, and cholesterol, or covalently bound to the surface of corneocytes. Their unique structure promotes the formation of tightly packed and highly organized lipid lamellae with low permeability, known as the long periodicity phase, while corneocyte-bound acylCer form the corneocyte lipid envelope, which supports lipid organization. Consequently, depletion of acylCer, as observed in several skin diseases, impairs the skin barrier and compromises its function.

In this study, we developed an efficient, high-yielding, and scalable synthesis, enabling the preparation of a diverse panel of ω -hydroxylated ceramides and acylCer. These compounds were used to develop (i) a library of analytical standards for improved subclass-specific LC–MS/MS quantification and (ii) a physiologically relevant acylCer tracer for investigating topical delivery and skin penetration without perturbing the native lipid organization. The label-free acylCer tracer with an odd chain length was evaluated in an imiquimod-induced mouse model of psoriasis to demonstrate targeted delivery of topically applied acylCer. This work provides an optimized and cost-effective synthetic route to acylCer from readily available starting materials, improves the quantitative accuracy of skin lipid analysis by LC–MS/MS through the use of subclass-specific internal standards, and introduces a label-free tracer that enables monitoring of acylCer delivery in the stratum corneum, supporting its application in the development of topical therapies for skin barrier disorders.

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Sex-related differences in the lipidome of young, old and gonadectomized rats revealed by MALDI-TOF imaging mass spectrometry

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Sex is a key biological variable influencing lipid metabolism, yet molecular data on sex-related differences in different tissue remain limited. This study aimed to identify and compare sex-specific differences in the liver, kidney and skeletal muscle molecular profiles of Sprague Dawley rats across three groups — young, old, and gonadectomized — using matrix-assisted laser desorption/ionization time-of-flight imaging mass spectrometry (MALDI-TOF IMS). Analyses were performed within two m/z ranges (300–700 Da and 700–1000 Da). Sex-related differences between male and female samples were observed in both young and old animals, across the analyzed mass ranges and tissues. In young rat liver, most significant 300–700 Da m/z signals ($FDR < 0.05$, $|\log_2FC| > 1$) were increased in females and tentatively annotated as phosphatidylcholine- and diacylglycerol-related features. In the 700–1000 Da range, most significant signals were increased in males, including phosphatidic acid-, diacylglycerol-, phosphatidylserine-, and triacylglycerol-related features, while a sphingomyelin-related signal was increased in females. A similar sex-related separation pattern was observed in old rats' liver, with most significant 700–1000 Da signals increased in females. In skeletal muscle, tentatively annotated statistically significant metabolites were detected in the 300–700 Da range in young and old animals, whereas no statistically significant m/z signals were observed in the remaining groups or range. In gonadectomized animals' liver and muscle, results showed group separation, but fewer individual m/z signals reached statistical significance, suggesting a sex-related molecular signature after gonadal hormone removal. Preliminary kidney IMS analysis showed group separation, suggesting possible differences in the renal molecular profile, while tentative metabolite annotation is still ongoing. MALDI-TOF IMS analysis revealed sex-related differences in the lipidome of gonadally intact rats. Results suggest that gonadal hormones contribute to shaping sex-specific lipid profiles. All tentative metabolite annotations require confirmation by MS/MS.



Brain crosstalk sphingolipids and oxylipins in chickens fed with fumonisins

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Alterations in sphingolipids (SL) are central in fumonisins (FBs) toxicity. However, despite FBs are neurotoxic their effects on SL in brain are poorly characterized. In a first study feeding 20.8 mg FB1+FB2/kg for 4, or 9 days to chickens resulted in an unchanged sphinganine concentration in the brain, while deoxysphinganine, sphingosine, C14-C20 ceramides (Cer), and C14-C20 sphingomyelins (SM) increased. A complementary study conducted by feeding 14.6 mg FB1 + FB2/kg for 14 and 21 days revealed that sphingosine, N-acetyl-sphingosine, sphingosine 1-phosphate (So1P), Cer, and SM were increased. The Cer/SM ratio was elevated at 14 days but normalized by 21 days, whereas the So1P:Cer ratio rose at 14 days and continued to increase at 21 days. FB1 was not detected in the brain at 4 and 9 days, became detectable at 14 days and continued to rise at 21 days. All these effects were evocative of an inflammatory response occurring in the brain that was independent of the inhibition of Cer synthase. Complementary analysis of cytokines revealed only weak variations in IL-1, IL-6 and TNF α . By contrast several change in the concentration of oxylipins (OL) occurred. At 4 days, the most discriminative OL were from ω 3-PUFAs, via the LOX pathway. By 9 days, several OL derived from arachidonic acid, spanning all enzymatic pathways, became discriminative. A modest rise in pro-inflammatory arachidonic acid-derived COX metabolites was observed at 14 days, while OLs derived from COX and LOX pathways, including PGE2, 15-HETE, and 17-HDHA, increased significantly at 21 days. These changes coincided with elevated PLA2c and COX2 activities. Altogether, these results suggested an indirect action of FBs on the chicken's brain with strong effects on SL and OL leading to mild neuroinflammation.



Proteins from the edible oyster mushroom as selective tools for the detection and targeted destruction of urothelial cancer cells

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Aegerolysin proteins, such as ostreolysin A6 (OlyA6) and its single-point mutant E69A, specifically recognize and interact with mammalian membrane nanodomains enriched in sphingomyelin and cholesterol. In nature, they recruit membrane-attack complex/perforin (MACPF)-domain partners to form transmembrane pores leading to cell lysis. Here, we explored these proteins as innovative tools for the targeted detection and elimination of urothelial cancer cells. We evaluated the diagnostic potential of fluorescently tagged aegerolysins on various *in vitro* urothelial cell models. Both OlyA6-mCherry and E69A-mCherry selectively labelled non-invasive (RT4) and highly invasive (T24) human cancer cells, while normal primary porcine urothelial (NPU) cells remained completely unlabeled. This selectivity correlates directly with urothelial differentiation. Highly expressed membrane uroplakins in normal cells act as a protective steric shield; conversely, the loss of uroplakins and extensive membrane remodeling in cancer cells expose their target lipid domains. Furthermore, malignant transformation increases membrane lipid raft abundance, providing more aegerolysin targets. Notably, E69A exhibited stronger binding to invasive cells due to its broadened sphingomyelin recognition, confirming its robust diagnostic potential. Additionally, we investigated the therapeutic potential of two-component cytolytic complexes consisting of OlyA6 or E69A combined with MACPF-partners pleurotolysin B (PlyB) or pulmolysin B (PulB). These complexes demonstrated exceptional efficacy in selectively destroying cancer cells while completely preserving healthy, uroplakin-shielded NPU cells. Specifically, the OlyA6/PlyB complex proved optimal for targeting the RT4 phenotype, whereas E69A/PlyB was superior against the aggressive T24 phenotype. Multiparametric analysis unequivocally confirmed that this effect is driven by rapid necrotic lysis, effectively bypassing common cellular resistance to apoptosis. Altogether, our findings present a strong experimental foundation for developing novel targeted strategies for urothelial cancer.



Ceramide- and sphingoid base-centered metabolic reprogramming in high- and low-grade gliomas

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Introduction: Sphingolipids are integral membrane components and important regulators of cellular stress responses, proliferation, and apoptosis. Ceramides occupy a central position within sphingolipid metabolism, connecting de novo sphingolipid synthesis, sphingoid base metabolism, and sphingosine-1-phosphate (S1P)-mediated signaling. Dysregulation of this metabolic network is increasingly recognized in gliomas. However, sphingoid base profiles across glioma grades and in paired tumor and peritumoral tissues remain less extensively investigated than other sphingolipid classes. We analyzed sphingoid bases, ceramides, and S1P in paired tumor and peritumoral tissues from patients with high-grade gliomas (HGG) and low-grade gliomas (LGG), together with corresponding patient and healthy control sera, and evaluated metabolically informative sphingolipid ratios.

Results: Tumor tissue showed increased levels of the d18:0 sphingoid base compared with matched peritumoral tissue, with the most pronounced differences observed in the HGG group and supported by glioblastoma and IDH-wildtype subgroup analyses. Additional changes included increased d16:1 in glioblastoma and IDH-wildtype tumors and increased d20:0 in HGG tumor tissue. This was accompanied by significantly higher d18:0/total ceramide and d18:0/d18:1 ratios in HGG tumor tissue. In contrast, d20:1 showed an opposite pattern, with higher levels in peritumoral tissue than in tumor tissue across both HGG and LGG, supported by a significantly increased d20:1/d18:0 ratio in peritumoral tissue. Measured ceramide species and the global S1P/total ceramide ratio showed no major tumor–peritumor differences. Exploratory serum analysis showed less pronounced sphingoid base alterations relative to healthy controls, including increased d18:0 in HGG serum samples and decreased d16:1 in LGG serum samples.

Conclusion: Gliomas exhibit distinct sphingolipid metabolic changes across tumor grades, characterized primarily by sphingoid base remodeling, with a relatively stable ceramide pool and no marked shift in the global S1P/total ceramide ratio. The opposing d18:0 and d20:1 patterns suggest a tumor-associated shift toward shorter-chain sphingoid base metabolism and support sphingoid base profiling as a potentially informative approach for gaining further insight into glioma biology.



Regulation of necroptosis specific to ceramide species

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Introduction: Ceramides are bioactive lipids that exist as various molecular species with an acyl chain on a sphingoid base. It has become clear that ceramide molecular species function as second messengers in the cell death of cancer cells; however, understanding their involvement in necroptosis remains limited. Necroptosis is a form of regulated cell death executed by RIPK3-mediated activation and oligomerization of the poreforming protein MLKL. Previously, we revealed that a nanoliposome formulation of short-chain C_{6:0}-ceramide induces MLKL-dependent necroptosis independently of RIPK3. In this study, we attempted to identify ceramide molecular species that induce necroptosis and to elucidate their detailed molecular mechanisms. **Results:** In a cell-free system, it was found that ceramides bind to the Cterminal region of MLKL. In a fat blot assay, MLKL interacted with C_{24:0}ceramide. When exogenous very long-chain ceramides were delivered to cells overexpressing ceramide-binding full-length MLKL or non-binding mutant, only the former cells showed MLKL re-localization to the plasma membrane and cell death. These results suggest the involvement of very long-chain ceramides in necroptotic cell death. Interestingly, defects in CERS2, which is responsible for the preferential biosynthesis of very long chain ceramides, suppressed necroptotic oligomerization of MLKL and cell death, but not apoptosis, in response to cisplatin treatment.

Conclusions: Ceramides directly interact with MLKL, and very long-chain ceramide molecular species are considered to be necroptosis-inducing molecules. The induction of necroptosis by selective ceramide species may lead to the development of novel cancer therapies.



Ceramide 1-phosphate suppresses cigarette smoke-induced MCP-1 release and migration in human eosinophils

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Introduction: Ceramide 1-phosphate (C1P) is an important regulator of cell survival, inflammation, and cell migration. Concerning inflammation, eosinophils are important because they act as multifunctional effector and regulatory cells within the immune response, particularly in type 2 (Th2)-mediated inflammation such as allergy, asthma, and parasitic infections. Eosinophils are recruited to sites of inflammation in response to cytokines and chemokines, underscoring the importance of regulating their migration as a means of controlling inflammatory responses. We have investigated the impact of cigarette smoke (CS), which is composed of many toxic compounds that can cause inflammation, on human eosinophil migration and tested whether C1P might be able to overcome this action.

Results: CS-conditioned medium from A549 lung adenocarcinoma cells stimulated human eosinophil migration at concentrations that did not alter cell viability. C1P completely blocked CS-conditioned medium-induced migration of eosinophils. By contrast PA, which is a natural structural analog of C1P and has pro-inflammatory properties, failed to reduce eosinophil migration. CS induced the release of macrophage chemoattractant protein- 1 (MCP-1) in the A549 lung cancer cells, and MCP-1 was able to stimulate eosinophil migration.

Conclusions: C1P inhibits MCP-1-stimulated cell migration, suggesting that the mechanism whereby C1P blocks CS-induced eosinophil migration involves inhibition of this chemokine.

Characterization of ceramide kinase from basolateral membranes of kidney proximal tubules: kinetics, physicochemical requirements, and physiological relevance

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Ceramide kinase (CerK) catalyzes the phosphorylation of ceramide to ceramide1-phosphate (C1P), a bioactive sphingolipid with diverse signaling roles. While CerK has been identified in several cellular compartments, its presence and functional significance in kidney proximal tubules remain unexplored. Herein, we report the first characterization of CerK activity in basolateral membranes (BLMs) from porcine proximal tubule cells. We demonstrate that BLM fractions contain neutral and acidic sphingomyelinases, providing local substrate for CerK, which efficiently generates C1P under physiological pH (6.5–7.2) and temperature (30– 37 °C) conditions. Enzyme activity was stimulated by cAMP in a protein kinase A-dependent manner but was not affected by angiotensin II. Lipidomic analysis confirmed the presence of C1P in human proximal tubule (HK-2) cells under basal conditions and revealed changes during ischemic stress. Transcriptomic analysis of kidney biopsies from patients with chronic kidney disease further uncovered coordinated remodeling of sphingolipid metabolism genes, with increased expression of ceramidases (ASAH1 and NAAA) and downregulation of ceramide synthases (CERS4, CERS5), consistent with adaptive regulation of the Cer/CerK/C1P axis. Together, these findings identify for the very first time CerK activity in renal BLM, establish its biochemical requirements, and highlight its potential role in modulating transporter function and sphingolipid signaling in physiology and kidney disease.

Identifying and validating ceramide transfer protein binding partners with a focus on the methodology

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Sphingolipids (SLs) are crucial membrane components with key roles in signal transduction and cell metabolism. A central step in SL metabolism is the production of ceramides (Cer), which are abundant in cell membranes and play crucial roles in cellular functions such as stress response, senescence, apoptosis, cell cycle, differentiation, and neuronal maturation. The ceramide transfer protein (CERT) transports Cer from the endoplasmic reticulum to the Golgi apparatus for further modification into more complex SLs. CERT has two isoforms, CERTL and CERT, which differ slightly in their middle region and are both composed of a pleckstrin homology domain, a middle region, and a StAR-related lipid transfer domain. CERT is expressed ubiquitously but is particularly important in the central nervous system, where it is associated with embryogenesis and brain development, and can also bind C1q to activate the complement cascade, highlighting a role in immune responses. Despite its significance, CERT's molecular mechanisms and broader interactome remain poorly understood. To identify CERT interactors, we employed a yeast two hybrid screen analyzing each region of the protein individually. Candidate interactions are being validated through complementary approaches, including BioID and NanoBRET. Using these assays, we confirmed the known interactor VAP-A, supporting the validity of our screening and validation pipeline. We also identified additional candidate interactors spanning functions such as mitochondrial activity, apoptosis, cell growth, vesicle transport, and protein degradation, including ORMDL1, a regulator of serine palmitoyltransferase and de novo ceramide biosynthesis, and RTN1, an ER-shaping protein implicated in apoptosis and neuronal differentiation. Validation of these candidates is ongoing. These findings lay the groundwork for future research to clarify CERT's molecular mechanisms and interactome, and its potential as a therapeutic target in diseases related to ceramide metabolism.

YM-07, a novel ceramide mimetic, induces MLKL-dependent necroptosis and overcomes cisplatin resistance in head and neck cancer

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Introduction: Cisplatin resistance limits therapeutic efficacy in head and neck cancer, often due to impaired apoptotic signaling. Induction of necroptosis mediated by mixed lineage kinase domain-like protein (MLKL) may eliminate apoptosis-resistant tumors. This study aimed to synthesize ceramide mimetics (RT-25, RT-71, and YM-07) and evaluate their necroptosis-inducing activity in cisplatin-resistant head and neck squamous cell carcinoma (HNSCC) cells.

Results: RT-71 and YM-07 reduced cell viability, whereas RT-25 was inactive. YM-07 selectively induced MLKL-associated necroptotic cell death with minimal apoptotic features in FaDu cells and promoted MLKL oligomerization/activation in a cell-free system. Importantly, YM-07 retained cytotoxicity in cisplatin-resistant cells despite reduced MLKL expression.

Conclusions: YM-07 is a novel MLKL-activating necroptosis inducer effective against cisplatin-resistant HNSCC cells, supporting necroptosis-based strategies to overcome cisplatin resistance.

Comparative interest of different biomarkers of fumonisins in feed

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Fumonisin (FBs) are among the most prevalent *Fusarium* mycotoxins contaminating cereal-based feeds used in poultry production. Because exposure can occur at low concentrations in feed, sensitive biomarkers are required to identify exposed animals. Two complementary studies investigated the tissue persistence of FBs and the usefulness of sphingolipid biomarkers in broiler chickens and turkeys exposed to diets containing 7.5 mg FB1 + FB2/kg feed, together with 2.5 mg deoxynivalenol (DON)/kg and 0.6 mg zearalenone (ZEN)/kg, for 14 days, followed by up to 4 days of mycotoxin free feeding. FB1 in liver was identified as the major FBs residue in both species. Liver concentrations declined progressively after withdrawal of the contaminated diet, with a half-life of elimination of 66 and 124 hours in chickens and turkeys, respectively. Analysis of sphingolipids (SLs) in liver and plasma revealed that the C22-24:C16 ratio measured across ceramides, monohexosylceramides and sphingomyelins were sensitive indicator of FBs exposure. By contrast, the measurement of sphinganine-to-sphingosine (Sa:So) ratio remained unchanged. A percentile approach used to reveal animals exposed to FBs provided satisfactory results in both species, even by using the Sa:So ratio. Multivariate analyses of the sphingolipidome was the most sensitive method to discriminate exposed animals from controls, even four days after exposure had ceased. Overall, these studies demonstrate that the measure of FB1 in liver was the most sensitive biomarker of FBs exposure, while SL profiling and particularly the C22-24:C16 ratio, provides robust and sensitive approach to reveal FBs effects.



Investigation of new biomarkers of fumonisins effects in chickens and pigs

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Fusarium mycotoxins are major contaminants of human and animal diets that have numerous effects on health. Among them fumonisins (FBs) are recognized inhibitors of ceramide synthase that increase the sphinganine-to-sphingosine ratio (Sa:So) commonly used as a biomarker of exposure. This first effect is responsible for strong alterations in sphingolipids (SLs) metabolism that are considered to play a key role in FBs toxicity. Different studies with FBs in feed at level close to the maximum recommended level by the EC in chickens and in pigs were conducted. SLs in plasma and tissues were dosed using targeted LC-MS/MS analysis. Main results revealed that, beyond changes in total SL concentrations, FBs induced a specific shift in the chain-length profile of ceramides and complex SLs. This alteration was characterized by an increase in the C22-24:C16 ratio, observed across several classes of SLs (ceramides, sphingomyelins, monohexosylceramides) in different tissues (liver, kidney, lung) and in plasma. This increase was mainly driven by a selective decrease in C16 SLs, whereas C22-C24 species remained stable or even increased. In chickens, comparative results obtained with FBs alone and with FBs in combination with deoxynivalenol (DON) and zearalenone (ZEN) revealed strong interactions between mycotoxins on the SL concentrations. By contrast, both the Sa:So ratio and the C22-C24:C16 ratios were only altered by the presence of FBs. In pigs, the use in feed of AlgoClay, a mycotoxin-binding agent, prevented the specific decrease in C16 SLs induced by FBs. Overall, these findings revealed that the C22-24:C16 ratios measured on different classes of SLs are robust and specific biomarkers of FBs effects.



Deficiency of Toll-like receptor 2 is associated with compositional and structural changes of membrane lipids

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Toll-like receptor 2 (TLR2) is a transmembrane pattern recognition receptor crucial for cellular innate immunity responses. TLR2 roles in the brain are related to cellular differentiation during neurodevelopment and neuroinflammation accompanying diverse neuropathological disorders. Due to its localization in lipid rafts (LRs), actions of TLR2 are associated with functional cross-talk with sphingolipids and cholesterol, the most abundant LRs residents. We investigated the influence of TLR2 deficiency on the composition of membrane lipids in brain and performed detailed lipidomic analysis of cortical tissue samples and isolated membrane fractions derived from TLR2-deficient female and male mice (TLR2D-F, TLR2D-M). Methodological approach encompassed: isolation and separation of gangliosides by high performance thin layer chromatography; ion mobility and collision-induced dissociation mass spectrometry (IMS-MS, CID-MS/MS); sterol liquid-chromatography mass spectrometry (LC-MS); LRs isolation. The main findings of the study indicate sex-related differences in brain ganglioside pattern, presence of unique ganglioside modifications, along with higher structural heterogeneity and diversity of glycan and ceramide moiety in TLR2D vs control mice. Also, using IMS CID MS/MS, we described the structure of GD1b isomer detected specifically in brain tissue of TLR2D-M. Analysis of sterols revealed lower concentration of total cholesterol and selected cholesterol synthesis intermediates in TLR2D, more prominently in TLR2D-F. At the membrane microdomain level, redistribution of GM1 and GD1a within rafts and non-rafts was noticed in TLR2D-F, while more dispersed distribution of cholesterol within isolated membrane fractions was detected in both control and TLR2D-F when compared with male counterparts. In conclusion, we demonstrate that distinct and sex-specific brain glycolipidomic and sterolomic profiles and rearrangements of membrane lipids occur in TLR2 deficiency, implying intricate and yet undescribed structural and functional interrelationships of cholesterol, specific gangliosides species and TLR2.

Off-target sphingosine 1-phosphate receptor 1-3 antagonism by sphingosine kinase 1 inhibitors and its impact on endothelial barrier function

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Introduction: Sphingosine 1-phosphate (S1P) regulates vascular endothelial barrier integrity and is generated from sphingosine by sphingosine kinase type 1 and 2. Our goal was to explore the impact of SphK1 inhibitors on endothelial barrier function, underlining the mechanisms how SphK1 inhibitors influence the integrity of the vascular endothelium.

Results: First, SphK1 enzymatic activity in the endothelial cell line EA.hy926 could be inhibited by SphK1 inhibitors (SKI-II and PF543). Next, we tested the functional consequences in electrical cell-substrate impedance (ECIS) measurements. Enhanced barrier resistance was observed after treatment with S1P, S1P receptor type 1 agonist and S1PR2/3 antagonists. Surprisingly, SKI-II and PF543 also stabilized the endothelial barrier despite reduced S1P production due to SphK1 inhibition. Importantly, when EA.hy926 cells were pretreated with the S1PR modulator FTY720, there was no increase of resistance after stimulation with S1P or SphK1 inhibitors, demonstrating the involvement of S1PRs. Furthermore, a partial block of the barrier protective effect of S1P and SphK1 inhibitors was also observed with the S1PR1 antagonist, W146. The rat hepatoma tissue culture-4 cell line HTC4 with stable S1P receptor subtype expression was used for intracellular calcium flux measurements to investigate a potential effect of SKI-II and PF543 on S1PRs. Intriguingly, both SKI-II and PF543 inhibited calcium mobilization by S1P via S1PR1-3. The inhibition of calcium mobilization in S1PR3 expressing HTC4 cells was relatively stronger than in those expressing S1PR1 or S1PR2. In addition, the inhibition was dose dependent for S1PR3.

Conclusion: SKI-II and PF543 are off-target antagonists for S1PR1-3. They regulate endothelial cell barrier integrity mainly by inhibiting S1PR3 signal activity, which is independent of its SphK1 inhibitory function.



Sphingosine 1-phosphate-driven interplay between neutrophils and endometriotic cells sustains endometriotic pathophysiologic traits: a role for neutrophil extracellular traps

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Introduction: Endometriosis is a chronic disease affecting millions of women worldwide, characterized by the presence of ectopic endometrial-like tissue. While the formation of neutrophil extracellular traps (NETs) has been linked to endometriosis progression, the underlying implicated molecular mechanisms remain poorly understood.

Results: Immunofluorescence of patient-derived lesions revealed a significant increase in NET markers compared to controls. NET treatment induced fibrosis as well as inflammation and invasion in endometriotic epithelial and stromal cells. Mechanistically, in endometriotic cells NETs modulated the S1P signaling axis by increasing the expression of sphingosine kinase-1 (SK1), one of the two isoforms responsible for S1P biosynthesis, the S1P transporter Spns2, and S1P2 and S1P3 receptors. Notably, the pathological traits elicited by NETs rely on S1P signaling via a mechanism dependent on both SK and S1PRs, with a primary role played by the SK1/S1P3 axis. To add further layers of complexity, conditioned media from endometriotic cells where S1P metabolism or export were modulated, potentially influenced neutrophil NETosis demonstrating that endometriotic cells stimulated neutrophils to release NETs via S1P, creating a pathological feedback loop. The relevance of the S1P/NET axis in endometriosis was supported by the positive correlation of the plasma levels between S1P and the NET marker myeloperoxidase in patients.

Conclusions: Our study identifies S1P signaling as the molecular hub linking NETosis and endometriotic cell dysfunction, uncovering a previously unrecognized pathogenic feedforward loop that promotes disease progression. By demonstrating that S1P signaling orchestrates both the cellular response to NETs and the ability of endometriotic cells to stimulate NET formation, these findings identify S1P signalling as an attractive non-hormonal therapeutic target. Indeed, targeting this pathway may simultaneously attenuate inflammation, fibrosis, invasion and NETosis, offering a multifaceted strategy to interrupt disease perpetuation and improve endometriosis management.



Assessing phospholipase pretreatment for enhanced immunohistochemical visualization of major gangliosides and lipid raft markers

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Gangliosides are glycosphingolipids highly enriched in neural tissue, where they are major components of lipid rafts and play important roles in cell signaling and membrane organization. Their immunohistochemical detection is often inconsistent. Our previous work suggested that this variability may partly result from steric masking of ganglioside epitopes by surrounding membrane phospholipids. Supporting this hypothesis, enzymatic phospholipid removal with phospholipase A1 significantly improved ganglioside visualization in the mouse cerebellum and spinal cord. Phospholipase A2 (PLA2) and phospholipase C (PLC) remove membrane phospholipids through distinct enzymatic mechanisms and may therefore further improve ganglioside detection. This study will evaluate whether PLA2 and two distinct PLC enzymes, applied individually or in combination, enhance immunohistochemical detection of the major gangliosides (GM1, GD1a, GD1b and GT1b) and lipid raft markers, including flotillin (raft) and the transferrin receptor (non-raft) in the spinal cord of wild-type (WT) and *St3gal5* knockout (KO) mice. WT and KO tissue will be compared to test the uniformity of GM1 and GD1a distribution in these models, as both gangliosides are highly expressed in WT and KO spinal cord. Cryosections of spinal cord tissue from WT and *St3gal5* KO mice will be treated with PLA2 and PLC enzymes individually or in combination, followed by immunohistochemical analysis of gangliosides and lipid raft markers. Based on our previous results with phospholipase A1, we expect PLA2 and PLC treatments to reduce phospholipid-mediated steric masking and increase ganglioside and lipid raft marker signal, with combined treatment potentially producing an additive effect. If confirmed, these findings would support phospholipase treatment as a useful approach for improving lipid immunohistochemistry in neural tissue.



Discovery of first-in-class small-molecule inhibitors of ceramide-1-phosphate transfer protein through an integrated biophysical, cellular, functional, and in silico drug discovery pipeline

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Ceramide-1-phosphate transfer protein (CPTP) is a cytosolic lipid transfer protein that mediates the intracellular transport of ceramide-1-phosphate (C1P), thereby regulating sphingolipid homeostasis and downstream signaling pathways that govern inflammation, cell survival and autophagy. Despite its emerging biological significance, no small-molecule inhibitors of CPTP have been reported to date, highlighting the need for chemical probes to elucidate its biological functions and therapeutic potential. To identify novel CPTP inhibitors, we established an integrated platform comprising complementary biophysical, biochemical, and cell-based assays. An annotated library of 2500 bioactive and clinically approved small molecules was initially screened using a differential scanning fluorimetry assay, yielding approximately 60 primary hits exhibiting significant target engagement. These hits were subsequently evaluated in a live-cell NanoBRET target engagement assay, followed by orthogonal validation using a fluorescence resonance energy transfer (FRET)-based liposomal lipid transfer assay to directly assess inhibition of CPTP-mediated C1P transport *in vitro*. Because CPTP has been implicated in autophagy regulation, a downstream cellular autophagy assay was developed to establish the functional consequences of CPTP inhibition on autophagy and to further validate the identified hits. Furthermore, structure-guided virtual screening was performed to design and prioritize novel derivatives with improved predicted binding affinity and pharmacological potential. Collectively, this study establishes a robust and orthogonal screening workflow for the identification and validation of small-molecule inhibitors of ceramide-1-phosphate transfer protein. The identified hit compounds and their rationally designed derivatives provide a foundation for the development of chemical probes to elucidate CPTP biology and facilitate future therapeutic discovery targeting sphingolipid signaling and autophagy.



Lactoferrin modulates acid sphingomyelinase expression in THP-1 monocytes

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Monocytes play a central role in the innate immune system and inflammatory responses, which are tightly regulated by bioactive lipid signaling. The sphingolipid metabolic pathway is a key regulator of cellular signaling and inflammatory processes. In particular, acid sphingomyelinase (aSMase), encoded by SMPD1, has been extensively investigated in macrophages. Evidence also indicates that its expression is regulated during monocytic differentiation, suggesting a role in monocyte biology (1). Lactoferrin (Lf) is an iron-binding glycoprotein with recognized immunomodulatory properties and has been shown to modulate several functions of monocytes, mainly through the downstream NF- κ B and MAPK signaling pathways. However, the role of sphingomyelinase in monocytic cells remains poorly characterized. Therefore, this study aimed to investigate the modulation of acid sphingomyelinase expression in THP-1 monocytic cells following different treatments.

THP-1 cells were treated with Lf, vitamin C and zinc – two other compounds with established immunomodulatory properties that have also been investigated in these cells– and with their combination. aSMase protein expression was evaluated by Western Blot analysis. Treatment with Lf reduced aSMase protein expression in THP-1 monocytic cells compared to untreated cells, whereas vitamin C and zinc alone did not produce appreciable changes in aSMase expression. Notably, combined treatment with Lf, vitamin C, and zinc also resulted in a reduction of aSMase expression comparable to that observed with Lf treatment alone. These findings suggest that Lf may be the main component responsible for the inhibitory effect on aSMase expression in THP-1 cells, thereby promoting the transition of cells toward an anti-inflammatory phenotype. Further studies are needed to determine whether the observed reduction in aSMase expression translates into functional modulation of inflammatory pathways and immune responses.

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Uncovering metabolic vulnerability in cardiac remodeling

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Introduction: Left ventricular hypertrophy reflects sustained cardiac remodeling, but its tissue metabolic and proteostatic features remain incompletely defined. We integrated myocardial proteomics and lipidomics in human left atrial appendage biopsies to characterize hypertrophic remodeling and Congo Red-reactive deposits.

Results: The cohort included 33 patients undergoing elective cardiac surgery, with 11 non-hypertrophic controls and 22 patients with left ventricular hypertrophy. Hypertrophic myocardium showed 193 nominally regulated proteins, mainly reduced, involving mitochondrial metabolism, fatty-acid utilization, protein folding, RNA regulation, cytoskeletal organization and membrane trafficking. Lipidomics identified 12 nominally significant myocardial species, including increased ether phosphatidylcholine O-34:1 and reduced phosphatidylcholine 40:8. Congo Red-reactive deposits occurred in 7 of 22 hypertrophic cases and 2 of 11 controls. Within hypertrophic myocardium, deposit-positive biopsies showed 78 differentially regulated proteins, with extracellular-matrix, complement and protease-regulation signals, and reduced proteostasis, trafficking and mitochondrial pathways. Canonical amyloid precursors were not diagnostically enriched. Deposit-positive lipidomics identified 61 increased tissue lipid species, mainly involving GM3-related glycosphingolipids, bis(monoacylglycerol)phosphates, cardiolipins and phosphatidylcholines.

Conclusions: Integrated tissue proteomics and lipidomics indicate that left ventricular hypertrophy is a state of metabolic, proteostatic and membrane remodeling rather than an exclusively anatomical phenotype. Congo Red-reactive deposits define a proteinopathy-like myocardial subgroup marked by impaired protein handling, extracellular-matrix activation, complement signaling and endolysosomal lipid remodeling, without molecular evidence for typed cardiac amyloidosis. Spatial deposit typing and independent validation should clarify the composition and clinical relevance of this phenotype.

Ceramide-driven mitochondrial stress in macrophages activates the NLRP3 inflammasome in coronary atherosclerosis

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Introduction: Macrophage-driven inflammation promotes coronary atherosclerosis and plaque instability. Although circulating ceramides strongly predict coronary artery disease and adverse cardiovascular outcomes independent of traditional risk factors, the mechanisms linking ceramide metabolism to macrophage inflammatory signaling remain poorly defined.

Results: Nogo-B, an endoplasmic reticulum membrane protein that restrains de novo ceramide biosynthesis, was highly expressed in human and murine macrophages. Myeloid-specific deletion of Nogo-B accelerated coronary atherosclerosis, increasing lipid accumulation, necrotic core formation, macrophage apoptosis, and features of plaque vulnerability. Nogo-B deficiency increased macrophage ceramide levels and enhanced activation of the NLRP3 inflammasome. Pharmacological inhibition of serine palmitoyltransferase attenuated inflammasome activation in Nogo-B-deficient macrophages, identifying ceramides as critical mediators. Mechanistically, inhibition of mitochondrial calcium uptake through blockade of voltage-dependent anion channels or the mitochondrial calcium uniporter abolished NLRP3 inflammasome activation, while scavenging mitochondrial respiratory chain-derived reactive oxygen species similarly suppressed inflammasome signaling.

Conclusions: Nogo-B restrains ceramide-driven mitochondrial calcium uptake, thereby preventing mitochondrial reactive oxygen species generation and NLRP3 inflammasome activation in macrophages. Loss of this pathway promotes macrophage apoptosis and plaque vulnerability, identifying macrophage Nogo-B-regulated sphingolipid metabolism as a potential therapeutic target for coronary atherosclerosis.



High-resolution mass spectrometry screening and fragmentation analysis of serum gangliosides for biomarker discovery in frontotemporal dementia

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Introduction: Frontotemporal dementia (FTD) is a heterogeneous neurodegenerative disorder for which reliable blood-based biomarkers are still unavailable. Given the central role of gangliosides in neuronal membrane organization and lysosomal homeostasis, comprehensive structural characterization of the serum gangliosidome by high-resolution mass spectrometry (HRMS) represents a promising strategy for biomarker discovery. This study aimed to establish an HRMS-based analytical workflow for screening, structural annotation, and preliminary fragmentation analysis of serum gangliosides associated with FTD.

Results: Serum gangliosides isolated from FTD patients and healthy controls were separated by HPTLC and analyzed by high-resolution nanoESI-MS in the negative-ion mode. HRMS enabled the detection of numerous ganglioside molecular species differing in glycan composition, degree of sialylation, and ceramide structure. Tandem MS fragmentation provided diagnostic glycosidic and ceramide-derived fragment ions, allowing preliminary structural annotation of the detected species. Comparative profiling revealed a marked remodeling of the FTD gangliosidome, characterized by increased abundances of GD2-, GD3-, and GM3-associated species and depletion of complex gangliosides, including GM1, GD1a, GD1b, GT1b, and GQ1b. In addition, HRMS data demonstrated distinct ceramide heterogeneity and altered sialylation patterns between FTD and control samples, supporting the discriminatory potential of serum gangliosides.

Conclusions: The developed HRMS/MS workflow provides sensitive molecular screening together with structural characterization of serum gangliosides and enables the identification of disease-associated molecular signatures in FTD. These preliminary findings demonstrate the utility of high-resolution tandem mass spectrometry for ganglioside biomarker discovery and establish a robust analytical platform for future in-depth structural characterization and clinical validation of FTD-specific serum biomarkers.



Parkinson's disease-derived dopaminergic neurons carrying new rare genetic variants revealed altered sphingolipid pathway

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Parkinson's disease (PD) is a complex neurodegenerative disorder caused by the progressive loss of dopaminergic neurons in the substantia nigra pars compacta. PD has a complex genetic background, and our group has previously demonstrated that multiple rare variants may contribute to disease onset. Recently, lipid metabolism has been implicated in PD pathophysiology due to the ability of lipids to bind α -synuclein, thereby promoting the accumulation of oligomers in dopaminergic neurons. Based on this evidence, the aim of this study was to identify metabolic alterations in hiPSC-derived dopaminergic neurons from PD patients carrying novel rare genetic variants and sex-matched healthy controls (HC). To this end, six PD and five HC iPSC lines were differentiated into midbrain dopaminergic (mdDA) neurons. After 45 days of differentiation, neuronal maturity was assessed by evaluating TH and MAP2 expression through immunofluorescence and qPCR, and by analyzing electrophysiological properties, which revealed distinct profiles within the PD-derived mdDA group. A multi-omics approach, including proteomic and lipidomic analyses, was then applied to uncover dysregulated pathways. Proteomic analysis revealed that PD-derived mdDA neurons exhibited altered expression of proteins involved in energy and mitochondrial metabolism, including glycolysis and the TCA cycle. Consistently, lipidomic profiling showed accumulation of lipids associated with energy metabolism, such as fatty acids (FAs) and acylcarnitines (CAR), in PD-derived mdDA neurons. Interestingly, we also detected alterations in sulfoglycosphingolipids (SHexCer), hexosylceramides (HexCer), and gangliosides (GM3), belonging to the sphingolipid class. Notably, increased levels of HexCer were also observed in plasma samples from a larger cohort of PD patients compared to HC. In addition, we found altered protein levels of SMS1, CERK1, and UGT2, and ongoing qPCR analyses are investigating the dysregulated expression of additional enzymes involved in sphingolipid metabolism. Ongoing scRNA seq analysis will better elucidate the presence of a specific alteration in genes related to Sphingolipids metabolism in PD-derived mdDA. Taken together, our results highlight a strong link between lipid metabolism alterations and PD at both cellular and systemic levels, supporting a role for the identified rare variants in neuronal dysfunction. These findings may contribute to future precision medicine strategies for PD diagnosis and progression monitoring.

A drug repositioning strategy targeting S1P-mediated signaling to counteract neuroinflammation in Rett syndrome

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Rett syndrome (RTT; OMIM 312750) is a rare and severe neurodevelopmental disorder primarily affecting female individuals, characterized by cognitive and motor impairments. RTT is mostly caused by mutations in the X-linked *Methyl-CpG-binding Protein 2* (*MECP2*) gene, which encodes an epigenetic factor crucial for nervous system functioning. To date, the pathogenic mechanisms of the disease are still not fully understood, and effective treatments are not yet available.

Our previous findings highlighted an unbalanced sphingolipid (SL) metabolism in both human and murine RTT models, which is attenuated by a treatment with RPC-1063 (Ozanimod), a sphingosine-1-phosphate (S1P) receptor modulating drug, able to cross the blood-brain barrier, and approved for the treatment of other neuropathologies. We hypothesize that the alteration of SL metabolism in RTT might contribute to the establishment of a neuroinflammatory state, which represents a hallmark of the disease. Accordingly, we observed increased levels of the pro-inflammatory glucosyl-ceramide and GT1b ganglioside, and decreased content of the anti-inflammatory GM1 ganglioside in the brain of *Mecp2*^{-/-} mice. Moreover, the inspection of transcriptomic profiles obtained from brain of *Mecp2*^{-/-} and WT mice, treated with Ozanimod or vehicle, underlined, in *Mecp2*^{-/-} mice, an aberrant expression of genes related to inflammation, including *Irak1*, *Il1b*, *Il33*, *S100a16*, *Mmp9*, and a modulation of their expression upon pharmacological treatment. We also analyzed the expression of additional pro-inflammatory and anti-inflammatory factors in a larger cohort of *Mecp2*^{-/-} and WT mice treated with Ozanimod or vehicle. Finally, our data highlighted that Ozanimod restores the physiological levels of the neuroprotective factors BDNF and IGF-1 in brain of *Mecp2*^{-/-} mice.

In conclusion, even though the molecular effects of Ozanimod are still poorly characterized, our preliminary findings suggest that the pharmacological modulation of S1P-mediated signaling by this drug has the potential to modulate neuroinflammation.



Proteomic and lipidomic signatures of synaptic dysfunction in Alzheimer's disease

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Alzheimer's disease (AD) is a neurodegenerative disorder characterized by progressive neuronal dysfunction followed by neuronal loss. Disease progression is associated with the spread of aggregated Tau across synaptically connected brain regions and a concomitant decline in synapse number and function. To characterize synaptic alterations in AD in greater detail, we purified synaptosomal fractions from two independent cohorts of human AD donors at different disease stages, as well as non-demented controls. Weighted gene co-expression network analysis (WGCNA) and module preservation analysis revealed disease stage-associated proteomic changes in synaptic fractions that were conserved across both human cohorts. Gene Ontology enrichment of preserved modules highlighted endocytosis, synaptic processes, and lipid metabolism, suggesting lipid-associated alterations in synaptosomes from AD brains. Sphingolipid changes are key features of multiple neurological disorders including AD, and lipidomics analyses showed increased levels of specific ceramide species in AD brain-derived synaptosomes. Further mechanistic analyses in a human iPSC-derived neuronal model revealed a correlation between tau aggregation and ceramide increase. Ongoing integrated analyses of the proteomic and lipidomic datasets are expected to further define disease-associated changes and may identify lipid associated pathways with therapeutic potential.



Region-specific dysregulation of sphingolipid metabolism during disease progression in PS19 mice

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Introduction: Alzheimer's disease (AD) is the leading cause of dementia worldwide, accounting for 60-70% of cases. Due to the increasing aging of the global population, identifying biomarkers for early AD detection has become one of the greatest global healthcare challenges. Alterations in sphingolipid metabolism are increasingly described in AD. In addition, this dysregulation has been shown to contribute to amyloid-beta processing, tau hyperphosphorylation, mitochondrial dysfunction, neuroinflammation, and synaptic impairment.

Therefore, this study aims to investigate changes in sphingolipid metabolism in a mouse model expressing slow-progressing tauopathy (PS19).

A 12-month follow-up was conducted in Wild-type (WT) and PS19 mice, consisting of cognitive tests, MRI studies, and plasma collection at multiple time points. Additionally, qPCR, Western blot (WB), and immunohistochemistry analyses were performed on the brain tissue of the animals.

Results: Longitudinal follow-up of PS19 and WT mice revealed a significant phenotypic divergence in PS19 mice beginning at 9 months of age. At this time point, PS19 mice exhibited the onset of cognitive impairment, accompanied by a reduction in hippocampal and cortical volumes. As expected, phosphorylated tau (pTau) levels were significantly increased in PS19 mice compared with WT controls.

Interestingly, the expression of several enzymes involved in sphingolipid (SL) metabolism was altered in specific brain regions, including the hippocampus, cortex, and cerebellum. In addition, the expression of sphingosine-1-phosphate receptors (S1PRs) was also dysregulated, suggesting that disturbances in sphingolipid signaling accompany the progression of tau pathology and may contribute to the neurodegeneration.

Conclusion: These findings suggest that dysregulation of sphingolipid metabolism could be an early event associated with cognitive impairment and neurodegeneration in the first steps of the disease.



Microglial ceramide transport shapes cognition: characterization of a microglial CERT knock-out model

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Ceramide transfer protein (CERT) mediates the principal non-vesicular transport of ceramide from the endoplasmic reticulum to the Golgi and therefore sits at a central point of cellular sphingolipid balance. Loss of CERT leads to ceramide accumulation, ER stress and mitochondrial dysfunction, while altered ceramide levels have been implicated in inflammatory signaling and neurodegeneration, including Alzheimer's disease. Lipid handling is increasingly recognized as central to microglial function, but the role of CERT-mediated ceramide transport remains unknown.

We characterize a microglia-specific CERT knock-out (CERTMG^{-/-}) model combining in vivo behavioral assessment of both sexes with in vitro work on isolated primary microglia. Transcriptomic and lipidomic profiling as well as functional assays on isolated microglia are ongoing. Preliminary data demonstrate that at six months of age, female CERTMG^{-/-} mice showed impaired hippocampus-dependent spatial memory in the object location task, indicating that disrupted ceramide transport within microglia is sufficient to affect cognitive function. These data identify CERT as a candidate regulator of microglial sphingolipid homeostasis with consequences for behavior. Ongoing lipidomic, transcriptomic and functional characterization aims to define the underlying mechanism.

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