SPHINGOLIPID CLUB

XV SPHINGOLIPID CLUB MEETING

3 -7 Sept 2024, Friedrich-Alexander-University Erlangen-Nürnberg

MEETING BOOK

ORGANIZERS

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PROGRAM

Tuesday, Sept. 3, 2024

16:00 - 20:00 *Registration*

Lecture theater, Medical Faculty, FAU Erlangen

18:00 - 18:20 *Welcome*
Riccardo Ghidoni

Opening remarks
Christian P. Müller, Liubov S. Kalinichenko

18:20 - 19:00 *Keynote Lecture presented by Christian P. Müller*
Thierry Levade (Toulouse, FRA)
Human genetic defects of sphingolipid biosynthesis

19:00 *Welcome buffet*

Wednesday, Sept. 4, 2024

Lecture theater, Medical Faculty, FAU Erlangen

09:00 - 11:30 ***Session 1 - Sphingosine-1-phosphate***

Chair: **Gerhild van Echten-Deckert** (Bonn, GER)

09:00 - 09:25 **Timothy Hla** (Boston, USA)

Sphingosine 1-phosphate in biology and medicine

09:25 - 09:50 **Sarah Spiegel** (Richmond, USA)

Sphingosine-1-Phosphate - from Bench to Clinic

09:50 - 10:15 **Dagmar Meyer zu Heringdorf** (Frankfurt, GER)

Regulation of calcium homoeostasis by sphingosine-1-phosphate: novel insights

10:15 - 10:40 **Eric Camerer** (Paris, FRA)

Zonation and mechanisms of engagement of S1PR1 signaling in the murine vasculature

10:40 - 11:05 **Webster Santos (**Blacksburg, USA)

Therapeutic assessment of blocking S1P transport

11:05 - 11:30 **Caterina Bernacchioni** (Florence, ITA)

Functional interplay between sphingosine-1-phosphate signaling and endocannabinoid system in endometriosis

11:30 - 12:00 *Coffee break*

Lecture theater, Medical Faculty, FAU Erlangen

12:00 - 12:40 *Keynote Lecture presented by Liubov S. Kalinichenko*

Jae-sung Bae (Seoul, KOR)

Alzheimer's - a disease beyond the brain: Insights from sphingolipid Metabolism

12:40 - 14:00 *Lunch*

Lecture theater, Medical Faculty, FAU Erlangen

14:00 - 14:15 **Joseph Pfeilschifter** (Frankfurt, GER)

*In memory of **Andrea Huwiler***

14:15 - 16:10 ***Session 2 - Sphingolipids in cancer I***

Chair: **Tiago Gil Oliveira** (Braga, PTG)

14:15 - 14:40 **Antonio Gomez-Muñoz** (Bilbao, ESP)

Control of inflammation and lung cancer cell migration by phosphatidic acid and ceramide-1-phopshate

14:40 - 15:05 **Marco Presta** (Brescia, ITA)

β-Galactosylceramidase: a novel player in physiological and pathological Angiogenesis

15:05 - 15:30 **Myles Cabot** (Greenville, USA)

Is the unwanted drug resistance chemotherapy efflux pump, P-glycoprotein, and asset in ceramide-directed therapies?

15:30 - 15:55 **Bruno Ségui** (Toulouse, FRA)

Targeting ceramide metabolism in melanoma to improve efficacy of immune checkpoint inhibitor therapy: from basic mechanisms to the clinic

15:55 - 16:10 **Michele Dei Cas** (Milan, ITA)

Differential expression of Lewis antigens linked to glycosphingolipid in pancreatic cancer

Seminar room, Medical Faculty, FAU Erlangen

14:15 - 16:05 ***Session 3 – Sphingolipids in neurological and psychiatric diseases***

Chair: **Iulia Zoicas** (Erlangen, GER)

14:00 - 14:25 **Tim Cox** (Cambridge, GBR)

Therapeutic understanding of the nervous system of Dr Thudichum

14:25 - 14:50 **Cosima Rhein** (Erlangen, GER)

Sphingolipids in psychosocial stress and the treatment of stress-induced disorders

14:50 - 15:15 **Alessandro Prinetti** (Milan, ITA)

Sphingolipid-dependent membrane organization and signaling orchestrating myelin repair

15:15 - 15:40 **Christiane Mühle** (Erlangen, GER)

Sphingolipid metabolizing enzymes as biomarkers for depression and alcohol use disorder

15:40 - 16:05 **Svjetlana Kalani-Bognar** (Zagreb, CRO)

Partnership of gangliosides and membrane proteins involved in synaptic plasticity and ion homeostasis – new insights and relevance for human neurological disorders

16:05 - 16:30 *Coffee break*

Lecture theater, Medical Faculty, FAU Erlangen

16:30 - 18:20 ***Session 4 - Sphingolipids in neurodegenerative diseases***

Chair: **Alessandro Prinetti** (Milan, ITA)

16:30 - 16:55 **Vittorio Maglione (**Pozzilli, ITA)

Glycosphingolipid pathways in Huntington's disease: new insights into molecular mechanisms and potential therapies

16:55 - 17:20 **Lola Ledesma** (Madrid, ESP)

Sphingomyelin-induced alterations in oligodendrocytes as a trigger for neurodegeneration in acid sphingomyelinase deficiency

17:20 - 17:35 **Lucia Centofanti** (Milan, ITA)

Investigating the impact of Epstein-Barr virus on sphingolipid composition in extracellular vesicles of multiple sclerosis patients

17:35 - 17:50 **Ludovica Pizzati** (Pozzilli, ITA)

Sphingomyelin homeostasis is disrupted in the striatum of HD mouse models and is restored after treatment with THI

17:50 - 18:05 **Giuseppe Pepe** (Pozzilli, ITA)

Treatment with the sphingolipid modulator THI, preserves hippocampal homeostasis and mitigates cognitive deficit in a HD mouse model

18:05 - 18:20 **Museer Lone** (Zurich, SUI)

Dysregulated sphingolipid metabolism in juvenile ALS

Seminar room, Medical Faculty, FAU Erlangen

16:30 - 18:30 ***Session 5 – Sphingolipids in cancer II***

Chair: **Marco Presta** (Brescia, ITA)

16:30 - 16:55 **Stephane Bodin** (Montpellier, FRA)

Flotillins, new players of the sphingolipid metabolism favoring S1P generation to deregulate the endolysosomal traffic in invasive breast cancer cells

16:55 - 17:20 **Sabine Grösch** (Frankfurt, GER)

Ceramide synthases in colon cancer development

17:20 - 17:45 **Miroslav Machala** (Brno, CZE)

Roles of transcription factors AHR, ZEB1 and SNAI1 in deregulation of SL and GSL metabolism during epithelial-to-mesenchymal transition in human bronchial cells

17:45 - 18:10 **Leyre Brizuela** (Lyon, FRA)

Targeting Sphingosine-1-phosphate metabolism as a therapeutic avenue for prostate cancer

18:10 - 18:25 **Mia Jurilj Sajko** (Zagreb, CRO)

LC-MS Characterization of phosphorylated and free sphingoid bases in high- and low-grade gliomas, peritumoral tissues, and serum samples

18:25 - 18:40 **Linda Montavoci (**Milan, ITA)

Lipid dysmetabolism in intrahepatic cholangiocarcinoma-derived tissue

18:40 - *Free Dinner time*

Thursday, Sept. 5, 2024

Lecture theater, Medical Faculty, FAU Erlangen

09:00 - 10:45 ***Session 6 – Sphingolipids in infection and immunity***

Chair: **Timothy Hla** (Boston, USA)

09:00 - 09:25 **Erich Gulbins** (Essen, GER)

Effects of sphingosine on Pseudomomas aeruginosa and Staphylococcus aureus

09:25 - 09:50 **Tsaffrir Zor** (Tel Aviv, ISR)

Sulfatides are endogenous inhibitors of the Gram-positive bacteria sensor TLR2

09:50 - 10:15 **Alexandru Movila (**Indianapolis, USA)

Dihydroceramide produced by periodontal pathogen Porphyromonas gingivalis promotes osteolysis by directly activating intracellular cathepsin B in osteoclast precursors.

10:15 - 10:30 **Anne Ninnemann** (Essen, GER)

Inhibiting acid ceramidase: a promising strategy against malaria

10:30 - 10:45 **Matteo Prisinzano** (Florence, ITA)

Identification of functionally expressed G protein-coupled receptors in endometriotic epithelial cells and characterization of their potential to trigger invasion

10:45 - 11:30 *Coffee break*

11:30 - 13:00 **Poster session**

13:00 - 14:20 *Lunch*

Lecture theater, Medical Faculty, FAU Erlangen

14:20 - 16:10 ***Session 7 – From biochemistry to therapy***

Chair: **Vittorio Maglione** (Pozzilli, ITA)

14:20 - 14:45 **Ashley Cowart** (Richmond, USA)

SPTLC3 and the atypical sphingoid bases in mitochondrial function

14:45 - 15:10 **Stefano Piotto** (Salerno, ITA)

The effect of hydroxylation on sphingolipid membranes: insights from molecular dynamics simulations

15:10 - 15:25 **Luca Mignani** (Brescia, ITA)

Brain accumulation of lactosylceramide characterizes β-galactosylceramidase deficiency in a zebrafish model of Krabbe disease

15:25 - 15:40 **Beatriz Gonçalvez Arede** (Maastricht, NLD)

Identifying and validating ceramide transfer protein binding partners

15:40 - 15:55 **Nadine Merz** (Frankfurt, GER)

Deregulation of ceramide synthases CerS4 and CerS5 affect ZDHHC expression, protein S-palmitoylation and the lipid network

15:55 - 16:10 **Sara Penati** (Milan, ITA)

Unveiling the biochemical secrets of ST3GAL3: a multi-approach characterization of the glycosphingolipid pathway

Seminar room, Medical Faculty, FAU Erlangen

14:20 - 16:20 ***Session 8 – Sphingolipid in organ systems***

Chair: **Erich Gulbins** (Essen, GER)

14:20 - 14:45 **Ewa Gurgul-Convey** (Hannover, GER)

The irreversible degradation of sphingosine-1-phosphate negatively regulates lipid storage capacity of pancreatic beta cells in response to free fatty acids and proinflammatory cytokines

14:45 - 15:10 **Mariana Nikolova-Karakashian** (Lexington, USA)

The sphingolipids of the lipid droplets: Novel functions of neutral sphingomyelinase 2 in the biogenesis of lipid droplets and peri-droplet mitochondria during non-alcoholic fatty liver disease

15:10 - 15:35 **Michael Risner** (Rochester, USA)

Glaucoma is associated with reduced sphingomyelin in human retinal ganglion cells

15:35 - 15:50 **Kylie Morin** (Lexington, USA)

Ceramide homeostasis at the hepatic plasma membrane during non-alcoholic fatty liver disease is regulated by the rate of nSMase2 palmitoylation

15:50 - 16:05 **Johnny Stiban** (Birzeit, PAL)

Fatty liver induction negatively affects mitochondrial respiratory function in female rats

16:05 - 16:20 **Romana Scheffel** (Erlangen, GER)

Deciphering the role of sphingosine-1-phosphate on blood-brain barrier function

16:20 – 16:50 *Coffee break*

Lecture theater, Medical Faculty, FAU Erlangen

16:50 – 18:00 ***Session 9 – Sphingolipid and cell function***

Chair: **Myles Cabot** (Greenville, USA)

16:50 - 17:15 **Thorsten Hornemann** (Zurich, SUI)

1-Deoxy(DH)Ceramides with very long chain acyls (24:0/24:1) are specific mediators of neurotoxicity

17:15 - 17:30 **Federico Fiorani** (Perugia, ITA)

A new role for sphingomyelin in regulating nuclear activity

17:30 - 17:45 **Sara Grassi** (Milan, ITA)

Role of ganglioside GM3 in myoblasts' metabolism and differentiation

17:45 - 18:00 **Susana Mesén-Porras** (San José, CRC)

Sphingolipid-based synergistic interactions to enhance chemosensitivity in lung cancer cells

Seminar room, Medical Faculty, FAU Erlangen

16:50 - 17:50 ***Session 10 – Sphingolipid in circulatory and cardiovascular system***

Chair: **Dragana Fabris** (Zagreb, CRO)

16:50 – 17:05 **Camillo Morano** (Milan, ITA)

The relationship of glucose intolerance/diabetes and overweight/obesity with sphingolipidome alterations

17:05 - 17:20 **Lisa Teresa Porschen** (Lund, SWE)

High-fat diet alters stroke-associated S1P signaling

17:20 - 17:35 **Lotte Vanherle** (Lund, SWE)

SphK2-S1P signaling as a target to lower elevated blood pressure

17:35 - 17:50 **Jiaxin Fu** (Jena, GER)

The impact of sphingosine kinase 1 (SphK1) on endothelial barrier function

19:30 *Bus to Gala Dinner*

20:00 *Gala Dinner and live entertainment, Erlangen*

24:00 *Bus Return*

Friday, Sept. 6, 2024

Lecture theater, Medical Faculty, FAU Erlangen

09:00 - 11:30 ***Session 11 - ISN Symposium: Brain sphingolipids driving psychiatric and neurological disorders: mechanisms and therapeutic targets***

Chair: **Christian P. Müller** (Erlangen, GER)

09:00 - 09:30 **Erhard Bieberich** (Lexington, USA)

When opponents become partners in crime: synergistic function of ceramide and sphingosine-1-phosphate in Alzheimer's disease

09:30 - 10:00 **Hee Kyung Jin** (Seoul, KOR)

Targeting ASM for novel pathogenesis and therapeutics in Alzheimer's disease

10:00 - 10:30 **Céline Galvagnion-Büll** (Copenhagen, DEN)

Lipidomic Profiling Reveals Shared Pathological Signatures in Sporadic Parkinson's Disease and GBA Mutation Carriers: Implications for Disease Mechanisms

10:30 - 11:00 **Tiago Gil Oliveira (**Braga, PTG)

Lipidomic insights into mood and neurodegenerative disorder mechanisms

11:00 - 11:30 **Liubov S. Kalinichenko** (Erlangen, GER)

Brain acid sphingomyelinase – a sex-specific mechanism for drug addiction

11:30 - 12:00 *Coffee break*

Lecture theater, Medical Faculty, FAU Erlangen

12:00 - 13:00 **Session 12 -** ***Sphingolipids in the brain***

Chair: **Christiane Mühle** (Erlangen, GER)

12:00 - 12:25 **Wiebke Herzog (**Erlangen, GER)

Sphingosine-1 phosphate signaling interactions regulating brain angiogenesis versus blood brain barrier formation

12:25 - 12:40 **Angel Gaudioso** (Madrid, ESP)

Acid sphingomyelinase deficiency alters sphingolipidome during neuronal development

12:40 - 12:55 **Daan Van Kruining** (Oxford, GBR)

The role of sphingolipids in GBA-Parkinson's disease

12:55 - 13:10 **Daria Chestnykh** (Erlangen, GER)
The brain sphingolipid system in Schizophrenia and its treatment

13:10 - 13.20 *Picture Time*

13:20 - 14:30 *Lunch*

15:00 *Bus to Bamberg*

16:00 - 18:00 *Guided City tour in Bamberg*

18:00 - 20:00 *Social time: free time in Bamberg*

20:00 - 23.00 *Dinner in Bamberg*

23:00 *Return to Erlangen*

Saturday, Sept. 7, 2024

Seminar room, Psychiatric Clinic, FAU Erlangen

09:00 - 10:00 *General Meeting of the Sphingolipid Club*

10:00 - 10:15 *Closing remarks*

POSTERS

1. **L. Brizuela** (Madrid, ESP) Sphingosine 1-phosphate as an important factor during vascular calcification in chronic kidney disease
2. **F. Cencetti** (Florence, ITA) Sphingosine 1-phosphate signalling is involved in fibroblast plasticity responsible for pulmonary fibrosis
3. **G. Grelle** (Rio de Janeiro, BRA) The role of JAK2/STAT3 on Sphingosine 1-phosphate (S1P) protective effect on human proximal tubule cells submitted to an *in vitro* ischemia model
4. **L. M. Volk** (Frankfurt, GER) The role of plasma membrane Ca^{2+} ATPases in regulation of cytosolic free Ca^{2+} concentrations by sphingosine-1-phosphate metabolism
5. **M. Majewska** (Hannover, GER) Sphingosine-1 phosphate phosphatase 1 overexpression protects against cytokine-mediated beta cell failure
6. **E. Meacci** (Florence, ITA) Sphingosine-1-Phosphate/Sphingosine-1-Phosphate Receptor-Mediated Signaling plays a role in Irisin expression/release in Skeletal Muscle cells
7. **G. Fabrias** (Barcelona, ESP) Targeted degradation of CERT1 with small molecule chimeras directed to the 26S proteasome
8. **D. Derkacz** (Wroclaw, POL) Alterations in *Candida albicans* sphingolipids hydroxylation corresponds with changed plasma membrane dynamics and azoles resistance
9. **H. Nurmi** (Turku, FIN) The glycolipid transfer protein GLTP as a possible regulator of ceramide transfer from the ER to cis-Golgi
10. **F. Fiorani** (Perugia, ITA) Effect of dietary Sphingomyelin and Vitamin D3 in rabbit brain
11. **F. Fiorani** (Perugia, ITA) Plasma acid sphingomyelinase and alteration of taste-smell as sign of long COVID in pregnant women
12. **E. Lassallette** (Toulouse, FRA) Use of whole blood to reveal LPS induced alterations of sphingolipids in lactating cow
13. **A. Pierron** (Toulouse, FRA) Variation of the plasma sphigolipidome in dairy cows during peripartum
14. **A. Vukasinovic** (Perugia, ITA) Sphingomyelin level in bovine meat from organic short supply chain
15. **C. Mühle** (Erlangen, GER) Characterization of neutral sphingomyelinase, acid and neutral ceramidase activities in human plasma and serum
16. **A. Mannini** (Florence, ITA) Ganglioside GD2 in the stem-like compartment of intrahepatic cholangiocarcinoma
17. **D. Capoferri** (Brescia, ITA) Knock-out of β-galactosylceramidase decreases the malignant potential of human melanoma cells
18. **R-M. Bradzis** (Erlangen, GER) Peripheral Upregulation of Parkinson's Disease-Associated Genes Encoding α-Synuclein, β-Glucocerebrosidase, and Ceramide Glucosyltransferase in Major Depression
19. **R-M. Bradzis** (Erlangen, GER) Acid Sphingomyelinase knock-out and overexpression models link synuclein genes expression and behavior in mice
20. **R-M. Bradzis** (Erlangen, GER) Impact of acid sphingomyelinase deficiency on other ceramide related enzymes in mice
21. **P. Hinc** (Wroclaw, POL) Ceramide-1-phosphate modulates binding of α-synuclein to the membrane
22. **M. Koster** (Maastricht, NED) APOE genotype-related hippocampal lipid changes are sex-dependent in a mouse model of familial Alzheimer's disease.
23. **C. P. Gonzales-Guerrero** (Erlangen, GER) Activity pattern of sphingolipid metabolizing enzymes in murine brain regions and peripheral tissues
24. **C. P. Gonzales-Guerrero** (Erlangen, GER) Effect of sphingolipid metabolizing enzymes on BDNF- trkb neurotransmission.

25. **C. P. Gonzales-Guerrero** (Erlangen, GER) Peripheral sphingolipid enzyme activities in masculine depression.
26. **A. Garcia-Ruiz** (Barcelona, ESP) Ganglioside GD3 and liver fibrosis: role of GD3 acetylation
27. **S. Şahoğlu-Göktaş** (Lund, SWE) Blood pressure lowering by sphingosine kinase 2 inhibition improves outcome after myocardial infarction
28. **C. Warden** (Rochester, USA) Visualizing sphingomyelin in the retinal ganglion cell layer.
29. **K. Liliom** (Budapest, HUN) Sphingosine inhibits calmodulin action: Implication for regulation of endothelial nitric oxide synthase-mediated vasorelaxation
30. **S. Naik** (Heidelberg, GER) Loss of Globotriaosylceramide in Kidney Protects Against Acute Cisplatin Injury but Worsens TGF-B1 mediated Epethilial-Mesenchymal Transition

ABSTRACTS

KL1

Human genetic defects of sphingolipid biosynthesis

T. Levade

Unité INSERM 1037, CNRS 5071, Université Toulouse III - Paul Sabatier, Cancer Research Center of Toulouse (CRCT), and Laboratoire de Biochimie, Institut Fédératif de Biologie, CHU Purpan, Toulouse, France

Genetic disorders of sphingolipid degradation, known as lysosomal sphingolipidoses, have been described more than six decades ago. A number of human rare monogenic conditions, transmitted as autosomal recessive or dominant traits, caused by disturbed biosynthesis of sphingolipids have now been identified. These disorders are due to variants in the genes that encode most of the enzymes involved in the synthesis of ceramides, sphingomyelins and glycosphingolipids. In many instances, these variants result in the loss of catalytic function of the mutant enzymes. But additional gene defects implicate the subcellular localization of the sphingolipid-synthesizing enzyme, the regulation of its activity, or even the function of a sphingolipid-transporter protein. As sphingolipids are ubiquitous lipids, some of them being abundant in the nervous system and the stratum corneum, the resulting metabolic alterations lead to two major, non-exclusive types of clinical manifestations : a neurological disease, more or less rapidly progressive, associated or not with intellectual disability, and an ichthyotic-type skin disorder. These phenotypes highlight the critical importance of sphingolipids in brain and skin development and homeostasis. The genetic alterations, biochemical changes and clinical symptoms of this newly individualized group of inherited metabolic disorders will be reviewed. We will also discuss the molecular pathophysiological

T1

Sphingosine 1-phosphate promotion of vascular health in retina and brain

T. Hla

Vascular Biology Program, Boston Children's Hospital, Department of Surgery, Harvard Medical School, Boston, MA 02115, USA

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 the vascular barrier function, blood flow and suppresses EC injury and inflammation. The central nervous system (CNS) endothelium found in organs such as retina and brain have specialized barrier, flow and transport properties to protect the neural retina and brain cells. During CNS vascular development, S1P receptors are needed for vascular maturation and organotypic specialization. In a model of pathological vascular growth that damages CNS parenchymal cells, namely oxygen-induced retinopathy, we showed that HDL-S1P/ EC S1PR1 signaling axis restrains pathological vascular leak and vasoproliferation. We also provide evidence to suggest that this pathway is therapeutically tractable with engineered S1P chaperones derived from ApoM. Inducible deletion of EC S1PR1 in adult mice led to tight junction dysfunction, abnormal vascular leak and cognitive defects without inducing excessive CNS inflammation. This suggests that circulating $ApoM^{+}$HDL-S1P/ EC S1PR1 signaling axis protects the CNS endothelium. In aging, plasma ApoM levels decline which is correlated with cognitive defects. This can be modeled in aged C57BL6 mice, which exhibit reduced plasma ApoM and vascular EC defects. We will present evidence which supports that this age-induced CNS defects may be therapeutically tractable by targeting the S1P signaling axis in the EC. These studies suggest new possibilities to promote the health of the EC which in turn enhances CNS function.

T2

Sphingosine-1-Phosphate from Bench to Clinic

S. Spiegel

Department of Biochemistry and Molecular Biology, Virginia Commonwealth University School of Medicine and the Massey Cancer Center, Richmond, VA, USA.

Sphingosine 1-phosphate (S1P) is a pleiotropic bioactive sphingolipid metabolite that regulates numerous processes important for health and diseases. S1P is generated intracellularly by two sphingosine kinases (SphK1, SphK2) and is exported out of cells by Spinster 2 (Spns2) to exert its effects through activation of five specific cell surface S1PRs in autocrine or paracrine manners. In addition, recent research led to identification of several novel intracellular targets of S1P. In this lecture, I will discuss what we used to know about S1P and what we know now, describe well-established concepts in S1P biology and actions. I will also highlight emerging and new concepts in S1P actions. Finally, I will discuss evolving concepts from bench to clinic of targeting the SphK/S1P/S1PR axis that suggest that it is a useful therapeutic approach for several human diseases.

T3

Regulation of Ca^{2+} homeostasis by sphingosine-1-phosphate: novel insights

D. Meyer zu Heringdorf

Institute of Pharmacology and Toxicology, Goethe-University Frankfurt am Main, University Hospital, Frankfurt am Main, Germany

The bioactive lipid, sphingosine-1-phosphate (S1P) acts via five specific G-protein-coupled receptors (GPCR) to regulate a multitude of cellular functions, including migration, adhesion, proliferation, differentiation and survival. S1P-GPCR couple differentially to G_i, G_q and $G_{12/13}$ proteins to activate their complex downstream signalling pathways, including phospholipase C (PLC)/Ca^{2+} mobilization. However, early S1P research suggested that this mediator could release Ca^{2+} directly from the endoplasmic reticulum, and that sphingosine kinase (SphK)/S1P could serve as intracellular Ca^{2+} mobilization pathway. Further work has shown that S1P metabolism can cause long-term alterations in cellular Ca^{2+} homeostasis. For example, S1P lyase-deficient mouse fibroblasts had augmented $[Ca^{2+}]_i$ increases in response to agonists, elevated resting $[Ca^{2+}]_i$, and enhanced storage of Ca^{2+} in endoplasmic reticulum and lysosomes. This phenotype was caused by alterations in histone acetylation, which have also been observed in other models of S1P lyase depletion. Recently, we have revisited the role of SphK1 in Ca^{2+} signalling, using cells with stable knockdown of SphK1. In this model, we observed a complex alteration in Ca^{2+} homeostasis caused by marked upregulation of the plasma membrane Ca^{2+} ATPases, PMCA1, PMCA4, and their accessory protein, basigin. Furthermore, deletion of SphK1 led to upregulation of SphK2 and S1P lyase and depletion of cellular sphingolipids via this pathway. Finally, the data again suggest a role for enhanced histone acetylation in upregulation of ATP2B1/PMCA1 and basigin. Thus, we show for the first time a transcriptional regulation of ATP2B1/PMCA1 by S1P metabolism, which is of potential functional relevance since PMCA1 is widely expressed and plays important roles in the cardiovascular and nervous systems.

T4

Zonation and mechanisms of engagement of S1PR1 signaling in the murine vasculature.

I. Del Gaudio, A. Nitzsche, E. Camerer

Paris Cardiovascular Research Center, INSERM U970, Paris, France.

Circulating levels of sphingosine 1-phosphate (S1P), a ligand for the endothelial cell (EC) protective S1P receptor-1 (S1PR1), are reduced in disease states associated with endothelial dysfunction. Yet as S1PR1 has high affinity for S1P and can be activated by EC-autonomous S1P production, it is unclear if relative reductions in circulating S1P impact endothelial function. It is also unclear how S1PR1 insufficiency, whether induced by ligand deficiency or by S1PR1-directed immunosuppressive therapy, affects different vascular subsets.
To address these questions, we first mapped the zonation of EC S1PR1-ß-arrestin coupling in the murine vasculature and then superimposed cell type-specific and relative deficiencies in S1P production to define ligand source- and dose-dependence. To correlate receptor engagement to function, we assessed the zonation of vascular integrity defects in mice with EC-selective S1PR1 deficiency. In naïve blood vessels, despite broad expression, EC S1PR1 engagement was restricted primarily to resistance arteries, lung capillaries and high-endothelial venules (HEV). Similar zonation was observed for albumin extravasation in EC S1PR1 deficient mice, and loss of cerebrovascular integrity was reproduced with arterial EC-selective *S1pr1* deletion. While cell autonomous S1P production sustained S1PR1 signaling in HEV, hematopoietic cells sustained signaling in both resistance arteries and lung capillaries. S1PR1 signaling and endothelial function were both sensitive to relative reductions in plasma S1P with an apparent saturation threshold around 50% of normal levels. S1PR1 engagement did not depend on sex or age, but modestly increased selectively in arteries in hypertension and diabetes. Sphingosine kinase (Sphk)-2 deficiency also increased S1PR1 engagement selectively in arteries. The latter could be attributed to Sphk1-dependent S1P release in the vessel wall rather than increased plasma S1P levels observed in Sphk2 deficient mice.
Our observations highlight vessel subtype-specific S1PR1 functions and mechanisms of engagement and supports the relevance of S1P as circulating biomarker for endothelial function.

T5

Therapeutic Assessment of Blocking S1P Transport

W. Santos,[1] R. Fritzemeier[1], K. Dunnavant[1], D. Foster[1], Y. Kharel[2], T. Huang[2], K. Lynch[2]

[1]*Virginia Tech, Department of Chemistry, Blacksburg, Virginia, USA*
[2]*University of Virginia, Department of Pharmacology, Charlottesville, Virginia, USA*

Sphingosine 1-phosphate (S1P) is a bioactive lipid that regulates the growth, survival, and migration of several cell types. Targeting the S1P pathway has resulted in the development of S1P1 receptor modulators (SRMs). While the immunosuppressive activity of SRMs has proved useful in treating autoimmune diseases such as multiple sclerosis and ulcerative colitis, the drug class is hampered b-target liabilities such as initial dose bradycardia. We hypothesize that targeting an upstream node of the S1P pathway may provide an improved adverse event profile. The extracellular S1P that binds to cell surface lymphocyte S1P receptors is provided by S1P transporters Mfsd2b and Spns2. Mice born deficient in spinster homolog 2 (Spns2) are lymphopenic and have low lymph S1P concentrations indicating another avenue to modulate immune cell positioning. Indeed, mice null with Spns2 are protected in the mouse model of multiple sclerosis (experimental autoimmune encephalomyelitis, EAE). In this presentation, we will discuss the development of novel Spns2 inhibitors, provide a molecular basis for binding, characterize their activity both *in vitro* and *in vivo*, and demonstrate their efficacy in the EAE model and kidney fibrosis mouse models. Overall, our studies demonstrate the therapeutic potential of blocking S1P transport with significantly improved safety profile.

T6

Functional interplay between sphingosine 1-phosphate signaling and endocannabinoid system in endometriosis

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Endometriosis is a chronic inflammatory gynaecological disease characterized by the ectopic implantation of functional endometrium outside the uterine cavity associated with pelvic pain and infertility. The pathogenesis of the disease is multifactorial, however the molecular mechanisms involved are complex and far to be fully elucidated.
We recently showed that the signaling of the bioactive sphingolipid sphingosine 1-phosphate (S1P) is deeply dysregulated in endometriosis being the expression of its specific receptors S1P1, S1P3 and S1P5 increased in endometriotic lesions.
The endocannabinoid system (ECS), consisting of the endocannabinoids anandamide (AEA) and 2-arachidonoylglycerol (2-AG), their receptors, CB1 and CB2, and their metabolic enzymes, plays a crucial role in modulating different processes including inflammation.
Here, the involvement of S1P and ECS signaling and their possible cross-talk in endometriosis has been investigated. CB1, CB2 and GPR18, an orphan cannabinoid-like receptor belonging to the class A family of G-protein coupled receptors, have been found to be expressed in endometriotic lesions both at mRNA and protein levels. Furthermore, the effect of 2-AG and AEA in the modulation of inflammation has been investigated in endometriotic epithelial cells. 2-AG, but not AEA, significantly augmented the expression of proinflammatory cytokines as well as COX2. Interestingly, 2-AG-induced increase of S1P3 expression is crucial for the biological action of the endocannabinoid. Indeed, S1P3 pharmacological blockade or its specific silencing impaired the pro-inflammatory action of 2-AG.
In conclusion, these findings demonstrate for the first time the occurrence of a functional interplay between S1P signaling and ECS in endometriosis, paving the way for innovative pharmacological approaches for the treatment of the disease.
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KL2

Alzheimer's - a disease beyond the brain: Insights from sphingolipid metabolism

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The global burden of Alzheimer‘s disease (AD), already the most common type of dementia, is expected to increase still further owing to population ageing. Current major challenges in AD include the lack of reliable biomarkers for its early diagnosis, as well as the lack of effective preventive strategies and treatments. Thus, increased understanding of the novel molecular pathogenesis of AD could lead to the development of improved diagnostic and therapeutic strategies. In this talk, we will discuss the development of therapeutics for AD in the context of novel neuropathological mechanisms including adult neurogenesis, inflammation, immune responses, impairment of autophagy, and vascular dysfunction related with sphingolipid metabolism. The novel therapeutic strategies currently in development based on biological principles, especially two kinds of sphingolipid enzymes such as sphingosine kinase1 (SphK1) and acid sphingomyelinase (ASM), will provide promise for the development of a new generation of therapeutics to prevent and treat AD.

T7

Control of inflammation and lung cancer cell migration by phosphatidic acid and ceramide 1-phosphate

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Initial studies showed that ceramide 1-phosphate (C1P) elicited chemotactic and pro-inflammatory actions in different cell types. However, it was later demonstrated that particularly in lung tissue, C1P was able to inhibit cigarette smoke (CS)-induced airway inflammation, and that it potently attenuated lipopolysaccharide-induced acute lung injury by preventing the activation of pro-inflammatory NF-kB. In the present work, we show that C1P potently inhibits the stimulation of human lung cancer cell migration that was elicited by phosphatidic acid (PA), a glycerophospholipid that has structural similarities to C1P. The mechanisms by which PA stimulates lung cancer cell migration includes prior phosphorylation (activation) of the MAP kinases ERK1-2, p38 and JNK, as well as upregulation of the phosphatidylinositol 3-kinase (PI3K)/Akt/mTOR, focal adhesion kinase (FAK)/Rac1, and JAK2/STAT3 pathways. However, C1P was unable to modify the activity of the latter kinases and proteins, suggesting that the inhibitory effect of C1P on PA-stimulated cell migration is independent of interaction of C1P with any of these pathways. Noteworthy, further investigation into the mechanism by which C1P inhibits lung cancer cell migration revealed that the sphingophospholipid significantly reduced the secretion of interleukin (IL)-8, which is a potent chemoattractant for lung cancer cells, thereby demonstrating that this is at least part of the mechanism whereby C1P blocks the chemotactic effect of PA. The latter findings may set up the basis for development of future therapeutic strategies to treat human lung cancer.

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T8

β-Galactosylceramidase: a novel player in physiological and pathological angiogenesis

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GALC is a lysosomal enzyme that cleaves β-galactose from β-galactosylceramide. Its genetic deficiency is causative of the neurodegenerative Krabbe disease. Previous studies demonstrated that GALC deficient mice and Krabbe patients are characterized by an impaired angio-architecture of brain vessels and that GALC deficient endothelium fails to respond to angiogenic factors *in vitro* and *in vivo*. Together, these data point to a role of GALC in physiological angiogenesis.
The angiogenic process plays a pivotal role in tumor progression. Data from our laboratory have shown that GALC may act as pro-oncogenic enzyme in human cutaneous melanoma, its overexpression in human melanoma cells (upGALC cells) leading to increased proliferation and migration *in vitro* and tumor growth *in vivo*. On this basis, we have investigated the angiogenic potential of GALC in melanoma, whose progression and dissemination are strictly linked to tumor neovascularization.
When upGALC cells were implanted s.c. in mice, upGALC tumor grafts showed a higher vascularization when compared to controls. By mimicking what occurs in tumor microenvironment where GALC is secreted by melanoma cells, the conditioned medium (CM) of upGALC cells exerted a significant angiogenic activity *in vitro* and *in vivo* that was hampered by inhibitors of its enzymatic activity. Accordingly, a catalytically inactive GALC mutant expressed in HEK293T or melanoma cells failed to show a significant angiogenic potential when compared to its wildtype counterpart. In addition, mannose 6 phosphate (M6P), an inhibitor of GALC uptake by M6P receptors, abrogated the angiogenic activity of GALC *in vitro* and *in vivo*. M6P and GALC inhibitors had no effect on the angiogenic potential of the prototypic angiogenic factors VEGF-A and FGF2, thus confirming their specificity. Finally, in keeping with its pro-angiogenic potential, the CM of upGALC cells activates the angiogenic tyrosine kinase VEGF receptor 2 and various downstream signaling pathways in human umbilical endothelial cells.
In conclusion, the data demonstrate for the first time that GALC produced and released by melanoma cells may play a paracrine role in tumor vascularization.

T9

Is the unwanted drug resistance chemotherapy efflux pump, P-glycoprotein, an asset in ceramide-directed therapies?

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Chemotherapy resistance is the leading cause of cancer treatment failure. Upregulated expression of the ATP-binding cassette transporters (ABC), such as P-glycoprotein (P-gp), is a prominent, causative feature in chemotherapy resistance. ABC transporters actively efflux chemotherapy drugs from within the cell, reducing therapeutic impact at the intended site. These transporters are classically plasma membrane localized; however, high concentrations are also present in Golgi, a key site in ceramide anabolic reactions. Ceramide, a sphingolipid (SL) with tumor-suppressor activity, is now recognized as a pivotal element in the cytotoxic mechanism of action of many types of chemotherapy drugs, including the ceramide nanoliposome (CNL). These drugs promote intracellular ceramide levels requisite for initiation of apoptosis. However, working against this benefit is dysfunctional SL metabolism, notably enhanced ceramide glycosylation. This promotes ceramide clearance, a prominent feature in chemotherapy resistant cancer that hijacks the anticancer properties of ceramide. Therefore, blocking ceramide glycosylation to elevate levels of apoptosis-inducing ceramides appears a favorable, therapeutic direction. Although ceramide glycosylation is catalyzed by glucosylceramide synthase (GCS), forming glucosylceramide (GlcCer), we show that the most therapeutically beneficial approach to upwardly manipulate ceramide levels is via the employ of P-gp antagonists and not GCS inhibitors. As many of the ABC transporters "influx" GlcCer into the Golgi for differential glycosphingolipid biosynthesis, targeting this trafficking point is crucial for enhancing ceramide-directed therapeutics. This a story about an unwanted protein being engaged in a therapeutically serviceable manner via use of P-gp antagonists that have been employed in the clinic for decades. (NIH/NCI P01 CA171983)

T10

Targeting ceramide metabolism in melanoma to improve efficacy of immune checkpoint inhibitor therapy: from basic mechanisms to the clinic

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Cutaneous Melanoma represents the main cause of death among malignant skin neoplasms. Immune checkpoint inhibitors (ICI), such as anti-PD-1 or anti-CTLA-4 blocking antibodies, are successful only in a subset of metastatic melanoma patients due to primary or adaptive resistance mechanisms. Abnormal lipid profiles are often associated with an altered metabolic phenotype in tumor cells, which is a hallmark of cancer. Our results led to the identification of ceramide metabolism dysregulation [i.e., downregulation of neutral sphingomyelinase 2 (nSMase2) and upregulation of sphingosine kinase 1 (SK1)] in immune escape and resistance to ICI in murine melanoma models. Reprogramming ceramide metabolism by re-expressing nSMase2 or downregulating SK1 in melanoma cell lines enhances the CD8 T cell-dependent immune response and overcomes the resistance to ICI. Mechanistically, whereas nSMase2 expression increases the small extracellular vesicle immunogenicity, SK1 downregulation reduces the production of key immunosuppressive molecules such as PGE2 and TGFβ, thus limiting the accumulation of tumor-infiltrating regulatory T lymphocyte (Treg). Our results indicate that TNF, which impairs the anti-melanoma immune response and confers resistance to ICI in mice, upregulates sphingolipid production, including glycosphingolipids, in melanoma cell lines. Lastly, analysis of plasma samples from melanoma patients treated with ICI, with or without anti-TNF therapy, revealed significant predictive sphingolipids. Notably, the top 8 predictive sphingolipids, including glycosphingolipids, were associated with a poor response to immunotherapy. When co-administered with anti-CTLA-4 and anti-PD-1, anti-TNF decreased the monohexosylceramide plasma level and increased the total sphingomyelin plasma level. Collectively, our results highlight that reprogramming ceramide metabolism in melanoma enhances the efficacy of ICI therapy in preclinical melanoma models, and ceramide metabolism pattern in plasma can predict clinical outcome.

T11

Differential Expression of Lewis Antigens linked to Glycosphingolipid in Pancreatic Cancer

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Tumor-deranged glycosylation is common in cancer cells, supposedly due to dysregulation of glycosyltransferase or glycohydrolase expression. Lewis antigens are fucosylated cell-surface oligosaccharides that can be carried by both glycoproteins and glycosphingolipids. Their importance in carcer is due to the ability of sialylated Lewis antigens sialyl-Lewis x and sialyl-Lewis a (the epitope of CA 19.9 antigen) to act as ligands for E-selectin promoting cell adhesion, angiogenesis, and cancer invasion. Circulating CA 19.9 is often elevated in gastrointestinal cancers as well as in various benign conditions. Though commonly used for managing pancreatic ductal adenocarcinoma (PDAC), CA 19.9 has limitations due to low specificity, sensitivity, and predictive value. Circulating Lewis antigens like CA 19.9 are carried by mucins, but the role of sphingolipid-linked Lewis epitopes in cancer is still unknown. A preliminary study on matched normal and cancer specimens from PDAC patients (n=10) undergoing surgery revealed no significant difference in mRNA expression of most relevant glycogenes (FUT1, FUT2, B3GALT5, ST3GAL4, ST3GAL6) between normal and tumor tissues, as determined by RT-qPCR. FUT2 expression increased slightly in tumors, while B3GALT5 was downregulated. ST6GALNAC6, FUT3, and ST3GAL3 were significantly deregulated in tumor tissues, with ST6GALNAC6 increased and FUT3 and ST3GAL3 significantly altered. The most striking result seems to be the statistically significant difference between the matched normal and cancer biopsies in lacto- (or neolacto-) glycosphingolipids series, as determined by mass spectrometry. The tumor counterpart of the resections displayed increased levels of different lacto- (or neolacto-) series glycosphingolipids but not in other glycosphingolipid series (e.g. ganglio- or globo- series) or in the simple glycosphingolipids, such as dihydroceramide, ceramide, glucosylceramide, or sphingomyelins. The increase in PDAC biopsies was particularly remarkable on the Lewis antigens such as Lewis A and B; on the contrary the sialyl-Lewis a was found to be undetectable in any conditions. In conclusion, the alteration in the pattern of glycosphingolipids in PDAC patients may be related to the pathological condition and useful to enrich the data brought by glycoproteins.

T12

Therapeutic Understanding of the Nervous System of Dr Thudichum

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Nature is generous in her senseless but cruel experiments on humankind: the inborn errors of sphingolipid metabolism have proved informative for biochemists, cell biologists and physicians alike - they have also greatly enriched the modern pharmaceutical world.
Thudichum chose the ox and human brain for his enduring research on sphingolipids but hitherto practically all spectacular therapeutic applications related to his discoveries relate to their rôle in immunity and diseases affecting the bone marrow and visceral organs. This is exemplified by the approval of therapies in three classes for one orphan disease; two attract generous 'blockbuster' scale revenues.
Many sphingolipid diseases affect the brain but despite extensive studies into their pathogenesis, nearly all therapeutic initiatives have been unavailing. Even when obstacles for drug delivery across blood-brain barrier are overcome, it is clear that so far as the biochemistry of sphingolipids and related disorders is concerned, the brain is, simply put: not the same!
In this presentation we consider disparities in the metabolism of sphingolipids in different compartments, pitfalls for identifying and accessing disease targets in sphingolipid diseases. Current examples will include in classical rare as well as common diseases of humankind.

T13

Sphingolipids in psychosocial stress and the treatment of stress-induced disorders

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Chronic stress is a risk factor for developing stress-induced mental disorders like posttraumatic stress disorder and major depression. Low-grade inflammatory processes seem to mediate this association. The sphingolipid metabolism was shown to play an important role in the pathophysiology of affective disorders and inflammation. We conducted an explorative trial to investigate the effect of intensive psychosomatic - psychotherapeutic treatment of stress-induced disorders on the biological level. Before and after eight weeks of treatment, blood plasma of 67 patients was analyzed for sphingolipid levels and their metabolizing enzymes. Symptom severity of depression (PHQ-9), anxiety (GAD-7), and somatization (PHQ-15) was assessed in parallel. During psychosomatic - psychotherapeutic treatment, symptom severities decreased significantly. Enzymatic activities of secreted acid sphingomyelinase (S-ASM), neutral sphingomyelinase (NSM), and neutral ceramidase (NC) increased significantly upon treatment. The molar ratio of ceramide species Cer16:0 and Cer18:0 decreased upon treatment, whereas sphingosine and S1P levels increased significantly. Thus, psychosomatic – psychotherapeutic treatment impacts the sphingolipid metabolism towards increased sphingosine and S1P levels, and reduces the ratio of specific ceramide species, potentially resulting from increased activity of sphingolipid metabolizing enzymes. Stress-induced mental disorders might be associated with disturbed sphingolipid levels that seem to be balanced during psychosomatic treatment. This study offers a further piece of evidence that the sphingolipid metabolism could be involved in the pathophysiology of stress-induced disorders, and its analysis could be helpful for treatment monitoring.

T14

Sphingolipid-dependent membrane organization and signaling orchestrating myelin repair

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Recombinant human IgM22 (rHIgM22) binds to myelin and oligodendrocytes (OLs) and promotes remyelination in all of the mouse models of multiple sclerosis. However, its molecular and cellular targets antigen and its mechanisms of action are still unclear.
We showed that 1) rIHgM22 binds to sulfatide and lysosulfatide, but also to phosphatidylinositol, phosphatidylserine and phosphatidic acid; 2) changes in the composition of the lipid microenvironment of the target antigen can modulate the affinity of the antibody, suggesting that reorganization of lipid membrane microenvironment might be relevant in its biological activity.
rHIgM22 induced proliferation in rat mixed glial cells (MGCs), with the most significant response associated with astrocytes, and increased the production and release of sphingosine 1-phosphate (S1P). rHIgM22 treatment did not induce changes in the release of S1P in pure astrocyte or OPCs cultures, but it increased S1P release in BV-2 microglia cells, suggesting that rHIgM22 indirectly influences astrocytes proliferation via microglia-released S1P.
rHIgM22 had no effects on glycosphingolipids in MGCs and pure astrocytes, while in OPCs, OLs and BV-2 microglia we observed a significant increase in the levels of GM3 and GD3 gangliosides. Thus, we hypothesize that rHIgM22 myelin-repair activity could be at least in part mediated by alterations of lipid-dependent membrane organization in OPCs, OLs and microglia. The finding that rHIgM22 treatment affected in opposite ways two sphingolipid metabolic enzymes further supports this hypothesis. rHIgM22 treatment in differentiated OLs reduced the activity of the acid sphingomyelinase (a key mediator for the detrimental effects of ceramide observed in mouse models of MS). Noteworthy, treatment with the chemokine fractalkine, able to induce oligodendrogliogenesis, significantly reduced ceramide levels in OPCs and OLs. On the other hand, S1P treatment in MGCs induced an increased expression of the galactocerebrosidase (known to be important to preserve the efficiency of myelin repair).
In conclusion, rHIgM22 exerts its protective effects by acting directly or indirectly on different glia populations involved in the mechanism of myelin repair, with sphingolipids being always key players.
In conclusion, rHIgM22 exerts its protective effects by acting directly or indirectly on different glia populations involved in the mechanism of myelin repair, with sphingolipids being always key players.

T15

Sphingolipid metabolizing enzymes as biomarkers for depression and alcohol use disorder

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Sphingolipids and their metabolizing enzymes are gaining increasing attention in neuropsychiatric disorders. Our research focuses on the activities of ceramide-centered enzymes, including acid and neutral sphingomyelinase, acid and neutral ceramidase, and sphingomyelin synthase, to better understand their roles in physiological and pathophysiological processes and assess their potential as biomarkers in alcohol addiction and major depressive disorder in both human clinical studies and animal models.
The role of sphingolipids and acid sphingomyelinase in depression has been well-documented in various studies, including those using knockout and transgenic mice, which exhibit reduced and increased levels of depression-like behavior, respectively. Elevated ceramide levels and acid sphingomyelinase activity have been observed in depressed patients, with many antidepressants acting as functional inhibitors of this enzyme. Initially increased enzyme activities were linked to greater improvements in depression severity in patients during a three-week treatment. Notably, leukocyte activity and gene expression levels of sphingomyelin synthase were also elevated in depressed patients, suggesting this enzyme as a potential target for fast-acting antidepressants via autophagy induction.
Several studies have demonstrated increased lysosomal and secretory acid sphingomyelinase activity in alcohol-dependent patients of both sexes, which decreases within days of detoxification and correlates with markers of alcohol consumption, such as liver enzyme activities and myelosuppression. First data also suggest similar elevations in neutral sphingomyelinase activity in these patients. Additionally, in a recent study, we observed a correlation between serum acid sphingomyelinase activity and binge drinking markers in female depressed patients.

We are expanding our activity assays to include additional enzymes and biomaterials, aiming to deepen our understanding of disease mechanisms and identify novel therapeutic targets in the field of sphingolipids.

T16

Partnership of gangliosides and membrane proteins involved in synaptic plasticity and ion homeostasis – new insights and relevance for human neurological disorders

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Gangliosides, membrane glycosphingolipids, display high structural diversity, abundance and specific expression patterns in the mammalian brain. Gangliosides are known as culprits for lysosomal storage diseases arising from inefficient ganglioside catabolism. In early 2000s, new evidence has revealed that altered biosynthesis of gangliosides may also lead to a disease - a rare familial epilepsy. In addition, altered metabolism of brain gangliosides has been evidenced in Alzheimer's and Parkinson's disease. Majority of (patho)physiological effects of gangliosides is related to nervous system, as shown by investigating phenotypes of genetically modified mouse models with disrupted ganglioside synthesis. Being membrane constituents, localized mostly within organized lipid microdomains, some of the functions of gangliosides include modulation and fine-tuning of variety of membrane proteins. Here we present research which clarifies influence of specific ganglioside environment on the positioning and functions of proteins important for synaptic plasticity and maintenance of ion homeostasis: neuroplastin (Np) whose brain-specific isoform is expressed in synapses and participates in long-term potentiation; plasma membrane calcium ATPase (PMCA) which controls cellular concentration of calcium ions and requires neuroplastin as an auxiliary subunit; Na^+/K^+-ATPase (NKA) which maintains the membrane potential. By combining several methodological approaches for analysis of brain tissue samples derived from GM2/GD2 synthase-deficient mice compared with control samples, and cultured rat hippocampal neurons, we found that: 1) ganglioside GM1 colocalizes with neuroplastin in lipid rafts and stabilizes functional Np/PMCA assembly by maintaining calcium transients; 2) changed ganglioside composition affects catalytic activity of NKA and its redistribution within membrane. In conclusion, we propose that specific gangliosides act as essential partners of here analyzed proteins involved in membrane ion transport and molecular events underlying learning and memory. Further investigation of the exact nature of structural and functional interactions between gangliosides, ATPases and neuroplastin within neuronal membranes may add to understanding of biochemical basis of epilepsy and neurodegeneration.

T17

Glycosphingolipid pathways in Huntington's disease: new insights into molecular mechanisms and potential therapies

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Huntington's disease (HD), a fatal genetic and rare neurodegenerative disorder, is characterized by a progressive striatal and cortical neurodegeneration, associated with motor, cognitive and behavioral disturbances.
Among all the molecular mechanism defective in HD, perturbed metabolism of gangliosides, glycosphingolipids with a plethora of functions in the brain, has been reported to play a critical role in the pathogenesis of the disease.
In the last few years, our research group has extensively demonstrated that the metabolism of other sphingolipids, such as Sphingosine-1-phosphate (S1P), is defective in HD, even at an early stage of the disease. We have also shown that modulation of S1P axis is beneficial in different HD preclinical models.
More recently, we also discovered an interesting link between the metabolism of S1P and the accumulation of glucosylceramide (GluCer) in HD pre-clinical models.
Our findings demonstrate that pharmacological interventions aimed at modulating S1P levels are able to normalize GluCer content in a HD animal model. This is associated with an amelioration of disease phenotype e with a modulation of neuroprotective pathways.
Collectively, our findings support the concept that the alteration of (glyco)sphingolipid pathways may contribute to HD pathogenesis and may be eventually pharmacologically targeted.
Our thought is supported by the evidence that some drugs, whose molecular targets belong to these pathways, are already in clinical trial for different other diseases and could be eventually repurposed for the treatment of neurodegenerative conditions and/or serve as a tool for the development of new ones.

T18

Sphingomyelin-induced alterations in oligodendrocytes as a trigger for neurodegeneration in the acid sphingomyelinase deficiency

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Acid Sphingomyelinase Deficiency (ASMD) is a lysosomal storage disorder (LSD) caused by mutations in the gene encoding the acid sphingomyelinase (ASM), leading to cellular accumulation of sphingomyelin (SM). Pathological hallmarks of the fatal infantile neurovisceral form of ASMD include demyelination, microgliosis and progressive neurodegeneration, with an early death of cerebellar Purkinje Cells.
Myelination in the central nervous system relies on mature oligodendrocytes (OLs), which develop a modified plasma membrane that wraps axons, providing them support and protection and controlling the nervous conduction. Defects in myelin have been traditionally assigned a secondary pathological role in LSDs. However, recent studies from our laboratory pointed to demyelination as the triggering factor for microglia dysfunction and neuronal death in ASMD. Deciphering the causes of demyelination is thus important to understand and treat ASMD pathology.
To this aim we have used the mouse model for the disease, which lacks the ASM (ASMko). Our results *in vitro* and *in vivo* evidenced defects in OL differentiation caused by SM accumulation. RNA sequencing analysis of OL precursor cells (OPCs) and mature OLs from ASMko mice identified defects in different pathways in ASMko OPCs and mature OLs. Components of the extracellular matrix binding implicated in OL inhibition stood out from the deregulated genes. Pharmacological attempts to ameliorate OL differentiation in ASMko conditions will be discussed.

T19

Investigating the Impact of Epstein-Barr Virus on Sphingolipid Composition in Extracellular Vesicles of Multiple Sclerosis Patients

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Background. Multiple sclerosis (MS) is a chronic autoimmune disease characterized by the immune system's attack on the central nervous system, leading to demyelination and neurodegeneration. Epstein-Barr virus (EBV) has been recognized as a crucial risk factor in the development of MS, as almost all MS patients show evidence of past EBV infection. Extracellular vesicles (EVs) have been proposed as key players in the development of MS, due to their immunomodulatory potential and ability to cross the blood-brain barrier. Moreover, an increase in EBV-related proteins has been found in the circulating EVs of MS patients. Recent studies suggested that sphingolipid metabolism may be disrupted in MS, contributing to disease process. In light of this evidence, our aim was to study a possible link between MS and EBV, by assessing selective changes in EV sphingolipids.
Methods. Plasma-derived EVs from an exploratory cohort (n=24) of MS patients, on anti-CD20 or on other immunomodulating therapies, and healthy controls, were isolated by ultracentrifugation. Size and number of EVs was determined by nanoparticle tracking analyzer (NTA). Sphingolipids from patients' plasma and EVs were analyzed by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). The same approach was carried out on EVs from EBV- and EBV+ B lymphocyte-derived cell lines.
Results. EVs produced by EBV+ cell lines showed a significant increase in the concentration of dihydroceramides, ceramides, hexosylceramides, gangliosides GM3 and globosides GB3 (the last two precursors of the ganglio and globo series respectively) (all, $p<0.05$). No significant changes were observed in the plasma of MS patients, while a significant increase in sphingomyelins and hexosylceramides was seen in EVs from patients in anti-CD20 therapy compared to those from patients on other immunomodulating therapies (both, $p<0.05$).
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T20

Sphingomyelin homeostasis is disrupted in the striatum of HD mouse models and is restored after treatment with THI.

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Sphingolipids are essential components of biological membranes and play key functions in the homeostasis of the central nervous system.
Huntington's disease (HD), is a rare inherited disorder with no definitive cure, characterized by degeneration of specific brain areas, such as the striatum and cortex.
Previous studies have shown that HD is characterized by profoundly altered levels of certain sphingolipids, such as gangliosides and sphingosine-1-phosphate (S1P), and that their metabolism may be a potential therapeutic target.
In this study we investigated whether these alterations also involved sphingomyelin (SM), one of the most abundant sphingolipids in brain cells.
Both WT and R6/2 mice were treated with either vehicle or THI (0.1mg/kg) for five weeks. Lipidomic analyses was performed on the striatal tissue of the mice with LC-MS/MS.
The results obtained indicate that sphingomyelin levels are higher in the striatum of a mouse model of HD (R6/2 transgenic mice) than in controls and this correlate by aberrant gene and protein expression of the enzymes that metabolize it. Interestingly alteration in SM metabolizing enzymes, were found also in heterozygous zQ175 mice. Furthermore, infusion of THI, an inhibitor of S1P degradation, modulates sphingomyelin levels and the expression of genes/proteins involved in its metabolism in the striatum of R6/2 mice.
In conclusion, these data further support the idea that alterations in sphingolipid metabolism may be a crucial factor in the etiopathogenesis of the disease and possibly represent a potential therapeutic target.

T21

Treatment with the sphingolipid modulator THI, preserves hippocampal homeostasis and mitigates cognitive deficit in a HD mouse model

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A number of evidence, demonstrates that defective (glyco)sphingolipid pathways may contribute to the pathogenesis of Huntington's disease (HD). In particular, aberrant metabolism/levels of both simple (Sphingosine-1-Phosphate - S1P -) and complex (Glucosylceramide and Gangliosides) sphingolipids has been reported in both HD pre-clinical models and human post-mortem brains from patients.
In this study, we investigate the ability of THI, an inhibitor of S1P degradative enzyme SGPL1, in counteracting cognitive dysfunctions in HD mice.
Both WT and R6/2 mice were treated with either vehicle or THI (0.1mg/kg) for five weeks. Cognitive deficits were assessed by Novel Object Recognition test and Y-Maze Test. Western Blot and qPCR analyses were performed to assess protein and gene expression profiles, respectively.
THI treatment mitigated mouse cognitive deficit, modulated (glyco)sphingolipid pathways and preserved hippocampal homeostasis. The compound also stimulated the autophagic flux and the endo-lysosome system, facilitating the reduction of mutant huntingtin aggregates.
This study confirmed the ability of THI to counteract the disease phenotypes observed in HD mice, and further support the notion that the modulation of (glyco)sphingolipid homeostasis may represent and effective therapeutic option for the disease.

T22

Dysregulated sphingolipid metabolism in juvenile ALS

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Amyotrophic lateral sclerosis (ALS) is a devastating neuromuscular disorder, characterized by motor neuron degeneration and muscle wasting, leading finally to paralysis and death. Etiologies for ALS are heterogeneous but rarely genetic. We recently reported specific mutations in *SPTLC1* as a cause for juvenile ALS. SPTLC1 is an essential subunit of the serine palmitoyltransferase (SPT) enzyme. SPT catalyzes the first rate-limiting step in the de novo sphingolipids (SL) synthesis. SPT conjugates palmitoyl-CoA with L-serine to form long chain bases (LCB), the identifying structural moieties of SL.

SPT interacts with regulatory, ORMDL-1, -2 and -3 proteins that inhibit SPT activity in response to cellular SL levels. A single SPTLC1 transmembrane domain (TMD) assists SPT interaction with ORMDLs. Previously, mutations in SPTLC1 catalytic domain were associated with the Hereditary Sensory and Autonomic Neuropathy type 1 (HSAN1). The HSAN1 mutations shift substrate specificity of SPT enzyme from serine to alanine that leads to the formation of neurotoxic 1-deoxysphingolipids (1-deoxySL). In contrast, SPT-ALS mutations occur in the TMD, and alter SPT-ORMDL interaction, therefore, disrupting the complex formation. Using HEK293 SPTLC1 knockout cells, we showed that ALS mutations impair feedback inhibition of the enzyme. SPT-ALS mutations thus lead to unregulated canonical SL synthesis.

We also deduced lipid signatures specific to SPT-ALS and HSAN1 mutations in HEK293 cells, patient plasma, and patient derived primary fibroblasts. These SL species could serve as markers for identifying the disease form in patients carrying SPT mutations. Targeting genetically the mutant mRNA for degradation, restored feedback inhibition and SL profiles in patient derived fibroblasts. Most interestingly, relative serine and alanine availability in SPT-ALS expressing cells increased shifted the SL profile from an ALS to an HSAN1-like signature. This effect was corroborated in an SPTLC1-ALS pedigree in which the index patient uniquely presented with increased 1-deoxySL levels, and an L-serine deficiency. Even when the family members inheriting the same mutation developed ALS, the index patient showed sensory phenotypes observed in HSAN1. These data demonstrate how pathogenic variants in different domains of SPTLC1 give rise to distinct clinical presentations that are nonetheless modifiable by substrate availability.

T23

Flotillins, new players of the sphingolipid metabolism favoring S1P generation to deregulate the endolysosomal traffic in invasive breast cancer cells.

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Overexpression of flotillins is observed in many invasive tumors and is a marker of poor prognosis in breast cancer. Flotillins 1 and 2 are functionally non-redundant., they form hetero-oligomers at the cytosolic leaflet of membranes that scaffold sphingolipid rich domains. Upregulation of flotillins promote cell invasion by exacerbating an endosomal trafficking pathway called the upregulated flotillin-induced trafficking (UFIT) pathway. We showed that overexpression of flotillins, favoring their oligomerization, promotes the formation of endocytic sites at the plasma membrane and the accumulation of flotillins in endolysosomes harboring a secretory function. MT1-MMP and AXL are cargoes of the UFIT-pathway. In cells overexpressing flotillins, flotillin-mediated endocytosis drives MT1-MMP towards flotillin-rich endolysosomes, from which it is efficiently secreted to promote extracellular matrix degradation and cell invasion. We also identified the UFIT-pathway as a new mechanism participating in AXL overexpression and downstream oncogenic signaling.
The molecular mechanisms of the UFIT-pathway remain elusive. Flotillins bind sphingosine (Sph) and we observed an accumulation of Sph in flotillin-positive endolysosomes. Our study showed that flotillin-mediated deregulations of the endosomal trafficking are coupled to deregulations of the sphingolipid metabolism. Comparing the sphingolipidomic profiles of two parental breast cancer cell lines (Hs578T and MDA-MB-231) endogenously expressing high flotillin levels with their flotillin-deficient counterparts, revealed that high flotillin levels influence the Sph1-phosphate (S1P)/ceramide rheostat in favor of S1P production at the expense of ceramide levels. Considering the emerging roles of S1P in membrane remodeling and endolysosomes properties, we investigated the involvement of Sph-kinases (SphK) in the UFIT-pathway. We demonstrated a preferential role of SphK2 over SphK1. SphK2 is recruited at flotillin-endocytic sites and at flotillin-rich endolysosomes. Its inhibition abolishes the fast flotillin-mediated endocytosis of AXL and its overexpression in cancer cells. We are currently investigating how flotillin accumulation on endolysosomes can, via S1P generation, modulates their cholesterol content and their interaction with the endoplasmic reticulum, two parameters known to influence the endolysosome secretory function.

T24

Ceramide Synthases in Colon Cancer Development

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Colon cancer is one of the most common cancers worldwide. Various molecular pathways have been identified to be involved in the development of colon cancer. However, most times gene- or protein-expression data are used but cellular functions depend also on cellular membranes either as shelters from the environment or involved in intracellular compartmentalisation and signalling. Sphingolipids are important membrane compounds and influence membrane physicochemical properties by their chain length and grade of saturation, accordingly, changes in different sphingolipids impact membrane proteins and their activity. Ceramide synthases 1-6 (CerS 1-6) are central enzymes of the sphingolipid pathway and responsible for the synthesis of ceramides with different chain length. Human primary colon tumor data and protein Atlas data indicate that CerS5 and CerS4 are prognostic markers for late stage colon cancer patient outcome. CerS4 and CerS5 decrease after hypoxia in colon tumor cells and depletion of both enzymes enhance tumor proliferation *in vitro* and *in vivo* in the nude mice model. Lipidomic, proteomic and metabolomics data from CerS4- and CerS5-depleted human colon cancer cells indicate that CerS4 depletion enhanced the Warburg effect in aggressive growing colon cancer cells, whereas CerS5 abatement leads to an enhanced tumor growth by upregulation of oncogenes in lipid raft fractions in these cells. Furthermore, proteome data give a comprehensive overview about changes in protein expression after sphingolipids deregulation and uncover new correlations between the lipid homeostasis and metabolic changes in colon cancer cells. So downregulation of CerS4 and CerS5 in late-stage colon cancer induces various cellular changes linked to hallmarks of cancer progression. Therefore, reduced expression of CerS4 and CerS5 in colon tissue of late-stage tumors are predictive markers for patient outcome.

T25

Roles of transcription factors AHR, ZEB1 and SNAI1 in deregulation of SL and GSL metabolism during epithelial-to-mesenchymal transition in human bronchial cells

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INTRODUCTION. of the alterations of expression of genes linked to sphingolipid (SL) and glycosphingolipid (GSL) metabolism and changes in sphingolipidomic profiles during cancer development and progression still remain only partially characterized. Here, we evaluated potential roles of three transcription factors contributing to deregulation of SL/GSL metabolism occurring during epithelial-to-mesenchymal transition (EMT) of normal bronchial epithelial cells HBEC-12KT, which was induced by a chronic exposure to environmental carcinogen benzo[a]pyrene (BaP). The aryl hydrocarbon receptor (AHR) is a transcription factor well-known for its roles in xenobiotic metabolism; however, it may also belong among important regulators of endogenous lipid metabolism and tumor development. ZEB1 and SNAI1 are two transcriptional regulators of EMT, which help to maintain mesenchymal status of tumor cells.
RESULTS. When modulating chemically the AHR transcriptional activity in wild type HBEC-12KT cell line, we observed upregulation of sphingosin-1-phosphate and glucosylceramides after AHR activation, which may contribute to the induction of early phase of EMT by BaP. Using siRNA-mediated silencing of ZEB1 or SNAI1 we observed significant modulations of SL/GSL gene expression and SL/GSL metabolism in the BaP-transformed mesenchymal-like HBEC-12KT-B1 cells. The ZEB1 knockdown significantly altered expression of SPHK2, SGPL1, GBA2, B4GALT5, B4GALT6, ST8SIA4, B4GALNT1, A4GALT, HEXA, HEXB and several other genes, accompanied with significant changes in SL and GSL levels. The SNAI1 knockdown indicated that this transcription factor may support increased biosynthesis of several SL species, including sphingosine-1-phosphate and sphingosine, and it had a partial impact on control of GM and GD synthesis pathways. The CERK, B4GALT5, ST3GAL5, ST8SIA5 and A4GALT were among the most deregulated genes in cells with silenced SNAI1.
CONCLUSIONS. While the AHR may contribute to the changes in SL/GSLs during early stages of EMT, both ZEB1 and SNAI1 seem to play important, and partly complementary, roles in deregulation of a series of genes related to SL/GSL metabolism in fully transformed mesenchymal-like HBEC-12KT-B1 cells. Deregulation of SL and GSL metabolism plays various functional roles in diseases including carcinogenesis and malignant tumor progression. The present data indicate the need to further explore the mechanisms regulating SL/GSL levels and functions.
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T26

LC-MS Characterization of Phosphorylated and Free Sphingoid Bases in High- and Low-Grade Gliomas, Peritumoral Tissues, and Serum Samples

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Background: Gliomas are the most common primary brain tumors, with glioblastoma being the most aggressive one. Sphingoid bases (SBs) and phosphorylated SBs are important signaling molecules that influence cell survival, proliferation, mobility, and play a significant role in the pathogenesis of gliomas. The aim of this study was to analyze and compare the content of free SBs (d16:1, d17:0, t18:0, d18:0, d18:1, d20:0, d20:1) and phosphorylated SBs (d18:0-P, d18:1-P) in 14 adult-type diffuse gliomas samples: 7 low-grade gliomas, LGG (oligodendrogliomas, IDH^{mut}, grade 2, and astrocytomas, IDH^{mut} grade 2) and 7 high grade gliomas, HGG (GBM, IDH^{wt}, grade 4, gliosarcomas, grade 4 and astrocytomas, IDH^{mut}, grade 3), corresponding peritumoral tissues (PTs) and patients' serums (PSs). **Methods:** Free SBs and phosphorylated SBs were extracted from tissue homogenates and serum samples and analyzed by Agilent 6550 iFunnel Q-TOF LC/MS by MRM analysis. **Results:** The free SBs d18:1, d18:0, and d20:1 were quantified in all tissue samples, with sphingosine (d18:1) being the most abundant base. In HGG samples, d18:1 was detected in higher concentrations compared to LGG samples, while its concentrations were higher in LGG PT than in HGG PT samples. In HGG PSs d18:1 was quantified in significantly higher proportions in comparison to LGG PSs. d18:1-P was detected in all tissue samples in significantly lower concentrations than in PSs, while d18:0-P was quantified only in PSs, in lower concentrations than d18:1-P. In HGG PSs d18:1-P was quantified in significantly higher concentrations than in LGG PSs, while d18:0-P was quantified in approximately same concentration in both HGG and LGG PSs. The levels of d18:1-P were higher in HGG samples than in HGG PT samples, while lower in LGG samples compared to LGG PT samples. Additionally, d18:1-P levels were higher in LGG compared to HGG samples and significantly higher in LGG PT than in HGG PT samples. **Conclusion:** The distinct sphingolipid profiles between high-grade and low-grade gliomas may provide insights into the sphingolipid rheostat imbalance underlying their pathogenesis, especially with further analysis of ceramide content.

T27

Targeting Sphingosine 1-Phosphate Metabolism as a Therapeutic Avenue for Prostate Cancer

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Prostate cancer (PC) is the second most common cancer in men worldwide. More than 65% of men diagnosed with PC are above 65. Patients with localized PC show high long-term survival, however with the disease progression into a metastatic form, it becomes incurable, even after strong radio- and/or chemotherapy. Sphingosine 1-phosphate (S1P) is a bioactive lipid that participates in all the steps of oncogenesis including tumor cell proliferation, survival, migration, invasion, and metastatic spread. The S1P-producing enzymes sphingosine kinases 1 and 2 (SK1 and SK2), and the S1P degrading enzyme S1P lyase (SPL), have been shown to be highly implicated in the onset, development, and therapy resistance of PC during the last 20 years. First, I will present the most important studies demonstrating the role of S1P and S1P metabolic partners in PC. Second, I will explain the different *in vitro, ex vivo*, and *in vivo* models of PC that were used to demonstrate the implication of S1P metabolism. Third, I will summarize the most efficient molecules targeting S1P metabolism that are under preclinical and clinical development for curing PC. Finally, I will discuss the possibility of targeting S1P metabolism alone or combined with other therapies in the foreseeable future as an alternative option for PC patients.
Keywords: bone metastasis; prostate cancer; sphingosine 1-phosphate; sphingosine kinase.

T28

Lipid dysmetabolism in intrahepatic cholangiocarcinoma-derived tissue

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Introduction: Intrahepatic cholangiocarcinoma (iCCA) is the second most common liver cancer. Cancer cells often alter their metabolism to support growth, and lipids are essential for cell membranes, energy, and signaling. Information on the iCCA lipidome profile is still lacking, but it is known that fatty acid (FA) synthesis is reduced while FA uptake is increased via specific transporters. Several studies demonstrated that tumors address FA to cell proliferation and energy storage through lipid droplets (LDs), whose have been suggested to be associated with cancer cell growth, resistance, and stem cell functionality. In this study, we investigated the iCCA lipidome in paired iCCA and non-tumour (NT) liver tissue and serum from iCCA patients vs healthy controls (HC), focusing on fatty acid metabolism.
Results: The untargeted lipidome profile, performed with liquid chromatography-tandem mass spectrometry, showed a clear-cut separation between iCCA and NT tissues as well as between iCCA and HC sera. Indeed, FA accumulated both in iCCA tissue and patients' sera. Expression of Acetyl-CoA Carboxylase Alpha *ACACA*, the rate-limiting enzyme of *de novo* lipogenesis, was higher in iCCA tissue than in NT tissue. *FABP5* expression was higher in iCCA tissue while the expression of *FABP4* and *CD36* was down-regulated in iCCA tissue. Expression of Acyl-CoA Dehydrogenase Medium chain *ACADM*, which catalyses the initial step of FA β oxidation, was lower in iCCA tissue compared with NT tissue. LD were found in the cytoplasm of hepatocytes and macrophages of non-neoplastic parenchyma. Conversely, only small amounts of LD were found in neoplastic cells, while clusters of small droplets were found in the extracellular matrix and in intratumoral fibrous tissue. Several species of phospholipids and sphingolipids, the main components of plasma membranes, were upregulated in iCCA compared with NT tissues.
Conclusions: These findings suggest that FA accumulation could promote iCCA aggressiveness by supporting membrane biogenesis and generation of bioactive lipids. Differences in lipidome serum profile between iCCA patients and HC suggest a mean to identify individuals with iCCA by liquid biopsy.

T29

Effects of sphingosine on *Pseudomomas aeruginosa* and *Staphylococcus aureus*

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We have shown that sphingosine kills pathogenic bacteria such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Acinetobacter baumanii*, *Escherichia coli*, *Haemophilus influenzae* and *Burkholderia species*. Here, we demonstrate that sphingosine also kills Mycobacteria such as *Mycobacteria abscessus* or BCG. Sphingosine induces rapidly kills these pathogens *in vitro*, but also eliminates *P. aeruginosa*, *S. aureus* and *BCG* in the lungs of infected mice or pigs *in vivo*. We further show that sphingosine targets both, extracellular and intracellular bacteria, and accumulates in the pathogens. Mechanistically, we demonstrate a direct binding of sphingosine to cardiolipin present in bacterial membranes and thereby resulting in bacterial permeabilization. In addition, sphingosine also alters biochemical aspects of the bacteria that also contribute to killing of the bacteria. In summary, our data show that sphingosine kills pathogenic bacteria *in vitro* and *in vivo* by a combination of biophysical and biochemical alterations.

T30

Sulfatides are endogenous inhibitors of the Gram-positive bacteria sensor TLR2

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Introduction: Toll-like receptor 2 (TLR2) senses pathogen-associated molecular pattern (PAMP) molecules derived from Gram-positive bacteria, e.g. lipopeptides, and combines with TLR1 or TLR6 to initiates inflammation. Additionally, TLR2 is also thought to recognize endogenous danger-associated molecular pattern (DAMP) molecules released from stressed or dying cells, leading to autoinflammation and autoimmunity. Yet, the identity of such endogenous TLR2 ligands is still poorly understood. We recently reported that three molecules of the endogenous lipid C16-sulfatide (3-O-sulfogalactosylceramide) can activate mouse TLR4/MD-2 by mimicking LPS, its known ligand – a PAMP derived from Gram-negative bacteria. In contrast, C16-sulfatide inhibits LPS activity in human macrophages. In the current research, we examined the activity of sulfatides toward TLR2.
Results: Short (C16) and long (C24) fatty acid chain sulfatides competitively inhibit the activity of the TLR2/6 agonist Pam2Cys in human macrophages. The inhibitory activity of C16-sulfatide towards hTLR2 occurs at concentrations that are more than an order of magnitude lower than those required for hTLR4 inhibition (K_I = 0.15 and 1.45 μM, respectively), and it is also demonstrated in mouse cells. Based on the observed competition and the partial chemical similarity with the lipopeptide, we hypothesized that sulfatides could mimic the TLR2 agonist. Indeed, molecular docking simulation has shown that a single sulfatide molecule fits into the hydrophobic pocket of TLR2, similarly to the lipopeptide.
Conclusions: Sulfatides, natural membrane glycosphingolipids in mammals, can act as potent endogenous antagonists of TLR2 in human and mouse macrophages. This activity may suppress inflammation induced by PAMPs and DAMPs acting at TLR2.

T31

Dihydroceramide produced by periodontal pathogen *Porphyromonas gingivalis* promotes osteolysis by directly activating intracellular cathepsin B in osteoclast precursors.

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Introduction. Cathepsin B (CatB), a lysosomal cysteine protease, plays a critical role in the fusion of osteoclast precursors (OCPs) induced by RANKL. Published observations demonstrated that intracellular eukaryotic ceramide sphingolipids directly activate CatB in OCPs thereby promoting inflammatory osteolysis. While ceramides are constantly produced by eukaryotic cells, only a limited number of oral bacterial species represented by the genera *Bacteroides*, e.g., *Porphyromonas gingivalis* synthesize structurally unique isoC17:0-Dihydroceramide-1-phosphoglycerol phosphoglycerol (PG-iso-DHC). We demonstrated that *P. gingivalis*-derived PGDHC accelerates RANKL-primed osteoclastogenesis *in vitro*. However, the possible impact of CatB on osteoclastogenesis elevated by non-eukaryotic *P. gingivalis*-dervided PG-iso-DHC is largely unknown. Therefore, this study aimed to evaluate crosstalk between host CatB and non-eukaryotic PGDHC on the promotion of osteoclastogenesis.
Results. Local injection of PG-iso-DHC in experimental periodontitis and calvarial osteolytic lesions dramatically accelerated bone loss. Using in vitro assays, we detected that PG-iso-DHC accelerates lysosomal membrane permeabilization leading to catB relocation from lysosomes to cytosol in RANKL-primed OCPs. According to a pulldown assay, the high affinity between PG-iso-DHC and CatB was observed in RANKL-stimulated OCPs-like RAW264.7 cells *in vitro*. It was also demonstrated that PG-iso-DHC promotes the enzymatic activity of recombinant CatB protein *ex vivo* and in RANKL-stimulated osteoclast precursors *in vitro*. Furthermore, no or little effect of PG-iso-DHC on the RANKL-primed osteoclastogenesis was observed in male and female CatB-*knock out* mice compared with their wild-type counterparts.
Conclusions. Altogether, these findings demonstrate that PG-iso-DHC produced by *P. gingivalis* elevate RANKL-primed osteoclastogenesis via direct activation of intracellular CatB in OCPs.

T32

Inhibiting Acid Ceramidase: A Promising Strategy against Malaria

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Introduction: Malaria remains one of the most life-threatening infectious diseases worldwide. Although primarily endemic in tropical and subtropical regions, climate change and globalization are expanding the distribution of *Anopheles* mosquitoes, the vectors for malaria-causing *Plasmodium* parasites and increasing parasite drug resistance necessitate the development of innovative approaches. Sphingolipids, such as ceramide, are crucial bioactive molecules that mediate fundamental cellular processes. Based on its ceramide hydrolysing activity, Acid ceramidase (Ac) plays a key role in regulating cellular ceramide levels. While Ac and ceramide have been implicated in various infectious diseases, their roles in malaria remain unclear. Thus, we aimed to investigate the impact of the Ac/ceramide system on *Plasmodium* infection.
Results: Using Ac-deficient mice with ubiquitously elevated ceramide levels, we elucidated the role of endogenous Ac activity during *Plasmodium yoelii* (*P. yoelii*) infection, a murine malaria model. Interestingly, ablation of Ac led to reduced parasitemia, associated with diminished T cell responses in the early phase of infection. Mechanistically, we identified dysregulated erythropoiesis in Ac-deficient mice, resulting in decreased frequencies of reticulocytes, the preferred host cells of *P. yoelii* parasites. Furthermore, we demonstrated that inhibiting Ac with carmofur in wild-type mice had similar effects on the course of *P. yoelii* infection and red blood cell development. Most importantly, therapeutic administration of carmofur after an established *P. yoelii* infection efficiently reduced parasitemia.
Conclusion: In summary, our study highlights the significant role of the Ac/ceramide axis in regulating *Plasmodium* infection. Thus, pharmacological inhibition of Ac might serve as a novel and effective therapeutic strategy to combat malaria and other infectious diseases caused by reticulocyte-prone pathogens.

T33

Identification of functionally expressed G protein-coupled receptors in endometriotic epithelial cells and characterization of their potential to trigger invasion

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Endometriosis is a chronic inflammatory disease characterized by the invasion of endometrial cells outside the uterine cavity, pain and infertility. Current interventions for the disease are unsatisfactory, relying on the surgical removal of the lesions and hormonal therapies with high symptom relapse and collateral effects, respectively. Aim of the present study was to expand our knowledge on the molecular mechanisms responsible for endometriosis pathogenesis and to exploit the rationale for G protein-coupled receptors (GPCR) as non-hormonal therapeutic targets in this disease. For this, human endometriotic epithelial 12Z cells were employed to study GPCR-mediated increases in intracellular Ca^{2+} concentrations ($[Ca^{2+}]_i$) using fluo-4. Cell invasion assays (Boyden chamber) were used to study the potential of the identified GPCR to trigger endometriotic cell invasion. The results show that 12Z endometrial epithelial cells express a number of GPCR that are linked to $[Ca^{2+}]_i$ increases, such as oxytocin, bradykinin, histamine, lysophosphatidic acid (LPA), and sphingosine 1-phosphate (S1P) receptors. Recently, we demonstrated that the signaling of the pleiotropic sphingolipid S1P is profoundly altered in endometriosis. In particular, $S1P_1$, $S1P_3$ and $S1P_5$ receptors were highly expressed in endometriotic lesions as well as 12Z cells. We show here that pretreatment with pertussis toxin significantly reduced S1P-dependent $[Ca^{2+}]_i$ increases in 12Z cells, highlighting the involvement of G_i-mediated signaling. Employing specific agonists and/or antagonists of S1P receptors isoforms, we demonstrate that $S1P_1$, $S1P_3$ and $S1P_5$, but not $S1P_2$ and $S1P_4$ mediated the $[Ca^{2+}]_i$ increase in these cells. Moreover, the aforementioned GPCR ligands were studied in cellular invasion assays. Interestingly, while bradykinin, histamine and oxytocin exerted a limited pro-migratory effect, the bioactive lipids LPA, and particularly S1P, acting via $S1P_1$, $S1P_2$ and $S1P_5$, effectively stimulated cell invasion. The data illustrate the importance of the S1P-GPCR, compared to other GPCR that are functionally expressed, in triggering the invasive phenotype of endometriotic epithelial cells. The project is financed within the PRIN 2022 PNRR D.D. 1409 14/9/2022 National Recovery and Resilience Plan, Mission 4 - Component 2, Investment 1.1 funded by the European Union - NextGenerationEU - CUP_ B53D23024590001.

T34

SPTLC3 and the atypical sphingoid bases in mitochondrial function

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Serine and palmitoyl-CoA serve as the canonical substrates for serine palmitoyltransferase (SPT), the initiating enzyme in sphingolipid biosynthesis. Condensation of these substrates by the enzyme yields an 18-carbon sphingoid base, which is the substrate for biosynthesis of ceramide and all downstream sphingolipids. This reaction is catalyzed by an oligomeric SPT complex containing the structural SPTLC1 subunit and the catalytic SPTLC2 subunit. More recently it has become appreciated that the SPT complex can utilize a wider range of substrates depending on its subunit composition. For example substitution of SPTLC2 with SPTLC3, a more recently identified SPT subunit, enables utilization of C14-C22-CoA substrates. We have found that, thought these substrates are of low abundance, they are concentrated in highly specific subcellular compartments and regulate cell functions including ATP production through the electron transport chain (ETC) in mitochondria.
We found that, in cardiomyocytes, 16-carbon sphingoid bases are required to generate a specific pool of lactosylceramides in the inner mitochondrial membrane, and without SPTLC3, these lipids are absent and the NADH oxidation activity of complex I is severely compromised. On the other hand, in hepatocytes, depletion of SPTLC3 reduces 19-carbon sphingoid base lipids including ceramides and complex sphingolipids. Though inherent Complex I enzymatic activity remains intact, ubiquinone levels are reduced, and consequently, electron transport from complex I to complex III is impaired. Together our data demonstrate that SPTLC3 serves a specific niche in generating a fundamental suite of non-canonical lipids with key roles in mitochondrial function. The contrasting mechanisms in distinct cell types indicate that subcellular niches filled by SPTLC3-derived sphingolipids contribute to the diversity of mitochondria function in distinct organs and tissues.

T35

The effect of hydroxylation on sphingolipid membranes: insights from molecular dynamics simulations

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The study investigates the effect of integrating 2-hydroxyoleic acid (2OHOA) into ceramide and sphingomyelin on the physical properties of sphingolipid membranes. 2OHOA, a synthetic oleic acid derivative currently under clinical evaluation for cancer treatment, modifies plasma membrane composition and can cross the blood-brain barrier. Within cells, 2OHOA activates sphingomyelin synthase 1 (SMS1), an enzyme crucial for maintaining sphingolipid levels, and reduces the activity of membrane-associated signaling proteins that promote tumor growth.
Introduction: We employed molecular dynamics simulations to analyze the effects of incorporating 2OHOA into ceramide and sphingomyelin on lipid membranes' physical properties. Model membranes composed of both hydroxylated and non-hydroxylated sphingolipids were compared.
Results: The findings reveal significant changes in membrane dynamics due to hydroxylation, providing insights into the role of hydroxylation in altering membrane properties. Notable changes were observed in lipid area, volume, and bilayer thickness, suggesting a more rigid membrane environment.
Conclusions: This research offers a preliminary framework for understanding how modifications in lipid composition influence membrane behavior. These insights have potential implications for future studies on antimicrobial peptides, anesthetics, gases, and temperature variations on membrane properties.[i]

T36

Brain accumulation of lactosylceramide characterizes β-galactosylceramidase deficiency in a zebrafish model of Krabbe disease

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Krabbe disease is a neurodegenerative disorder characterised by extensive myelin loss. It is caused by mutations in the *GALC* gene that encodes for β-galactosylceramidase (GALC), an enzyme that catalyses the removal of a β-galactose moiety from galactosylceramide and other sphingolipids. The absence of GALC activity induces the accumulation of the cytotoxic metabolite psychosine that contributes to demyelination, neuroinflammation, and oligodendrocyte loss. However, the mechanism involved in the pathogenesis of Krabbe disease is still poorly understood.
To investigate the impact of GALC deficiency in zebrafish, two KO lines were generated for the *GALC* zebrafish co-orthologues *galca* and *galcb* by CRISPR/Cas9. Interestingly, 4-month-old *galcb* KO animals were characterized by an impairment of swimming and feeding ability in parallel with a significant reduction (80%) of their enzymatic activity. In contrast, no behavioural alterations and loss of GALC activity were observed in *galca* mutants. Based on these results, the *galcb* KO line was further characterized. Adult *galcb* KO animals showed a reduced body length and weight, and a decreased brain weight. Microscopic, immunohistochemical, and RT-qPCR analyse showed the presence of severe demyelination, neuronal loss, neuroinflammation, and microglia recruitment. Target lipidomic analysis on KO *galcb* brains showed significant alterations in their sphingolipid profile. In particular, a remarkable upregulation of lactosylceramide (LaCer) levels was observed together with a less prominent increase of hexosylsphingosines, ceramides, dihydroceramides, GM3, and dihydrosphingomyelins. RT-qPCR analysis of various enzymes involved in sphingolipid metabolisms revealed a significant upregulation of *asah1a*, *asah1b*, *gm2a*, and *neu1* expression whereas *hexa*, *glb1*, *smpd1*, *gba1*, *galtV*, and *st3gal5* mRNA levels were unchanged.
Altogether these results indicate that *galcb* KO zebrafish may represent a novel model of Krabbe disease that recapitulates the main clinical features of the human pathology. Interestingly, at variance with the brain of Krabbe patients and other animal models of the disease, accumulation of LacCer, rather than psychosine, characterizes the zebrafish model. Thus, *galcb* KO zebrafish may provide novel insights about the role of LacCer in the pathogenesis of Krabbe disease.

T37

Identifying and validating ceramide transfer protein binding partners

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Sphingolipids (SLs), characterized by a sphingosine backbone, are crucial cell membrane components with key roles in signal transduction and cell metabolism. A central step in SL metabolism is the production of ceramides (Cer), made of sphingosine and fatty acid, and abundant in cell membranes. Cer have a crucial role in cellular functions, such as response to stress and extracellular stimuli. They are involved in signalling pathways linked to senescence, apoptosis, cell cycle, cell differentiation, and neuronal maturation.
The ceramide transfer protein (CERT) is responsible for the transportation of Cer from the endoplasmic reticulum to the Golgi apparatus, where it may be further modified into a more complex SL. CERT has three isoforms- CERTLΣ128, CERTL, and CERT—each composed of three regions- the pleckstrin homology domain, a middle region, and the StAR-related lipid transfer domain. CERTL and CERT differ slightly in their middle region and are both responsible for Cer transport. CERT is expressed ubiquitously but is particularly important in the central nervous system and is associated with processes in embryogenesis and brain development. Additionally, CERT can bind to C1q and activate the complement cascade, highlighting a role in immune responses.
Despite its significance, CERT's molecular mechanisms and roles remain poorly understood. To elucidate its physiological functions, identifying CERT interactors is crucial. Thus, we employed a yeast-two hybrid system, analysing each region of the CERT protein, to discover potential interactors. Our study uncovered several putative and validated protein partners, such as VAP-A and VAP-B, confirmed through immunofluorescence and proximity-ligation assays. Notably, we identified interactors involved in important cellular functions such as mitochondrial activity, apoptosis, cell growth, vesicle transportation, and protein degradation. These findings pave the way for future research to clarify CERT's molecular mechanisms and its potential as a therapeutic target in diseases related to ceramide metabolism.

T38

Deregulation of ceramide synthases CerS4 and CerS5 affects ZDHHC expression, protein S-palmitoylation and the lipid network

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Ceramide synthases (CerSs), key enzymes of the lipid metabolism, catalyze the formation of ceramides from a sphingoid base and acyl-CoA molecules. Their products, ceramides (Cer), are important for membrane integrity and stability, but are also essential for the formation and physicochemical properties of specialized membrane microdomains called lipid rafts. Many proteins translocate into lipid rafts, which are enriched in cholesterol and sphingolipids. By downregulation of CerS4 and CerS5 we alter the sphingolipid pathway and therefore the membrane composition and microenvironment for proteins to translocate into the membrane. Lipidation is a mandatory post-translational modification (PTM) for protein translocation to membranes. One post-translational lipidation of proteins is the reversible S-palmitoylation. It refers to the covalent attachment of a 16-carbon fatty acid to free cysteine thiols in proteins, called "thioester bond formation". Palmitoylation of proteins is catalyzed by palmitoyl-acyltransferases (PATs or ZDHHC-PATs). The abundance of palmitoylated proteins at a given time point can be visualized via the biorthogonal click chemistry approach. This method is based on the metabolic incorporation of a modified palmitic acid analog by cells, followed by CuAAC (copper-catalyzed alkyne-azide cycloaddition reaction) to provide reporter-labeled palmitoylated proteins. Here we show that in CerS4 and CerS5 downregulated HCT 116 cells, protein palmitoylation of several proteins is altered, which is accompanied by changes in the expression level of ZDHHC-PAT enzymes at the mRNA level. Furthermore, we follow the palmitic acid metabolism by lipidomic analysis. Using bioinformatic lipid network and moiety analysis (LINEX), we are able to reveal metabolic dysregulation and alteration of sphingolipid metabolism of CerS4 and CerS5 downregulated HCT 116 cells.
In conclusion, changes in sphingolipid balance in colon cancer cells affect cellular metabolism and signaling through alterations in protein palmitoylation and the lipid network.

T39

Unveiling the biochemical secrets of ST3GAL3: a multi-approach characterization of the glycosphingolipid pathway

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ST3GAL3 is a member of the sialyltransferase family able to transfer sialic acid to the C-3 position of galactose, terminating the oligosaccharide chain of both glycoproteins and gangliosides. It was supposed to be mainly dedicated to the biosynthesis of histo-blood antigens such as sialylated Lewis a, but the discovery of a rare disease caused by inactive variants of the enzyme revealed patients presenting predominantly neurological symptoms and expressing circulating CA19.9. Moreover, enzymatic studies in-vitro showed that ST3GAL3 prefers glycosphingolipid substrates presenting both the Galβ1,3GlcNAc (lacto-series) and the Galβ1,3GalNAc (ganglio- series) sequences. These evidences led to the hypothesis that ST3GAL3 may be crucial for the generation of a definite pool of minor brain gangliosides that could be essentials for the function of specific subsets of neurons. To prove this theory, we created a new method to assess enzyme activity using liquid chromatography coupled with mass spectrometry, which allowed us more sensitive detection avoiding the use of radioactive compounds. Then, plasma samples from patients have been analyzed by ultra-sensitive LC-MS/MS to study if and how the global levels of glycosphingolipids produced were affected by the variants. Results obtained for the enzyme activity assay conducted using LC-MS confirmed that most of the known pathogenic variants of ST3GAL3 lack enzymatic activity. The semi-quantitative LC-MS/MS analysis allowed us to detect lower levels of the putative main reaction products, sLc4 (Siaα2,3Galβ1,3GlcNAcβ1,4Galβ1,4Glc-Cer)/GM1b (Siaα2,3Galβ1,3GalNAcβ1,4Galβ1,4Glc-Cer), in plasma patients when compared to controls. Finally, we created and characterized an HCT-15 cell line, knock-out for the β4galnt1 gene, which is responsible for the biosynthesis of the ganglio- series gangliosides. This will be used for future studies to evaluate and confirm the substrates of choice for the ST3GAL3 enzyme, as well as a blank model for the evaluation of the variant activity. Considering the promising results obtained, further studies are required to study the distribution of glycosphingolipid in brain, in order to be able to connect the biochemical changes in patients to the clinical outcome.

T40

The irreversible degradation of sphingosine-1 phosphate negatively regulates lipid storage capacity of pancreatic beta cells in response to free fatty acids and proinflammatory cytokines

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Introduction: The failure of pancreatic insulin-secreting beta cells is linked to diabetes development. Chronic exposure to high concentrations of free fatty acids (FFA) is thought to induce beta cell failure during type 2 diabetes (T2D) development, while proinflammatory cytokines are well-described inducers of beta cell death during type 1 diabetes (T1D) onset. Beta cells are particularly vulnerable to the toxic effects of FFA and proinflammatory cytokines due to their well-known weak antioxidative and anti-inflammatory defence status. Accumulating evidence points also to an important role of intracellular sphingosine-1-phosphate (S1P) metabolism in beta cell failure during diabetes development. Here we investigate the impact of the irreversible S1P degradation on beta cell lipid storage capacity and fate in response to FFA and proinflammatory cytokines.
Results: We show that a high activity and expression of S1P-lyase (SPL) potentiates beta cell sensitivity to FFA. The high expression of SPL is associated with FFA-mediated apoptosis induction via potentiation of ER stress, oxidative stress and mitochondrial disturbances. This goes along with a reduced lipid storage capacity as evident by a lower lipid droplet content and size. We demonstrate that this reduced lipid storage capacity results from the inhibited lipid droplet biogenesis, while enhanced lipophagy. The high SPL expression provides protection against short-term exposure to cytokines by inhibition of ER and mitochondrial stress, which coincidences with prevention of mitochondrial ceramide accumulation. Interestingly, SPL overexpressing cells are more prone to cytokine toxicity upon chronic exposure. This goes along with a disturbed lipid storage capacity, similarly to the effects observed in FFA-treated cells.
Conclusions: We postulate that the turnover of S1P may be an overseen, but common, pathway that contributes to lipid storage disturbances and beta cell failure during T1D and T2D development.

T41

The sphingolipids of the lipid droplets: Novel functions of neutral sphingomyelinase 2 in the biogenesis of lipid droplets and peri-droplet mitochondria during non-alcoholic fatty liver disease

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Lipid droplets (LDs) have recently emerged as important metabolic regulators of cellular stress response that buffer excess free fats and protect cells from lipotoxicity. The LD hydrophobic core stores potentially toxic lipids and it is surrounded by a phospholipid monolayer decorated with dozens of peripheral proteins. In addition to providing an amphipathic border for LD formation, this phospholipid shell plays a role in LD biogenesis and contact with other organelles. Enzymes involved in glycerophospholipid metabolism, such as CTP:phosphocholine cytidylyltransferase (CCT1) and phospholipase D, have been also found on the LD surface, indicating that the phospholipid shell might undergo *in situ* remodeling; however, the possibility that sphingolipid-metabolizing enzymes are present and functional has been rarely addressed.
Neutral sphingomyelinase-2 (nSMase2) governs one of the several known pathways for ceramide generation along pro-inflammatory and pro-apoptotic signaling pathways. Recent data showed that *activation* of nSMase2 parallels the onset of the early stage of hepatic steatosis in animal models of NAFLD. Our earlier studies reported evidence that nSMase2 protein abundance increases during obesogenic diet-induced steatosis, the enzyme is palmitoylated and translocated to the plasma membrane in the fat-laden hepatocytes where nSMase2-generated ceramide interferes with insulin response. In the course of these experiments, we made the serendipitous finding that nSMase2 became also concentrated around the LDs of the steatotic liver. By employing lipidomics, indirect immunofluorescence, and mice carrying liver-specific deletion of, here we report that LDs isolated from livers of obese mice contain sphingomyelin, ceramide and a functionally active nSMase2. Furthermore, we find that LD-associated nSMase2 restricts the capacity of LDs to accumulate TAG, which in turn leads to lipotoxicity. The LD-associated nSMase2 has a negative impact on LD:mitochondria interactions and the segregation of mitochondria into peridroplet (PDMt) and cytosolic (CMt) subpopulations with distinct bioenergetic properties. Livers of obese mice with hepatic-specific deletion of nSMase2 exhibit larger lipid droplets and elevated propensity for LD: Mt interactions. LD-associated mitochondria purified from nSMase2 deficient mice had increased respiratory rates and suppressed rates of beta oxidation, indicating that LD-associated nSMase2 is essential part of the mechanism that control LD biogenesis and energy homeostasis of the cells during metabolic stress by regulating LDs: mitochondria interactions.

T42

Glaucoma is associated with reduced sphingomyelin in human retinal ganglion cells

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Introduction: Glaucoma is a blinding disease projected to affect 110 million people worldwide by 2040. Glaucoma causes irreversible blindness by targeting retinal ganglion cells (RGC) and their axons for degeneration. The pathogenesis of glaucoma is strongly associated with aging and oftentimes, elevated intraocular pressure (IOP). The disease is typically treated by IOP-lowering therapies, but despite treatment, vision continues to decline with time. Therefore, many investigators seek to identify targets for the treatment of glaucoma based on pathogenic signatures independent of IOP. Recently, a large-scale metabolomics study observed an inverse relationship between glaucoma diagnosis and sphingomyelin (SM) levels in blood plasma. Here, in this preliminary report, we sought to measure SM directly in RGCs of human donors with and without a diagnosis of glaucoma.
Methods: Human donor eyes from glaucoma patients (n=3) did not have a medical history of other ocular diseases (diabetic retinopathy, macular degeneration), and non-glaucoma donors (n=3) were free of ocular disease. Donor globes were enucleated 2.8- 6.9 h post-mortem, fixed with 4% PFA for 24 h at 4ºC, and transferred to 1% PFA at 4ºC. Whole retinas were removed from the globe and dissected, regarding orientation and eccentricity. To detect SM in RGCs of flat-mount retinas, we used green fluorescent protein conjugated to a non-toxic truncated form of lysenin (LysGFP) to detect SM and Brn3a to detect RGCs. In preparation for staining, retinas were incubated in 175 mM PBS for 4 h then 175 mM PBS and 50 mM glycine for 16 h. Retinas were then incubated in primary antibody, Brn3a (1:100), diluted in 170 mM NaCl and 10 mM PBS for 72 h. Afterwards, retinas were incubated in an appropriate fluorescent secondary antibody and LysGFP (10ng/µl) for 48 hrs. We recovered fluorescence signals by confocal microscopy and measured fluorescence using ImageJ.
Results and Conclusion: The time to preservation of eyes from glaucoma (5.2±0.85 hrs) and non-glaucoma (4±1 hrs) donors were not significantly different (p=0.41). Moreover, we did not detect a significant difference in age of glaucoma (76±4 yrs) and non-glaucoma (79±3 yrs) donors (p=0.5). We observed a significant reduction in Brn3a-positive RGCs in retinas of glaucoma patients (p=0.0006). Collectively, glaucoma reduced LysGFP binding to SM in Brn3a-positive cells versus controls (p=0.018); LysGFP signal did not differ in RGCs between non-glaucomatous donors (p=0.48). Interestingly, we observed a strong (R^2=0.97) positive correlation between RGC density and LysGFP fluorescence, indicating RGC density is linked to SM accumulation in glaucoma.

T43

Ceramide homeostasis at the hepatic plasma membrane during non-alcoholic fatty liver disease is regulated by the rate of nSMase2 palmitoylation

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Obesity-associated diabetes is linked to the accumulation of ceramide in various organs, including the liver. This accumulation has been linked to the onset of insulin resistance, however the underlying mechanisms that control ceramide homeostasis during obesity are not completely understood. Recently we found that plasma membrane-associated neutral sphingomyelinase 2 (nSMase2) controls ceramide increases in fat-loaded hepatocytes and drives the onset of insulin resistance. Specifically, we discovered that excess abundance of palmitic acid in hepatocytes causes increases in ceramide levels in parallel to increased rates of palmitoylation of nSMase2 in the Golgi apparatus followed by its transclocation though the secretory network to the plasma membrane. Experiments using the acyl-biotin exchange method to quantify extent of protein palmitoylation show that increased palmitate abundance doubles the proportion of nSMase2 that are palmitoylated (from 7% to 16%,). This change is sufficient to induce translocation to the PM, ceramide generation, and suppressed response to insulin, assessed by Akt phosphorylation. Site-directed mutagenesis reveals six Cys residues that are excessively palmitoylated. NSMase2 palmitoylation mutants that fail to translocate to the plasma membrane also do not induce accumulation of ceramide, underscoring the importance to investigate further the interplay between palmitate levels and nSMase2 palmitoylation. To this regards, we decided to identify the palmitoyltransferase responsible for nSMse2 palmitoylation in hepatocytes overloaded with fat. First, we used an animal model and mice were fed with diet rich in saturated fatty acids for 18 weeks. Bulk RNA sequencing (RNA-seq) of livers of these obese mice indicated that identified seven members of zDHHC (zDHHC 4,5,6,7,9,12,18) with expression levels exceeding 5 FPKM. These enzymes did not exhibit significantly higher expression in mice on a high fat diet compared to those on a standard diet. Reported expression levels in various tissues, was used to confirm that zDHHC enzymes with near 0 FPKM values were almost exclusively expressed in non-liver tissues. According to this database, hepatocytes may not have been the main contributor to zDHHC 6, 7, and 18 expression levels. Using RT-PCR we compared further the profile of expression of zDHHC mRNA in control and steatotic HepG2 cells, AML-12 cells and whole liver from lean and obese mice to define a common signature of zDHHC expression and identify specific members that regulate nSMase2 palmitoylation.

T44

Fatty liver induction negatively affects mitochondrial respiratory function in female rats

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Chronic liver disease (CLD) is one of the leading causes of death worldwide. Among the causes of CLD, non-alcoholic fatty liver disease which is now called metabolic-associated fatty liver disease (MAFLD) is one of the most common culprits, and the most common cases of MAFLD are due to a sedentary lifestyle, metabolic syndrome, and insulin resistance. MAFLD is a progressive, deteriorating disease that affects liver cell function. It is characterized by the accumulation of fats in hepatocytes and includes a range of histopathological findings. Until now, the mechanisms of liver function impairment by MAFLD are still a debatable and unresolved matter. In this research, we used two animal models of MAFLD to study the pathophysiology of MAFLD at the level of mitochondria, particularly oxidative phosphorylation. Acute NAFLD was induced by injecting female rats with ethionine, an analog of methionine that induces a rapid onset of triacylglycerol accumulation in the liver, and diet-induced MAFLD animals were prepared by feeding rats regular chow soaked in virgin olive oil for sixteen weeks. Both MAFLD models showed increased triacylglycerols, cholesterol, and sphingolipids in their livers compared to controls. Using Blue Native Polyacrylamide Gel Electrophoresis and UV-vis spectroscopy, we demonstrated that both models of MAFLD produced direct and indirect inhibitory effects on mitochondrial respiratory complexes, particularly complexes I and II. These effects were corroborated by biochemical enzymatic assays of complexes from isolated mitochondria. The effects of fatty liver induction were observed at the transcriptional level as the levels of NDUFS1 subunit of complex I were significantly reduced in MAFLD models. Cardiolipin content was negatively affected by fat accumulation, and this led to poor functionality of respiratory complexes. This study highlights one mechanism of hepatocyte injury in response to high-fat accumulation in the liver.

T45

Deciphering the role of sphingosine-1 phosphate on blood-brain barrier function

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The blood-brain barrier (BBB) was found to be dysfunctional in many neurological disorders, like Alzheimer's disease, multiple sclerosis, or epilepsy, as well as in ischemic conditions like stroke. Hence it is crucial for modern therapies to understand the linkage between BBB state and cerebrovascular injury or regeneration. The role of Sphingosine 1-phosphate (S1p) on endothelial tightness is well established yet the downstream mechanisms of how this pathway fine tunes barrier properties is focus of current research. We are combining *in vitro* models of the BBB and *in vivo* live imaging of the zebrafish (*Danio rerio*) to investigate the effects of S1p signaling on BBB function and possibilities to manipulate endothelial tightness.
Using the human cerebral microvascular cell line hCMEC/d3 we observed differential effects of S1p stimulation on cell-cell junctions depending on signal duration. As fast response cells react with a weakening of cell junctions which was followed by long-term barrier strengthening. To identify possible downstream effectors of S1p signaling we have performed a phospho-proteomic screen and found the scaffolding protein IQGAP1 to be phosphorylated within 5 minutes after S1p addition. This phosphorylation site is known to affect the affinity of IQGAP1 to activated Rac1. Indeed, we could prove by co-immunoprecipitation that the interaction of IQGAP1 and Rac1 is significantly reduced by short-term activation of the S1p pathway but not 2 hours after S1p treatment. We are now further assessing the role of IQGAP1 downstream of S1p signaling on cytoskeletal rearrangements and cell-cell junctions using phospho-mimetic and phospho-deficient variants of IQGAP1.
Furthermore, we provide evidence that loss of the zebrafish ortholog *iqgap2* severely impairs the development of brain capillaries. The observed phenotype resembles the previously described effects of pre-mature overactivation of the S1p receptor 1 (S1pr1)/Rac1 pathway in the zebrafish. To investigate the role of Iqgap2 in the S1p/Rac1 axis *in* vivo we are establishing transgenic zebrafish overexpressing Iqgap2 as well as CRISPR/Cas9 mediated knock-outs and observe vascular development by live confocal imaging.

T46

1-Deoxy(DH)Ceramides with very long chain acyls (24:0/24:1) are specific mediators of neurotoxicity

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1-Deoxysphingolipids (1-deoxySL) are atypical sphingolipids that are formed when the key synthetic enzyme Serine-Palmitoyltransferase (SPT) metabolizes Alanine, instead of its canonical substrate Serine. 1-DeoxySLs are neurotoxic and cause peripheral sensory neuropathies, such as HSAN1 or the diabetic neuropathy (DPN). Although the toxic effects of these lipids are known, are the mechanisms and enzymes involved little understood.
We performed a CRISPRi screen to identify enzymes responsible for 1-deoxySL-mediated toxicity. The hits were validated by functional assays
The screen revealed highly significant hits for ELOVL1, CerS2, HSD17B12, ACACA, and PTPLB. All identified genes are involved in the metabolism of very-long-chain fatty acids (VLC-FA). ELOVL1 is responsible for the synthesis of VLC-FAs (22:0, 24:0, 24:1), while CerS2 conjugates VLC-FA to a sphingoid base forming VLC-Ceramides. We compared 1-deoxySL toxicity in ELOVL1, CerS2, and CerS5/6 deficient cells. ELOVL1 and CerS2 deficiency, but not CerS5/6 deficiency, greatly reduced 1-deoxySL toxicity. Furthermore, we identified 1-deoxyDHCer (m18:0/24:1) as the main mediator of 1-deoxySL-mediated toxicity, while free 1-deoxy sphingoid bases and LC-deoxyCer (m18:1/16:0) are not or significantly less toxic. Supplementing VLC-FA (24:0, 24:1), but not LC-FA (16:0), to ELOVL1 deficient cells recovered toxicity. VLC-deoxySL toxicity was associated with a loss of mitochondrial function. Our data suggest, that 1-deoxySL manifest their toxic effects by impairing mitochondrial function and that this toxicity depends on a) the structure of the sphingoid base and b) the length of the conjugated N-acyl chain.

T47

A new role for sphingomyelin in regulating nuclear activity

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Sphingomyelin (SM) is the most abundant sphingolipid in the cell nucleus, localized in the nuclear membrane, nuclear matrix, and nucleolus. Nuclear SM plays both structural and functional roles including chromatin assembly, DNA replication and RNA transcription. Additionally, SM, together with cholesterol (CHO), forms particular microdomains in the inner nuclear membrane (NLM) associated with a double strands and/or circular RNAs. The aim of the work was to demonstrate the protective role of SM-CHO on double strands/circular RNA and to clarify all RNA composition linked to nuclear microdomain in embryonic hippocampal cell (HN9.10e cell line). The results showed that the RNA present in the NLM was the 20% of the total RNA in nucleus and this RNA was resistant to RNase treatment performed during the microdomain extraction. By incubating the cells with increasing concentration of SM-CHO, RNAse-resistant RNA increased. The opposite result was obtained treating the cells with neutral sphingomyelinase.
In order to understand which types of RNA were protect by SM, we isolated total RNA from NLM and we found different species of RNA like protein coding RNA, miRNA, miscRNA, retained intron, snRNA, lincRNA, nonsense-mediated decay RNA and small nucleolar RNA. We focused only on miRNA and mRNA because they were more abundant in NLM than whole nuclei. Regarding miRNA, 22 were expressed in different statistically significant manner compared to the nucleus, 8 showing higher expression and 14 showing lower expression. All overexpressed miRNAs were correlated with neuro-psychological diseases as chronic epilepsy, amyotrophic lateral sclerosis and psychological disorders. About mRNA, 20 mRNAs were differentially concentrated in NLMs respect to nuclei, all involved in chromatin remodeling and in the development of nervous system. To justify the high amount of miRNA in NLM, we investigated the possible presence of EIciRNA known to act as sponges for miRNA. The results showed the presence of 18 significant EIciRNAs.
In conclusion, this study reveals a new role for SM in NLM of embryonic hippocampal cells. The data demonstrate that SM can regulate nuclear activity by protecting and maintaining the double-strand and/or circular structure of different RNA types involved in chromatin organization and brain pathophysiology

T48

Role of ganglioside GM3 in myoblasts' metabolism and differentiation

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Ganglioside GM3 plays a crucial role in myogenesis. Not only its level and species change during myogenic differentiation, but it also modulates signaling pathways essential for correct myogenesis. Moreover, the acyl-chain length influences the process with long chain GM3 negatively affecting myogenesis compared to short chain GM3. Interestingly, different GM3 species are also capable of differently modulating toll-like receptor 4 (TLR4), a receptor whose stimulation by LPS inhibits myogenic differentiation and alters myoblasts' metabolism.
To understand the mechanism by which different GM3 species can modulate myoblasts' differentiation and to establish whether a different activation of TLR4 occurs and whether this can impact myoblasts' metabolism, myoblasts of the C2C12 cell line were treated with different species of GM3 (C12:0, C18:0, C24:0) in presence or absence of a TLR4 activator.
Sphingolipids levels in cells treated with GM3 C12:0 show no difference respect to untreated control cells, whereas the treatment with either GM3 C18:0 or C24:0 determines a significant increase in the level of both neutral sphingolipids (GalCer, GlcCer and LacCer) and gangliosides, in particular GM3 and GM1. Interestingly, while cells treated with LPS, an activator of TLR4, also exhibit increased levels of gangliosides GM3 and GM1, the combined treatment with LPS and GM3 leads to a reduction in ganglioside levels respect to cells treated with LPS, showing GM3 and GM1 levels similar to those of untreated control cells, suggesting that exogenous GM3 could inhibit TLR4 activation.
Treatment with GM3 C12:0, C18:0 and C24:0, alone or in combination with LPS, also leads to a decrease in total cholesterol levels respect to control cells.
Considering all this, we propose that exogenous GM3 could affect myoblasts' differentiation and metabolism by alterations of lipid-dependent membrane organization and/or signaling and that the mechanism underlying this effect could be involved in the pathogenesis of muscular pathologies such as muscular dystrophy.

T49

Sphingolipid-Based Synergistic Interactions to Enhance Chemosensitivity in Lung Cancer Cells

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Abstract: Tumor heterogeneity leads to drug resistance in cancer treatment with the crucial role of sphingolipids in cell fate and stress signaling. We analyzed sphingolipid metabolism and autophagic flux to study chemotherapeutic interactions on the A549 lung cancer model. Loaded cells with fluorescent sphingomyelin analog (BODIPY) and mCherry-EGFP-LC3B were used to track autophagic flux and assess cytotoxicity when cells are exposed to chemotherapy (epirubicin, cisplatin, and paclitaxel) together with sphingolipid pathway inhibitors and autophagy modulators. Our cell model approach employed fluorescent sphingolipid biosensors and a Gaussian Mixture Model of cell heterogeneity profiles to map the influence of chemotherapy on the sphingolipid pathway and infer potential synergistic interactions. Results showed significant synergy, especially when combining epirubicin with autophagy inducers (rapamycin and Torin), reducing cell viability. Cisplatin also synergized with a ceramidase inhibitor. However, paclitaxel often led to antagonistic effects. Our mapping model suggests that combining chemotherapies with autophagy inducers increases vesicle formation, possibly linked to ceramide accumulation, triggering cell death. However, the in silico model proposed ceramide accumulation in autophagosomes, and kinetic analysis provided evidence of sphingolipid colocalization in autophagosomes. Further research is needed to identify specific sphingolipids accumulating in autophagosomes. These findings offer insights into potential strategies for overcoming chemotherapy resistance by targeting the sphingolipid pathway.

T50

The Relationship of Glucose Intolerance/Diabetes and Overweight/Obesity with sphingolipidoma Alterations

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The CA.ME.LI.A (CArdiovascular risks, MEtabolic syndrome, LIver, and Autoimmune disease) epidemiological study aimed at exploring the associations between cardiovascular, metabolic, hepatobiliary, and autoimmune diseases in a representative population from Northern Italy (n=2545, 1251 men), with the primary goal of identifying risk factors for cardiovascular disease. In this work, we examined whether a unique sphingolipid profile, potentially a new biomarker for *diabesity*, could be associated with overweight/obesity and glucose intolerance/diabetes.
A random sample of 368 individuals (n=367, 217 men, 150 women) underwent plasma lipid extraction and LC-MS/MS analysis and was stratified according to BMI (NBW < 25 kg/m^2, OWO ≥ 25 kg/m^2) and fasting glycemia (NFG <100 mg/dl, IFG 100 – 125 mg/dL, DM ≥ 126 mg/dL). By combining these two variables, six groups were created: 1. NFG/NBW; 2. NFG/OWO; 3. IFG/NBW; 4. IFG/OWO; 5. DM/NBW; 6. DM/OWO.
The most notable changes were observed when glucose intolerance occurred alongside overweight or obese individuals. Specifically, dihydroceramides were found to be higher in the IFG/OWO group (0.39±0.18 uM) compared to NGT/OWO (0.35±0.18 uM) and DM/OWO (0.32±0.2 uM), suggesting that the accumulation of these molecules may serve as an early marker of glucose intolerance. In diabetic patients, both those with obesity (DM/OWO, 13.9±7.2 uM) and those of normal weight (DM/NBW, 16.6±6 uM), hexosylceramides were significantly lower compared to the other groups (NGT/NBW, 19.9±6 uM). Foremost, sphingosine-1-phosphate, a biologically active sphingolipid with a remarkable signaling activity, was significantly reduced in IFG/OWO (1.8±0.5 uM) and DM/OWO (1.8±0.7 uM) patients compared to NGT/OWO (2.2±0.7 uM).
This study highlights notable shifts in plasma sphingolipid levels among individuals with diabetes and glucose intolerance, highlighting their role as promising biomarkers, particularly when glucose intolerance coincides with overweight or obesity, suggesting a potential synergistic effect. Moreover, these alterations may be identifiable even at glucose levels as low as ≥126 mg/dL (7.0 mmol/L), underscoring their diagnostic potential.

T51

High-fat diet alters stroke-associated S1P signaling

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Stroke is a common, multifactorial cardiovascular disease linked to several risk factors, including lifestyle factors and cardiovascular co-morbidities. Specifically, metabolic syndrome characterized by obesity, insulin resistance and increased blood pressure associates with impaired cerebrovascular function and increased risk of stroke. Evidence emerged that sphingosine-1-phosphate (S1P) signaling is altered in cardiometabolic diseases. This study investigates S1P signaling alterations occurring post-stroke and how they are affected by high-fat diet (HFD) in a murine ischemic stroke model of permanent middle cerebral artery occlusion (pMCAo).
Mass spectrometry-based analyses revealed lower S1P plasma levels 1-day post-pMCAo, which was corroborated in acute ischemic stroke in humans. Similarly, S1P plasma levels increased over time in mice exposed to HFD, verifying higher S1P plasma levels detected in obese individuals compared to lean controls. In our co-morbid stroke model, diet intervention diminished the plasma S1P responses to stroke, while post-stroke brain S1P levels were lower in HFD mice compared to control diet (CD). S1P-generating enzyme SphK1 brain expression increased post-stroke but not with diet, whereas SphK2 expression levels decreased with diet but was unaffected by ischemia. Furthermore, S1Pr3 brain expression rapidly increases in the vascular compartment and in astrocytes within 24-hours post-pMCAo, which associates with blood-brain-barrier impairment and astrocyte activation. Interestingly, HFD exposure mitigated this acute increase of S1Pr3 post-pMCAo, showing higher ipsilateral S1Pr3 levels only at later time points post-stroke. Similarly, astrogliosis marker were lower acutely post-stroke in HFD mice, elevating only at later time points. In accordance, HFD mice presented with larger infarcts at 3-days post-stroke compared to CD-fed mice.
In conclusion, S1P signaling is significantly altered post-stroke and in the presence of increased cardiometabolic risk (i.e. metabolic syndrome), requiring further investigation in stroke pathology.

T52

SphK2-S1P signaling as a target to lower elevated blood pressure

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Hypertension is the most common preventable risk factor for cardiovascular disease and the leading contributor to all-cause mortality. Over the last years, more evidence of immune system involvement in the development and progression of hypertension has emerged, yet there are still no immune-targeting treatment options available. Our previous work in humans shows that higher plasma levels of the bioactive phospholipid sphingosine-1-phosphate (S1P) are associated with increments in blood pressure (BP) and are correlated with biomarkers of inflammation. In an experimental model, hypertension-associated plasma S1P elevation was linked to the S1P generating enzyme sphingosine kinase 2 (SphK2) and enhanced systemic inflammation evidenced by a higher frequency of circulating T-cells. The current study exploits this new concept by investigating the potential efficacy of therapeutic SphK2 inhibition on attenuating hypertension-associated immune system activation and thereby lowering elevated BP. Upon established experimental hypertension (at four weeks after initiation of Angiotensin II (AngII) infusion), mice were treated with the SphK2 inhibitor (SphK2i) ABC294640 or vehicle for two weeks. Pharmacological SphK2i treatment resulted in a normalization of BP levels, irrespective of alterations in plasma S1P levels or vascular response alterations that are typical of hypertension (i.e., increased vasoconstriction and impaired vasodilation compared to those from normotensive mice). SphK2i-mediated BP lowering was associated with a reduction in circulating $CD4^+$ T-cell counts. Interestingly, in mice lacking T-cell receptor alpha (i.e., with significantly lower $CD4^+$ T-cell populations), SphK2i therapy did not elicit the same BP lowering effects as in hypertensive wild-type mice. Our data indicates that SphK2i-mediated BP normalization during AngII-induced hypertension is mediated through immune-based mechanisms irrespective of direct vascular effects or plasma S1P levels. Taken together, our work identified first evidence for SphK2i as promising immune-based therapy in hypertension.

T53

The Impact of Sphingosine Kinase 1 (SphK1) on Endothelial Barrier Function

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Introduction

Sphingosine kinase 1(SphK1), a cytosolic enzyme, is responsible for the phosphorylation of sphingosine to Sphingosine 1-phosphate (S1P). S1P is a potent signaling sphingolipid secreted by endothelial cells, erythrocytes and platelets. It is also one of the regulators of vascular endothelial barrier integrity and function by promoting cytoskeletal rearrangements and strengthening cell-cell junctions. High level of SphK1 activity and S1P production are associated with enhanced endothelial barrier integrity. However, our previous animal study shows that no difference was observed in vascular endothelial cell barrier stability between $Sphk1^{-/-}$ mice and wt mice, and $Sphk1^{-/-}$ mice got improved survival rate compared to wt mice. So we want to explore the intricate relationship between SphK1 and endothelial barrier function, underlining the mechanisms how SphK1 influences the integrity of the vascular endothelial barrier under pathological conditions.

Results

Electric cell-substrate impedance sensing (ECIS), a technology monitoring trans-endothelial electrical resistance, was used to measure the change of monolayer cell barrier after the addition of stimulants. A previous study in our group has already proved that human endothelial cell line EA.hy926 is suitable for endothelial barrier study. Higher resistance indicates an improved barrier function. Enhanced barrier resistance was observed in the treated cell model with S1P, S1PR1 agonist, sphingosine kinase inhibitor 2 (SKI II), while the opposite results presented with the SphK1 inhibitor PF-543. Both S1P and the S1PR1 agonist SEW2871 can induce a rapid increase of resistance at the early time, but decrease back to baseline within around 3 hours. SKI II initially triggered a moderate level increase in resistance and maintained it even in a longer time. When the EA.hy926 cells were pretreated with FTY720, there was no increase of resistance after stimulation with S1P, S1PR1 agonist or SKI II.

Conclusions

Sphk1's ability to regulate endothelial cell barrier integrity underscores its importance in vascular function and disease. Targeting SphK1 could offer new therapeutic method for treating diseases characterized by endothelial barrier disruption.

T54

When opponents become partners in crime: synergistic function of Ceramide and Sphingosine 1-Phosphate in Alzheimer disease

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The classical ceramide-sphingosine-1-phosphate (S1P) rheostat, which predicts opposite roles for pro-apoptotic ceramide and pro-survival S1P in cancer cells, takes on a distinct function in neurodegenerative disease. Research in our laboratory shows that ceramide and S1P mutually amplify their pathogenic function in Alzheimer's disease (AD). We found that Aβ binds to ceramide-rich extracellular vesicles (CREVs) that are secreted by astrocytes. The Aβ-associated CREVs are taken up by neurons and transported to mitochondria, ultimately leading to mitochondrial damage and neuronal cell death. We also found that Aβ induces the interaction of microglial S1P receptor 1 (S1PR1) with toll like receptor 4 (TLR4), which leads to the activation of microglia that secrete neuroinflammatory cytokines and fail to clear Aβ. In turn, S1P induced suppression of Aβ clearance in microglia and astrocytes and ceramide-mediated Aβ mitotoxicity in neurons synergistically drive AD pathogenesis. We tested several pharmacological approaches to disrupt this pathogenic synergy of ceramide and S1P and mitigate AD pathology. Using the 5XFAD familial AD mouse model, we inhibited Aβ-induced CREV secretion by astrocytes with the neutral sphingomyelinase 2 inhibitor GW4869 and restored Aβ clearance by microglia with the S1PR1 antagonist Ponesimod. These interventions significantly improved AD pathology and memory function in 5XFAD mice. Currently, we are testing additional drugs to target the synergy of ceramide and S1P, aiming to develop new approaches for AD therapy. This work is supported by NIH grants R01AG064234, 1RF1AG078338, and R21AG078601, and VA grant I01 BX003643.

T55

Targeting ASM for novel pathogenesis and therapeutics in Alzheimer's disease

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Alzheimer's disease (AD) is characterized by complex, multifactorial neuropathology, suggesting that small molecules and antibody-based immunotherapy targeting multiple neuropathological factors are likely required to successfully impact clinical progression. Acid sphingomyelinase (ASM) activation has been recognized as an important contributor to these neuropathological features in AD, leading to the concept of using ASM inhibitors for the treatment of this disorder. Here we report the identification of small compound (KARI 201), a direct ASM inhibitor evaluated for AD treatment. KARI 201 exhibits highly selective inhibition effects on ASM, with excellent pharmacokinetic properties, especially with regard to brain distribution. We also explore plasma ASM as a circulating factor that accelerates neuropathological features in AD by exposing young APP/PS1 mice to the blood of mice overexpressing ASM, through parabiotic surgery. Elevated plasma ASM was found to enhance several neuropathological features in the young APP/PS1mice by mediating the differentiation of blood-derived, pathogenic Th17 cells. Antibody-based immunotherapy targeting plasma ASM showed efficient inhibition of ASM activity in the blood of APP/PS1 mice and, interestingly, led to prophylactic effects on neuropathological features by suppressing pathogenic Th17 cells. Our data highlight the possibility of potential clinical application of KARI 201 and ASM-targeting immunotherapy as an innovative and multifaceted drug for AD treatment.

T56

Lipidomic Profiling Reveals Shared Pathological Signatures in Sporadic Parkinson's Disease and *GBA* Mutation Carriers: Implications for Disease Mechanisms

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Introduction: Parkinson's Disease (PD) is a neurodegenerative disease characterized by the deposition of protein inclusions, called Lewy Bodies (LBs), in the brains of patients. LBs are heterogeneous structures whose main constituent is the protein alpha-synuclein (αS) and that are also composed of lipid molecules. Disruptions in the levels of specific lipids, including sphingolipids, fatty acids, and cholesterol, have been associated with PD, suggesting a role of lipids in the emergence and spreading of αS and PD pathology. For example, mutations in the gene *GBA*, which encodes Glucocerebrosidase (GCase), are the most important risk factor for familial PD and have been associated with the accumulation of pathological αS and changes in the levels and properties of sphingolipids in human derived samples.
Results: Using a combination of shotgun lipidomics and biochemistry, we have shown that long sporadic PD and *GBA* risk PD are associated with the same changes in lipid profile in post-mortem brain samples, including brain homogenates and isolated LBs and small aggregates. In particular, our results show that long disease duration led to a decrease in GCase activity levels and an increase in those of pathological αS as well as changes in the levels of specific lipids, including sphingolipids in the sporadic cases. Moreover, the levels of affected lipids were found to correlate negatively with GCase activity and positively with those of pathological αS. Finally, we found that, of the three groups of *GBA* mutations (severe, mild and risk) considered in this study, only risk *GBA* mutations led to changes in lipid levels, similar to those associated with long sporadic disease duration.
Conclusions: Our shotgun lipidomic analyses show that the same disruptions in lipid levels are associated with long sporadic PD and *GBA* risk PD. Together these results suggest the need for patient stratification in clinical trials of therapeutic interventions in GBA-PD and that successful therapeutics against GBA-PD should be considered for sporadic PD.

T57

Lipidomic insights into mood and neurodegenerative disorder mechanisms

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Lipids are one of the major constituents of the brain and current methodologies such as mass spectrometry lipidomics provide the possibility of studying lipid molecular signatures associated with physiologic and disease phenotypes. The most recent genome association studies implicate predominantly lipid metabolism and membrane trafficking associated genes to be particularly affected in Alzheimer's disease (AD). Accordingly, the major genetic risk factor for AD is associated with the lipid trafficking protein, Apolipoprotein E (APOE). The three common APOE variants ε2, ε3 and ε4, only found in humans, are differentially associated with AD risk: ε2 confers AD protection, ε4 increases AD risk and the ε3 allele is considered as AD-risk neutral. Therefore, understanding the lipidomic protection signatures associated with AD susceptibility, that rely on APOE ε4, has the potential to shed light on the mechanisms underlying AD pathogenesis and to provide new avenues for therapeutic targets. Additionally, environmental exposure to chronic stress has also been identified as a major risk factor for brain disorders, such as depression and AD. Therefore, we aim to understand how does chronic stress impact on the functioning of the hippocampus, a brain region relevant for learning and memory and emotional response. We studied the hippocampus at the subregional level along its longitudinal axis, since it was previously shown that the dorsal and ventral poles, in rodents, contribute differentially to spatial and emotional responses. The identification of these lipidomic signatures lead to candidate lipid signalling pathways, which we are currently testing with genetic rodent models at biochemical, functional and behavioural levels. Overall, the goal of these projects is to identify lipid pathways relevant for brain function and dysfunction with potential implications for the treatment and diagnosis of brain disorders, such as AD and mood disorders.

T58

Brain acid sphingomyelinase – a sex-specific mechanism for drug addiction

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Addiction is a chronic and severe mental disorder with high gender-specificity. However, the pathogenesis of this disorder is not fully elucidated, and no targeted pharmacotherapy is available. A growing body of evidence points out the potential involvement of the ceramide system in the pathophysiology of addiction. A pathogenic pathway for several mental disorders based on the hyperfunction of an enzyme of ceramide synthesis, acid sphingomyelinase (ASM), was recently proposed. Whole-body ASM overexpression is involved in the pathogenesis of depression and alcohol use disorder. However, it remains elusive whether central or peripheral ASM pool plays a crucial role in the pathogenesis of mental disorders. We showed a crucial role of the forebrain ASM for various types of addiction-related behaviour in a drug- and sex-specific way. In males, the forebrain ASM overexpression led to enhanced alcohol consumption in a free-choice paradigm. ASM overexpression diminished reinforcing properties of alcohol and cocaine, but not that of amphetamine, ketamine, or a natural reinforcer fat/carbohydrate-rich food in males. In females, forebrain ASM overexpression enhanced binge drinking, while moderate alcohol consumption was preserved. ASM overexpression in females contributed to conditioned place preference establishment for amphetamine, but no other psychoactive substances. Altogether, we showed a specific involvement of the forebrain ASM to the development of conditioned reinforcing effects of different types of substances with addictive properties in a sex-specific way. Our data enlarges the current knowledge on the specific molecular mechanisms of dependence from various drugs of abuse and might serve as a basis for the development of drug- and sex-specific targeted therapy.

T59

Sphingosine-1 phosphate signaling interactions regulating brain angiogenesis versus blood brain barrier formation

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The brain is a heavily protected site with the brain vasculature contributing to formation of the blood-brain barrier. Therefore brain vessels have specialized properties which also affect their angiogenesis. The blood-brain barrier (BBB) was found to be dysfunctional in many neurological disorders, like Alzheimer's disease, multiple sclerosis, or epilepsy, as well as in ischemic conditions like stroke. On the other hand a functional blood-brain barrier might prevent drug delivery into the brain.
While multiple signaling pathways are involved in generating and maintaining the BBB, we could recently show that Wnt and Sphingosine 1-phosphate (S1p) signaling functionally oppose each other by direct interaction during BBB development. Yet the downstream mechanisms of how these pathways fine tune barrier tightness remain elusive. We found that interrupting Wnt signaling results in pre-mature S1p receptor 1 (S1pr1) and downstream of it Rac1 activation and in turn severe defects in vascular development. However, we are focussing on how the interaction is mediated and which downstream components will balance it.
We are analyzing these interactions using zebrafish embryos as well as human cerebral microvascular cels (hCMEC/d3). IN zebrafish we use genetic and pharmacological impariment of signaling pathway components and micoscopical analysis. Using the cell line we performed a phospho-proteomic screen and identified a large set of differentially phosphorylated proteins. We are currently analyzing various GAP proteins involved in the regulation of Rac1 activation downstream of S1pr1.

T60

Acid sphingomyelinase deficiency alters sphingolipidome during neuronal development

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Acid sphingomyelinase deficiency (ASMD) is a lysosomal storage disorder due to mutations in the gene encoding acid sphingomyelinase (ASM). This lysosomal enzyme mediates the degradation of sphingomyelin (SM) yielding ceramide and phospho-choline. ASMD is therefore characterised by an aberrant accumulation of SM, and other classes of sphingolipids, in different cell types and tissues. The infantile neurovisceral form of ASMD, in which mutations lead to almost no ASM activity, is characterized by neurodevelopmental delay, cognitive impairment and rapid neurodegeneration.
We recently reported that, among the SM species accumulating in ASMD, SM 16:0 is the most abundant and toxic in ASM deficient mature neurons (Gaudioso et al., 2023). However, little is known about the sphingolipid changes that occur in developing neurons and how this is affected by ASM deficiency.
Using chromatography/mass spectrometry (LC/MS) we have analyzed the levels of different classes of sphingolipids at different developmental stages in primary cultured neurons from wild type and ASMko mice. This analysis has shown alterations in sphingolipid species at early stages of neuronal development in ASMko neurons. In addition, we have analysed the expression of enzymes involved in sphingolipid synthesis and degradation.
In summary, our results characterise in detail the sphingolipidome at early stages of neuronal development, and how this is affected in ASMD, unveiling new therapeutic targets for this fatal disease.

T61

The Role of Sphingolipids in GBA-Parkinson's Disease

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Mutations in the glucocerebrosidase (GBA) gene are the highest genetic risk factor for developing Parkinson's disease (PD) and glycosphingolipids potentially play a crucial role in this relationship. However, our understanding of how GBA mutations increase the risk of developing PD remains limited. Initial comparisons of plasma sphingolipid profiles from homozygous GBA loss of function Gaucher disease (GD) patients and PD revealed very different changes in sphingolipid profiles in these two diseases. This suggests differential changes in lipid metabolism occur in GD and PD.
Therefore, we aim to further explore this relationship by analyzing plasma samples from patients with and without different GBA mutations, correlating findings with iPSC models of PD, and investigating the expression of glycosphingolipid-related enzymatic pathways. Our analysis builds on our extensive experience in lysosomal storage diseases and success in developing therapies for some of these conditions. We use a refined, sensitive, and quantitative HPLC-based method to measure these lipids in plasma and CSF.
Additionally, we use bioinformatics to interrogate changes in the expression of key enzymes and cofactors involved in sphingolipid metabolism in GBA-PD versus controls in post-mortem human brains and larger blood-derived transcriptomic data sets. This includes the analysis of both snRNA and bulk RNA-sequencing data at the gene and transcript levels.
Taken together, this work has the potential to uncover the mechanistic implications of GBA mutations on sphingolipid metabolism specific to GBA-PD and pave the way for novel targeted therapeutic strategies.

T62

The brain sphingolipid system in Schizophrenia and its treatment

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Schizophrenia is a highly heterogeneous psychiatric disorder, which is elicited by multiple genetic and non-genetic factors. It remains insufficiently treatable with the current pharmaceutical approaches. In this study, we describe a new pathological mechanism and identify a new pharmacological treatment target with the use of a recently introduced ligand for it. An initial analysis of human gene polymorphisms and brain gene expression in schizophrenia patients identified an association of Smpd1 and Smpd3 genes coding for acid- (ASM) and neutral sphingomyelinase (NSM). In a rat model of amphetamine-induced psychosis, we found a locally restricted increase of ASM activity in the prefrontal cortex (PFC) that responds to psychosis-induction and its treatment. Short-term chronic haloperidol (HAL) treatment reversed behavioural symptoms and the ASM activity increase. A sphingolipidomic analysis confirmed a disrupted ceramide metabolism in the PFC during psychosis. Targeting enhanced ASM activity in a psychotic-like state with a selective ASM inhibitor reversed psychotic-like behaviour. While effective HAL treatment led also to a locomotor decline and cognitive impairments, selective ASM inhibitor did not.

P1

Sphingosine 1-phosphate as an important factor during vascular calcification in chronic kidney disease

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Patients with chronic kidney disease (CKD), and particularly those under hemodialysis (HD), will develop cardiovascular complications, mostly due to the exacerbation of vascular calcification (VC). VC relies on the transdifferentiation of vascular smooth muscle cells (VSMCs) into calcifying cells. Sphingosine 1-phosphate (S1P) is a pleiotropic sphingolipid that regulates proliferation, differentiation and angiogenesis. Sphingosine kinases 1 and 2 (SK1/SK2) catalyze the conversion of sphingosine into S1P. Because of the implication of S1P in cardiovascular and bone physiopathology, we explored if S1P metabolism participated in VC. 53 HD patients were recruited during 2019. We demonstrated for the first time that serum S1P is significantly increased in HD patients with mild abdominal aortic calcification (AAC) compared to healthy donors or HD patients without AAC. What's more, serum S1P was increased in CKD rats with mild VC. SK expression and activity were upregulated in calcified aortas from CKD rats and in calcified aortic explants. SK2 activity and S1P secretion, under the control of phospholipase D1 (PLD1), were enhanced in calcified VSMCs. Moreover, PLD1 KO mice showed significantly less circulating S1P in serum. Finally, the FDA-approved immunosuppressor drug fingolimod, a general modulator of S1P metabolism, strongly inhibited calcification of aortic explants and VSMCs. Fingolimod significantly reduced inflammation, attenuated metabolic syndrome and moderately inhibited aortic calcification in CKD rats. Our findings open an unexplored therapeutic option, targeting S1P metabolism, eventually with fingolimod, for the prevention and treatment of inflammation, metabolic syndrome and VC in CKD patients.

P2

Sphingosine 1-phosphate signalling is involved in fibroblast plasticity responsible for pulmonary fibrosis

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Pulmonary fibrosis (PF) is a chronic and progressive pathologic condition characterized by an unescapable decline of respiratory function with a limited survival expectance. The main hallmark of PF is represented by activation of myofibroblasts (MF), responsible for extracellular matrix (ECM) deposition that leads to respiratory failure, albeit the molecular mechanisms of the onset and progression of the disease have not been clarified yet. Recently, fibroblast plasticity has been addressed as a possible strategy to improve lung tissue regeneration. Indeed, upon injury lipofibroblasts (LF) differentiate into MF, which eventually de-differentiate back to LF in the case of PF resolution. Thus, it appears to be pivotal to boost the research towards new pathways that trigger the activation of LF phenotype. Sphingosine 1-phosphate (S1P) signalling has been found to be profoundly dysregulated in PF, although its role is so far unclear.
The specific aim of this study is to analyse the modulation of S1P signalling in fibroblast plasticity. The cellular model to study MF to LF switch was set up using IMR-90 human lung fibroblasts that differentiate into MF or transdifferentiate back into LF by the treatment with TGFβ1, in the absence or presence of rosiglitazone, respectively. The expression of fibrosis and lipogenesis markers was analysed together with electrophysiological parameters and lipid droplet content during MF-LF transition. Moreover, the expression pattern of S1P metabolic enzymes and S1P receptors was measured in MF and LF. Finally, to study the involvement of S1P anabolic enzymes, sphingosine kinases (SK1-2), and S1P receptors in the regulation of fibroblast plasticity, specific inhibitors and antagonists or RNA interference were employed.
Our recent findings point at a fundamental role of SK1 and S1P5 in TGFβ1-induced myofibroblast phenotype. Overall, these results could be crucial to identifying innovative and effective therapeutic strategies for the treatment of PF. The project has been founded by The Italian Ministry of University and Research-PRIN2022, Prot.2022NAFK8C.

P3

The role of JAK2/STAT3 on Sphingosine 1-phosphate (S1P) protective effect on human proximal tubule cells submitted to an *in vitro* ischemia model

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Acute kidney injury is a serious public health problem worldwide, being ischemia and reperfusion (I/R) the main lesion-aggravating factor that contributes to the evolution towards chronic kidney disease. Nonetheless, intervention approaches currently available are just considered palliative options. In order to offer an alternative treatment, it is important to understand key factors involved in the development of the disease including the rescue of the affected cells and/or the release of paracrine factors that are crucial for tissue repair. Bioactive lipids such as sphingosine 1-phosphate (S1P) have significant effects on the modulation of signaling pathways involved in tissue regeneration, such as cell survival, proliferation, differentiation, and migration. The main objective of this work was to explore the protective effect of S1P using human kidney proximal tubule cells submitted to a mimetic I/R lesion by ATP depletion. We observed that the S1P pre-treatment increases cell survival by 50% and preserves the cell proliferation capacity of injured cells. We also clearly observed a significant decrease in SK activity when cells were submitted to injury. On the other hand, administration of S1P prior to injury slightly sustains SK activity as an attempt to provide higher S1P production, thus suggesting a S1P-dependent S1P production. In general, we noted that the pre-treatment with S1P attenuated the ischemia-induced effects in response to the injury. All the S1P receptors are present in these cells and the protective and proliferative effect of S1P/S1P receptors axis occur, at least in part, through the activation of the SAFE pathway, as these effects were abolished by inhibition of JAK2/STAT3. To our knowledge, this is the first time that S1PR4 and S1PR5 are referred in HK-2 cells and also the first indication of JAK2/STAT3 pathway involvement in S1P-mediated protection in an I/R renal model.
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P4

The role of plasma membrane Ca^{2+} ATPases in regulation of cytosolic free Ca^{2+} concentrations by sphingosine-1-phosphate metabolism

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Introduction
Sphingosine-1-phosphate (S1P) is a small bioactive lipid which regulates various cellular processes, such as lymphocyte trafficking, angiogenesis or vascular barrier function. It is produced from sphingosine by sphingosine kinases, SphK1 and SphK2.
Aim of this study was to investigate the role of S1P for intracellular calcium homeostasis in cells in which SphK1 expression was supressed.
Results
Here, we show that basal intracellular free Ca^{2+} ($[Ca^{2+}]_i$) was significantly reduced in two independently generated SphK1 knockdown cell lines in EA.hy926 cells. This was true in the presence and absence of extracellular Ca^{2+}. In addition, Ca^{2+} increases induced by different agonists such as ATP, histamine, carbachol, S1P, lysophosphatidic acid (LPA) and the SERCA-inhibitor thapsigargin were significantly lower in EA.hy926 SphK1-KD. However, histamine-induced inositol phosphate production was not affected.
Expression analysis revealed that plasma membrane Ca^{2+} ATPase 1 (PMCA1) was strongly upregulated on mRNA and protein level, whereas PMCA4 was only upregulated on protein level. Furthermore, lipid measurements showed that S1P was reduced in SphK1 knockdown cells. Interestingly, the upregulation of PMCA1 could not be rescued by treating SphK1 knockdown cells with extracellular S1P, or specific S1P receptor agonists, respectively. Finally, the specific SphK1 activator K6PC-5 increased $[Ca^{2+}]_i$ and thapsigargin-induced Ca^{2+} increases and reduced PMCA1 protein expression.
Conclusion
These data show that SphK1 regulates intracellular calcium signalling by targeting PMCA1.

P5

Sphingosine-1 phosphate phosphatase 1 overexpression protects against cytokine-mediated beta cell failure

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Introduction: Type 1 diabetes (T1D) is an autoimmune disease, characterized by a progressive and specific death of pancreatic beta cells. During T1D development, activated immune cells infiltrate pancreatic islets and selectively destroy beta cells by induction of the integrated stress response through the action of proinflammatory cytokines IL-1□, TNF□ and IFN□. The predisposing mechanisms for beta cell sensitivity to cytokines remain unclear. Earlier studies indicate that beta cells are characterized by a unique expression pattern of enzymes regulating sphingolipid metabolism, which undergoes alternations upon exposure to cytokines. In the present study, we analyzed the role of sphingosine-1 phosphate phosphatases (SGPP) in rat INS1E beta cells. SGPP1, the enzyme related to antiapoptotic action in various cell types, is weakly expressed in beta cells in contrast to the abundantly expressed SGPP2, which action has been linked to proapoptotic effects in other tissues.
Results: Previous studies strongly indicate that the expression of SGPP2 is necessary for maintenance of beta cell proliferation (Taguchi et al JBC 2016) and we observed that a knockdown of SGPP2 disturbed INS1E cell survival. Interestingly, overexpression of SGPP1 resulted in a significant reduction of the endogenous SGPP2 expression. Moreover, INS1E-SGPP1 beta cells were significantly protected against cytokine-mediated toxicity under a short (24 h) and prolonged (72 h) exposure time. The protective effect was particularly strong in the cells treated with IL-1□. SGPP1 overexpression significantly inhibited cytokine-mediated nitrooxidative stress induction, as revealed by a reduced DCFDA oxidation and nitrite accumulation. This went along with a decreased expression of iNOS and MnSOD, two proteins crucial for the induction of nitrooxidative stress in beta cells. The reduced cytokine-mediated NO generation in INS1E-SGPP1 beta cells was followed by a diminished ER stress response. In line with a lower MnSOD expression a reduced formation of hydrogen peroxide in mitochondria was detected in cytokine-treated INS1E-SGPP1 cells.
Conclusions: This study suggests an essential role of the S1P recycling pathway in beta cells exposed to diabetogenic cytokines.

P6

Sphingosine-1-Phosphate/Sphingosine-1-Phosphate Receptor-Mediated Signaling plays a role in Irisin expression/release in Skeletal Muscle cells

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Irisin is a hormone-like myokine produced in abundance by skeletal muscle (SkM) in response to exercise. This myokine, identical in humans and mice, is involved in many signaling pathways related to metabolic processes and, recently, to synapse failure and memory impairment in Alzheimer's disease. Despite some evidence on the regulators of irisin and the relevance of sphingolipids (SLs) in neuronal and muscular system, the contribution of the bioactive lipid Sphingosine-1-Phosphate (S1P) on the modulation of myokines in SkM is only at the beginning..
Here, we show the existence of distinct intracellular pools of S1P able to affect the expression of the irisin precursor FNDC, irisin release and its function on myoblast proliferation and myogenic differentiation. Recently, we deposit a patent for a pharmaceutical composition based also on antisense oligonucleotides (A_A), which prevents SkM atrophy in myotubes treated with glucocorticoids as well as in SkM of C26-adenocarcinoma engrafted mice. Notably, A_A is able to enhance irisin secretion, and to potentiate the effect of trimetazidine, a compound which mimics exercise and limits the muscle functional impairment during aging. These findings may open new windows for potential therapeutic treatment of SkM atrophy associated to myokine impairment in aging.

P7

Targeted degradation of CERT1 with small molecule chimeras directed to the 26S proteasome

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Proteolysis targeting chimeras (Protacs) bind specific targets and E3 ubiquitin-ligases, promoting ubiquitination and degradation of targets by the proteasome. Multiple chimeras that degrade proteins relevant in several diseases have been developed, and the number is quickly increasing, indicating their therapeutic projection. Given the specificities of proteolytic pathways and limitations in E3-based Protacs, alternative strategies in target protein degradation are pursued. Herein we report a novel type of chimera based on ligands of USP14, a 26S-associated deubiquitinating enzyme involved in substrate processing and allosteric regulation of 26S activity, using CERT1 as model. The ceramide transporter CERT1, which conveys ceramides from the endoplasmic reticulum to the trans Golgi for sphingomyelin (SM) synthesis, has been reported as a therapeutic target in certain cancers. By increasing the intracellular levels of ceramides, CERT1 inhibition sensitizes cancer cells to anti-cancer drugs, which sustains the interest of developing CERT1-targeted Protacs (CERT1 Protacs), so far unreported. The CERT1 Protacs presented here feature ligands of USP14 and CERT1 bound with linkers of different lengths. The generated molecules have been characterized in cells, *in vitro* assays, by SPR and by docking studies and molecular dynamics simulations. Additionally, we provide evidence that targets are degraded by proteasomal colocalization in a ubiquitin-independent manner and that degradation involves the interaction of the ligand with the protein target. Remarkably, this work presents the first degrading chimera of a protein involved in sphingolipid metabolism, namely CERT1. We also show that two of the chimeras sensitize $HER2^+$ breast cancer cells to lapatinib and provide evidence suggesting that the increase in C14-C20Cer induced by the degraders may be involved in the sensitization.

P8

Alterations in *Candida albicans* sphingolipids hydroxylation corresponds with changed plasma membrane dynamics and azoles resistance

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Sphingolipids (SLs) play an essential role in maintaining the plasma membrane (PM) structure and functions. They are involved in fungal growth, signal transduction and pathogenesis. The presence of SLs-enriched domains in PM impact the resistance of fungal pathogens to commonly used antifungals – azole drugs. Recent studies also indicate that glucosylceramides and inositol-phosphoryl ceramides are pivotal for fungal virulence, survival in the host and provide fungal evasion of host immune response.
Candida albicans SLs are composed of acyl chain linked to long chain base (LCB) with amide bond and head group which can be mannosylated. Hydroxylation of acyl chain of fungal SLs can be crucial for interaction of SLs with other lipids and ergosterol, thus for activity of PM proteins and stability of domains. The enzymes responsible for hydroxylation of SLs acyl chains are Sur2p and Scs7p present in endoplasmic reticulum membrane. Here, we performed null deletion mutants of *C. albicans* and investigated impact of those deletion on azole resistance and PM dynamics. *C. albicans sur2Δ/Δ* display a diminished growth rate comparing to *wild type* and *scs7Δ/Δ* strains. The *C. albicans scs7Δ/Δ* strain exhibit increased resistance toward azole compounds (both for triazoles and imidazoles). The analysis of PM properties revealed its increased fluidity (fluorescence of Laurdan probe analysis), elevated level of unsaturated lipids with longer acyl chains (ATR-FTIR) and more ordered PM (FLIM analysis) of both *C. albicans sur2Δ/Δ* and *scs7Δ/Δ* strains.
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P9

The glycolipid transfer protein GLTP as a possible regulator of ceramide transfer from the ER to cis-Golgi

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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 for example proposed that it may also act as a sensor and regulator of glycolipid homeostasis in the cell, as expression levels of GLTP directly influence the cellular levels of many glycosphingolipid species. 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 or regulating vesicular transport in the cell. In this study, we propose a role of GLTP in regulating the transfer of ceramide from the ER to the Golgi apparatus and thereby indirectly regulating the glycosphingolipid homeostasis of the cell. 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 may provide a regulatory mechanism.

P10

Effect of dietary Sphingomyelin and Vitamin D3 in rabbit brain

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Sphingomyelin is the second most abundant phospholipid of the high-density lipoproteins and represents a fundamental constituent of cell membranes. Its metabolism is stimulated by vitamin D3 with the production of ceramide, sphingosine and fingosine-1-phosphate, known mediators of cellular signaling. Both sphingomyelin and vitamin D3 are essential for the development of nervous system and cognition. Milk, dairy products and eggs are rich in sphingomyelin. The aim of the work was to establish the effect of a normal rabbit diet enriched with sphingomyelin from egg yolk and vitamin D3. The diet was administered for four months. A blood sample was taken every month and, after 4 months, the animals were sacrificed and the brains were removed. The results showed an increase in plasma sphingomyelin already after 2 months of treatment. The brains of treated rabbits showed strong enrichment of GFAP in the subcortical white matter by both immunohistochemistry and immunofluorescence. The results suggest a positive effect of sphingomyelin and vitamin D on the nervous system structure/function
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P11

Plasma acid sphingomyelinase and alteration of taste-smell as sign of long COVID in pregnant women

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Persistent taste-smell alterations affect a substantial fraction of people after COVID-19 as an aspect of post-acute COVID-19 syndrome, also known as long COVID.
The sense of taste and smell is mediated by clusters of heterogeneous taste receptors cells that convey sensitive information from the oral cavity or nose to higher order brain centers via the gustatory sensory neurons. This presupposes the integrity of neuronal cells including myelin rich in sphingomyelin and cholesterol content. Thus, sphingomyelin is considered a biomarker of acquired demyelinating neuropathies. Degradation of sphingomyelin is due to acid and neutral sphingomyelinase activity. The aim of this study was to enroll women who had contracted the Covid-19 infection during pregnancy in whom signs of alteration of taste and smell persisted more than 1 year after the infection compared with pregnant women who had not had any taste and smell disturbance. Thus, the level of acid sphingomyelinase in the plasma of the patients included in the study was evaluated. The results showed that the level of plasma acid sphingomyelinase in women with taste and smell disorders was double that found in women without these disorders. It is possible to hypothesize that the persistence of disorders in long-Covid is linked to the reduction of sphingomyelin in the myelin sheath by sphingomyelinase with impairment of sensory activity
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P12

Use of whole blood to reveal LPS induced alterations of sphingolipids in lactating cow

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Bovine whole blood is increasingly used to characterize inflammatory, immune and infectious phenomena, and to reveal differences in susceptibility between animals. However, there are currently no data on its value in characterizing infectious or toxic effects on the sphingolipidome. The aim of this study was to characterize the effect of LPS on the sphingolipidome of the cell culture medium obtained from the whole blood of prime Holstein dairy cattle. As breeding and calving have a major impact on the sphingolipidome, this work was carried out on samples from animals considered pathology-free: no mastitis (milk cell count below 100,000), no abnormally high haptoglobin levels (<400 µg/mL), and no ketosis (score <1), from 3 different farms, before and after calving. Monovette blood samples taken from 45 cows on three farms (12, 23 and 10 cows respectively), were stimulated for 24h with LipoPolySaccharide (LPS) solubilized in PBS at 3µg/mL, with controls receiving only PBS. Targeted analysis of sphingolipids was conducted by UHPLC-MSMS. Cytokines were measured by a Merck-Millipore 15-plex bovine cytokine assay to confirm the efficiency of LPS. The effects of LPS on the sphingolipidome common to all three farms were dominated by an increase in sphingosine-1-phosphate (d18:1P). This effect ranged from 20.2% to 42.5% before parturition and from 14.9% to 25.6% after parturition. Significant effects of LPS were also observed on the bases sphingoids, dihydroceramides, sphingomyelins, dihydrosphingomyelins, monohexosylceramides, and lactosylceramides, but these effects were inconsistent depending on the farm. Similarly, parturition interfered with the determination of LPS effects on these analytes. The effects of LPS on most cytokines were significant, and not influenced by the farm of origin or farrowing. No correlation could be found between variations in sphingolipid and cytokine levels. Taken together, these results suggest that the sphingolipidome assay in whole blood is a good model for revealing the impact of pathologies that directly involve d18:1P, and dihydrosphingolipids. Further analyses are necessary to determine the effects of pathologies occurring in this study model.

P13

Variation of the plasma sphigolipidome in dairy cows during peripartum

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Measurement of the plasma sphingolipidome is increasingly used to reveal inflammatory phenomena. However, there is very little data on the impact of calving and mastitis on sphingolipid profiles in cattle. An initial analysis was carried out on 35 Prime Holstein dairy cattle whose blood was collected 30 days before and 7 days after calving. The animals, from 3 different farms, were in good fattening condition, with no apparent pathology on clinical examination, no ketosis (score <1), no mastitis (milk cell count below 100,000), and no abnormally high haptoglobin levels (<400 µg/mL). Targeted UHPLC-MSMS analysis of the sphingolipidome was carried out. Principal component analysis showed a strong breeding effect. After normalizing the values, a least squares discriminant analysis (PLS-DA) revealed an effect of parturition on the sphingolipidome ($Q^2 = 0.74$, specificity of 99% and sensitivity of 98%). This effect was similar in all three farms, and was characterized by a significant increase in sphingosine-1-phosphate (d18:1P) levels ranging from 37.8% to 51.9% depending on the farm, a 12.5% to 69% increase in dihydrosphingomyelin levels, and a 14.6% to 38.3% decrease in dihydroceramide levels. A second analysis was carried out on 31 cows of the same breed from 14 farms, divided into 3 groups consisting of 13 healthy cows, identified according to the above criteria, 10 mastitis-only cows, with a milk cell content above 300,000 and no sign of diffusion of the infectious process outside the mammary gland, and 8 intermediate cows. The effect of mastitis on the sphingolipidome assessed by ANOVA was not significant ($P>0.05$). Calculation of the ratios between analytes assayed and sphingosine revealed a significant increase for sphinganine, most sphingomyelins, hexosylceramides and lactosylceramides in mastitis cows alone. No effect on d18:1P or its ratio to sphingosine was observed. Taken together, these results show that plasma sphingolipidome variability in dairy cows is significant, with rearing and parturition having more marked effects than mastitis. The effects of mastitis were only revealed after normalization, allowing for individual variations. This last result is consistent with this pathology remaining localized to the mammary gland in this study.

P14

Characterization of neutral sphingomyelinase, acid and neutral ceramidase activities in human plasma and serum

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Sphingolipids and their metabolizing enzymes have received attention across a wide range of disorders. However, the detection of enzymatic activities has not yet become part of clinical routine. While sphingomyelinases and ceramidases, which catalyze reactions to either yield or reduce ceramide—the central sphingolipid hub molecule—are well characterized from cell and tissue sources, their characterization in blood, the most easily available human sample material, remains limited.

We used fluorescently labeled sphingomyelin and ceramide substrates, coupled with separation by thin-layer chromatography, to detect neutral sphingomyelinase and both acid and neutral ceramidase in human plasma and serum samples. We optimized reaction conditions to facilitate their application in clinical studies. These enzymes were characterized in terms of time- and sample volume-dependent conversion, pH profile, detergent preference, sensitivity to EDTA, and the effects of various cations on enzyme activity. Additionally, we assessed the sensitivity of these enzyme activities to temperature and freeze-thaw cycles in blood samples. Furthermore, we explored the impact of sex and age on these enzyme activities and conducted comparative analyses between human and rodent samples.

Our data broaden the spectrum of potential biomarkers that regulate ceramide levels in human liquid biopsies, providing additional tools to enhance our understanding of disease mechanisms and identify potential therapeutic targets in the field of sphingolipids.

P15

Ganglioside GD3 and liver fibrosis: role of GD3 acetylation

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Introduction: Fibrosis can be defined as the excessive accumulation of extracellular matrix, which occurs in most chronic liver diseases because of a persistent injury. These include viral infections, alcohol, autoimmune diseases, biliary obstructions or nonalcoholic steatohepatitis. The normal liver contains an epithelial component (hepatocytes), an endothelial lining, tissue macrophages (Kupffer cells) and the perivascular stellate cell. The activation of hepatic stellate cells (HSC) is the dominant event in fibrogenesis, these events refers to the conversion of quiescent cells vitamin A-storing cells into proliferative, fibrogenic, and contractile myofibroblasts. Active HSC are refractory to apoptosis. Ganglioside GD3 is a glycosphingolipid synthesized from GM3 by GD3 synthase that induces apoptosis by a dual mechanism involving mitochondrial targeting, eliciting mitochondrial membrane permeability transition, and inactivation of NF-κB-dependent survival program. Recent findings have shown that GD3 acetylation by O-acetyl disialogaglioside synthase (OAcGD3S), which acetylates GD3 to 9- O-acetyl GD3, antagonizes the proapoptotic actions of ganglioside GD3. It has been reported that human cirrhotic liver exhibit increased expression of OAcGD3S. **The aim** of this study was to investigate the status of GD3 acetylation during HSC activation, the susceptibility of HSC to GD3-mediated cell death and the role of OAcGD3S in HSC biology and activation in mouse models and human samples. **Methods:** Eight weeks old male C57BL/6J mice were fed a 60% high fat diet enriched in 0.5% with cholesterol (HFHC) from 1 week to three months. HSC from control and HFHC-fed mice were analyzed for a-SMA, Col1A1 TGFb, PDGF, GD3 levels and OAcGD3S expression. HSC apoptosis was examined by TUNEL and caspase 3 activity. The impact of OAcGD3S silencing was examined in HSC activation. GD3 immunohistochemistry analyses and OAcGD3S expression was monitored in human biopsies from subjects with chronic liver disease. **Results:** Animals on HFHC showed steatosis and hepatomegaly, starting at seven days after feeding. HSC from HFHC mice showed increased mChol deposition which correlates with increase alpha-SMA activation, and a-SMA, Col1A and PDGFR expression at six days of feeding. HFHC diet increased the expression of GD3 synthase and acetylase enzymes and correlated with the progression of fibrosis. GD3 incubation lead to HSC apoptosis in a time-dependent manner. Furthermore, silencing of OAcGD3S, prevented HSC differentiation and fibrosis progression **Conclusion:** Targeting of OAcGD3S could be a new therapeutic target for the treatment of liver fibrosis.

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P16

Ganglioside GD2 in the stem-like compartment of intrahepatic cholangiocarcinoma

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Introduction: Gangliosides (GS), sialic acid-containing glycosphingolipids, could play an important role as markers of cancer stem cells (CSC). In particular, GD2 has been investigated for its involvement in the malignant phenotype of several cancers and in cancer-stem like cells, but there are no data about human cholangiocarcinoma (CCA). Our study aims to provide a GS profiling of both stem-like subsets and their parental cells in intrahepatic CCA (iCCA). **Methods**: Stem-like subset of two iCCA cells was enriched by sphere culture (SPH) and compared to monolayer parental cells (MON). iCCA GS profiles were evaluated by chromatographic analytical procedures, after feeding with radioactive sphingosine. Plasma membrane GD2 expression was revealed by FACS, while GS biosynthesis enzymes were analyzed by RT-qPCR during spherogenesis. The modulation of stem features by GS and *in vitro*/*in vivo* tumorigenic properties were studied using a) PPMP (glucosylceramide synthase inhibitor), b) iCCA GD3S-transfected cells, and c) transcriptomic analyses. **Results**: In both iCCA lines, compared to MON, SPH showed severe changes in GS composition. In contrast to MON, iCCA-SPH exhibited increase content of GM3 and reduction of GM2, and, among complex GS, strongly increase of GD1a and appearance of GD2, findings corroborated by high levels of GM3 synthase as well as GD3- and GM2/GD2 synthases expression. By bioinformatic investigations, it has emerged that cancer-stem features related on GD2 availability are not due to the GM2/GD2 synthase, but depend on the GD3S, the synthase that provides the precursor (GD3) to produce GD2. iCCA cells stably transfected with GD3S highlighted *in vitro* enhanced sphere-forming ability, invasive properties as well as higher drug resistance compare to the transfected control. *In vivo* experiment, CCLP1 overexpressing GD3S developed a tumor mass volume 2-3 fold greater compared to the control. By global transcriptomic analysis, ontology investigations identified 74 processes shared by the cell lines, with an enrichment for development and morphogenesis processes, signaling, in particular MAPK pathway and locomotion. **Conclusions**: We demonstrate for the first time that the iCCA stem-like properties are related to GS synthetic pathway and patterns. GD3 synthase and GD2 ganglioside could represent potential markers for iCCA stem phenotype.

P17

Knock-out of β-galactosylceramidase decreases the malignant potential of human melanoma cells

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β-galactosylceramidase (GALC) is a lysosomal acidic hydrolase that exerts its action by removing β - galactosyl moieties from β -galactosyl sphingolipids. During the last decade GALC expression in melanoma was shown to be positively correlated to the stage of the disease, pointing to a role of this enzyme in promoting tumor progression and metastatization.
Using CRISPR/Cas9 genome editing, we induced GALC knock-out in melanoma A2058 and A375 cell lines. KO cells were characterized by a decrease in their proliferative potential in vitro and in vivo. This was paralleled by a decrease in the energic metabolism of A2058 GALC-KO cells, together with an increased production of mitochondrial reactive oxygen species and reduced glutathione levels.
At present, Omics data analysis of GALC KO cells is in progress to elucidate the mechanism by which GALC may affect the energetic metabolism of melanoma cells, thus hampering their tumorigenic potential. The results will shed new light about the role of sphingolipids in melanoma and may provide information for the development of novel therapeutic strategies.

P18

Peripheral Upregulation of Parkinson's Disease-Associated Genes Encoding α-Synuclein, β-Glucocerebrosidase, and Ceramide Glucosyltransferase in Major Depression

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Given the high comorbidity of Parkinson's disease (PD) with major depressive disorder (MDD) and the role of sphingolipids in both, we explored the peripheral expression levels of three genes primarily associated with PD: α-synuclein (*SNCA*), lysosomal enzyme β-glucocerebrosidase (*GBA1*), and UDP-glucose ceramide glucosyltransferase (*UGCG*) in a sex-balanced MDD cohort. Notably, *GBA1* and *UGCG* enzymes are involved in sphingolipid turnover.

We utilized quantitative PCR to determine normalized gene expression in MDD patients (unmedicated n = 63, medicated n = 66) and controls (remitted MDD n = 39, healthy subjects n = 61). Our analysis revealed that *SNCA* ($p = 0.036$), *GBA1* ($p = 0.014$), and *UGCG* ($p = 0.0002$) expression levels were significantly higher in currently depressed patients compared to controls and remitted patients. Notably, the expression levels of *GBA1* and *UGCG* decreased in patients undergoing medication therapy over three weeks. Moreover, subgroup analyses demonstrated a positive correlation between gene expression and the severity of depression and anxiety. We also identified correlations between gene expression levels and PD-related laboratory parameters.
These findings suggest that analyzing *SNCA*, *GBA1*, and *UGCG* could be crucial in identifying biomarkers for MDD and elucidating the overlapping pathological mechanisms underlying neuropsychiatric disorders.

P19

Acid Sphingomyelinase knock-out and overexpression models link synuclein genes expression and behavior in mice

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Considering the high comorbidity between Parkinson's disease and major depressive disorder, as well as the involvement of sphingolipids in both conditions, we explored the gene expression levels of α-, β-, and γ-synuclein in mouse models with alterations in acid sphingomyelinase (ASM). ASM catalyzes the degradation of sphingomyelin to ceramide, and it has been associated with behavioral parameters.
We investigated twelve brain regions in an ASM knock-out mice model (homozygous ASM-/-, n=7; heterozygous ASM+/-, n=7, wildtype n=5) and six brain regions in a mouse model with ASM overexpression restricted to the forebrain (ASM-tg^{fb}, heterozygous n=19, wildtype n=19). Quantitative PCR was used to determine the normalized gene expression of the three synuclein genes *Snca*, *Sncb* and *Sncg*. In the ASM knock-out model, the expression of all three synuclein genes was brain-region specific but independent of the genotype. However, in the ASM-tg^{fb} model, a genotype significant effect was found for *Snca* in the dorsal mesencephalon and ventral striatum, and for *Sncg* in the dorsal mesencephalon. Notably, β-synuclein exhibited overall higher expression levels. Furthermore, we discovered correlations between the synuclein gene expression levels and depressive-/anxiety-like behavior, as well as locomotor activity.
These results suggest that the analysis of synuclein genes could be valuable in identifying biomarkers and understanding the common pathological mechanisms underlying various neuropsychiatric disorders.

P20

Impact of acid sphingomyelinase deficiency on other ceramide related enzymes in mice

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Alterations in sphingolipid metabolism are believed to play a role in the development of major depressive disorder. Specifically, the sphingolipid rheostat, which balances levels of ceramide and its metabolites, is crucial for regulating cell survival and apoptosis, potentially influencing depressive symptoms. We investigated enzymes that increase or decrease ceramide levels in a model deficient for one enzyme—acid sphingomyelinase, which catalyzes the hydrolysis of sphingomyelin to ceramide—to determine its feedback or regulatory effects.
We measured the enzymatic activity of acid and neutral sphingomyelinases (ASM, NSM), acid and neutral ceramidases (AC, NC), and sphingomyelin synthase (SMS) in twelve brain regions, peripheral tissues, and serum of sex-mixed homozygous (ASM−/−, n=7) and heterozygous (ASM+/−, n=7) ASM-deficient mice, along with wild-type controls (ASM+/+, n=5) using fluorescently labeled substrates. While all ASM activities were in line with the corresponding genotype, none of the other enzymes showed consistent alterations due to reduced or absent ASM activity, except for SMS in the spleen, which was significantly increased in ASM−/− compared to the other genotypes. We also discovered increased activity of these enzymes in multiple brain regions associated with anxiety- and depressive-like behavior, including the lateral septum, hypothalamus, and dorsal and ventral striatum.
These findings offer new insights into sphingolipid metabolism in the context of major depression and co-occurring anxiety.

P21

Ceramide-1-phosphate modulates binding of α-synuclein to the membrane

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Protein-membrane interactions play a crucial role in majority of cellular processes, yet have been explored in relatively limited range when compared to protein-protein and protein-DNA interactions. This study focuses on investigating the interactions between α-synuclein (αS) and lipid membrane models enriched with ceramide-1-phosphate (C1P), a signaling lipid integral to multiple cellular processes. αS, a key player in neurodegenerative disorders like Parkinson's disease, exhibits high affinity for lipid membrane containing acidic phospholipids, and the latter influences its function, conformation and aggregation propensity.
The study uses a combination of experimental techniques such as microscale thermophoresis and biophysical characterization, along with computational simulations to analyze the properties of C1P-enriched membranes. By correlating membrane parameters with protein-membrane interaction descriptors, the study aims to identify key factors governing αS-membrane interplay. This comprehensive approach enabled us to find that the binding of αS to the membrane does not simply depend on the presence of an anionic lipid and the surface charge it generates, but rather on the biophysical properties of the entire membrane that is shaped by this lipid. With this approach, it was shown that the strength of αS binding to the membrane depends mainly on the presence of defects in the membrane and interdigitation of acyl chains between the membrane leaflets, while the cooperativity of this binding depends on the degree of lipid order in the membrane and the resulting properties such as rigidity of lipid bilayer.
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P22

APOE genotype-related hippocampal lipid changes are sex-dependent in a mouse model of familial Alzheimer's disease

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The hippocampus, an area in the brain crucial for learning and memory functions, is affected early in the pathological course of Alzheimer's disease (AD). Changes in the brain's lipid metabolism are additionally seen at the initial stages of the disease and that accompanying lipid changes in the hippocampus are age-dependent and sex-specific. However, due to the heterogeneity of cell type and function in the hippocampal formation, AD-related lipid changes have been shown to vary by hippocampal subregion, particularly the cornu ammonis 1 (CA1) and dentate gyrus (DG) regions. Nevertheless, the extent to which these region-specific hippocampal lipid changes are influenced by major AD risk factors, such as APOE genotype, age, and sex, is currently unknown.
In this study, lipid distribution differences were assessed in the hippocampal formation and its subfields (CA1, CA3, DG, and subiculum (SC)) by matrix-assisted laser desorption/ionization imaging (MALDI) imaging in transgenic male and female 5xFAD mice carrying the human APOE3 (E3FAD) or APOE4 gene variant (E4FAD) at 2-3, 5-6, and 8-9 months respectively.
We found that various phosphatidylcholine (PC) and phosphatidylethanolamine (PE) lipid species were changed in the hippocampus between E3FAD and E4FAD, mainly in the CA regions and the SC. While various genotype-related lipid distribution differences were affected by sex in specific hippocampal subregions, no age-related interactions were observed. We observed considerable variation in amyloid pathology as earlier observed in this mouse model warranting cautious interpretation of the results, findings from this study indicate that early genotype-related lipid changes in the hippocampus in AD may be moderated by sex.
A thorough understanding of the lipid profiles within hippocampal subregions during early-stage AD, in relation to key disease risk factors, may provide tools to understand and modulate the functions of these specific regions, both in health and disease.

P23

Visualizing sphingomyelin in the retinal ganglion cell layer

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Purpose: Glaucoma causes blindness by targeting retinal ganglion cells (RGC) for degeneration. Evidence from metabolomics studies suggest that glaucoma pathophysiology involves degradation of sphingomyelin (SM), which is a lipid composing cell plasma membranes. However, no method exists to directly relate SM accumulation in specific cell classes in the retina. The purpose of this study was to develop a method to detect SM in RGCs and astrocytes in the RGC layer (RGCL) of intact retinas.
Methods: C57Bl/J mice were perfused with 175mM PBS plus heparin then 175mM PBS and 4%PFA followed by post-fixation for 2hrs Then, eye cups were incubated in 175mM PBS for 4hrs followed by incubation in 175mM PBS plus 50mM glycine for 16hrs. After that, retinas were incubated in primary antibodies against RBPMS (a RGC marker), GFAP (an astrocyte marker), and CD31 (an endothelial marker) for 72hrs at 4ºC. Afterwards, we applied secondary fluorescent antibodies and lysenin conjugated with green fluorescent protein (LysGFP). Lysenin is a protein that specifically binds to SM. Retinas were exposed to secondary antibodies and LysGFP for 48hrs at 4ºC. For validation, we performed the same experiments with GFP without lysenin. Indicator fluorescence was recovered by confocal microscopy. We measured fluorescence intensity and colocalization of LysGFP to RBPMS, GFAP, and CD31 using ImageJ.
Results: We observed robust immunoreactivity of RBPMS, GFAP, and CD31. We detected putative SM in the RGC layer and in the retina vasculature as indicated by LysGFP fluorescence. Application of GFP instead of LysGFP resulted in minimal signal. Based on measurements of colocalization (Pearson's R) between RBPMS to LysGFP, GFAP to LysGFP, and CD31 to LysGFP, LysGFP colocalized more in RGCs vs. astrocytes ($p=0.0032$) and more in the endothelial cells vs RGCs ($p = 0.0002$). Interestingly, LysGFP does not appear to collect in RGCs equally. Instead, we find that a small but significant positive correlation between LysGFP intensity and RGC soma area ($R^2=0.15$, $p<0.001$).

Conclusions: We have provided a method to detect SM in intact retinas immunolabeled to indicate distinct cell classes in the RGCL. Our preliminary results indicate SM accumulates more in RGC vs astrocytes and larger RGC collect more SM vs. smaller RGCs. We also found that SM accumulates more in the retina vasculature vs RGC. This method can be used to determine how glaucoma impacts SM accumulation in distinct retinal cells classes.

P24

Activity pattern of sphingolipid metabolizing enzymes in murine brain regions and peripheral tissues

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Major Depressive Disorder (MDD) is a highly prevalent and often chronic disease. Despite extensive research, the underlying mechanisms and optimal therapies remain unclear. Recently, the ceramide/acid sphingomyelinase (ASM) pathway has garnered attention. However, spatial and temporal data on ASM and other sphingolipid metabolism enzymes in mice are sparse. Therefore, we analyzed these activities at various ages (newborn to 18 months) in twelve different brain regions and five peripheral tissues of female and male C57Bl6 mice to provide a comprehensive overview.

The enzymatic activity assays for ASM, neutral sphingomyelinase (NSM), acid/neutral ceramidase (AC/NC), and sphingomyelin synthase (SMS) utilized fluorescently labeled substrates and thin-layer chromatography to quantify the product and remaining substrate, normalized to protein concentrations determined by the Bradford Assay.

Our preliminary data indicate region-/organ-, age-, and sex-specific activities for all five sphingolipid-metabolizing enzymes, with generally higher activities in the brain compared to the periphery, except for high SMS activity in the kidney. We observed distinct age-related changes, such as increasing NC activity in the frontal cortex versus declining NC activity in most other brain regions. The cerebellum's low NSM activity further decreased from 10 to 84 days of age. Sex differences were more pronounced in older mice, with females often showing higher enzyme activity.

These initial findings provide a detailed fingerprint of central and peripheral ceramide-related sphingolipid enzyme activities over time, highlighting distinctive roles of sphingolipids in specific brain regions and during development. Upon completion of age group analyses, these data will serve as a baseline for future studies on mouse models, enhancing the understanding of neuropsychiatric disorders and optimizing therapeutic approaches.

P25

Peripheral sphingolipid enzyme activities in masculine depression

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"Masculine depression" refers to a set of alternative symptoms of depression, including externalizing behaviors, emotional suppression, substance abuse, and increased risk-taking, which can occur in both men and women. Due to these atypical manifestations, sufferers are often underdiagnosed and inadequately treated, necessitating new diagnostic and therapeutic approaches. Recent research suggests a link between sphingolipid metabolism and depressive disorders.
In a study involving 176 healthy individuals and 163 patients with major depressive disorder, the activities of several enzymes involved in sphingolipid metabolism were examined in serum samples. The patient group was divided into those with higher (MD) or lower (NMD) masculine depression severity based on gender-specific medians of the Male Depression Risk Scale (MDRS). The activity of secreted acid sphingomyelinase (S-ASM) was measured using a fluorescently labeled substrate and thin-layer chromatography.
Since S-ASM activity was significantly higher in men than in women, non-parametric analyses were performed separately by gender. While no significant differences in S-ASM activity were found between the groups in men, healthy women had the lowest S-ASM activity, and MD women had the highest ($p<0.001$). Additionally, in healthy men, S-ASM activity showed positive associations with the Gotland Male Depression Scale (GMDS) and subscales of the MDRS. In depressed females, S-ASM activity was nominally significantly associated with binge drinking measures. In both healthy ($r=0.4$, $p<0.001$) and depressed ($r=0.3$, $p=0.002$) women, high S-ASM activity correlated with the MDRS alcohol subscale.
The observed group differences and correlations align with findings of increased ASM activity and ceramide levels in depressive as well as in alcohol-dependent patients. Further studies are required to explore whether sphingolipid enzymes can serve as potential biomarkers for (masculine) depressive disorders, inform new diagnostic and therapeutic procedures, and elucidate the pathomechanisms of depression. Additional enzyme activities in the serum and leukocytes of this cohort are currently being analyzed.

P26

Effect of sphingolipid metabolizing enzymes on BDNF- TrkB neurotransmission

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Sphingolipids are ubiquitously found in eukaryotic cells and are among the most abundant structural and functional components of cell membranes. Recent findings have highlighted the role of membrane lipids in cellular communication due to their signaling properties and their impact on the physical attributes of membranes, which enable transmembrane receptor proteins to function adequately.
Brain-derived neurotrophic factor (BDNF) and its high-affinity receptor TrkB have been repeatedly associated with various psychiatric disorders, including major depressive disorder. Sphingolipid-related enzymes are also altered in patients with depression and other psychiatric disorders. This study aims to evaluate whether changes in sphingolipid enzyme activities, and thus alterations in membrane sphingolipids, can affect the activation and phosphorylation of the TrkB receptor protein upon BDNF stimulation in vitro, suggesting a possible mechanism connecting these findings.
Primary rat cortical neurons were treated with fluoxetine, a functional inhibitor of acid sphingomyelinase (ASM), and specific commercial inhibitors of sphingomyelin synthase (SMS) isoforms at various dosages and time combinations to pharmacologically reduce enzyme activity. After additional stimulation with BDNF, cells were harvested and analyzed for enzyme activity using fluorescently labeled substrates and for total and phosphorylated TrkB receptor via Western blot.
While ASM and SMS2 were successfully inhibited in the treated cells, two SMS1 inhibitors reduced SMS activity only in cell lysates but failed to show an effect in living cells. The ongoing evaluation of total and phosphorylated TrkB has shown high variation. ELISA and Western Blot optimizations are in progress to supplement the data. Other's work has demonstrated a rapid antidepressant effect of SMS inhibitors in mice. The understanding of sphingolipid implications in depression can set the basis for faster acting and more effective therapies and contribute to the understanding of lipid-protein interactions in health and disease.

P27

Blood pressure lowering by sphingosine kinase 2 inhibition improves outcome after myocardial infarction

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Hypertension is a risk factor for cardiovascular disease. Particularly, the pro-hypertensive hormone Angiotensin II (AngII) mediates cardiac contractility and remodeling. Previous work has shown that sphingosine-1-phosphate (S1P), generated by hematopoietic sphingosine kinase 2 (SphK2), contributes to the development of hypertension. Moreover, SphK2 inhibition was conferred with therapeutic efficacy to lower elevated blood pressure (BP). Thus, this study examined if pharmacological SphK2-inhibition represents a promising cardioprotective anti-hypertensive strategy and improves outcome after myocardial infarction (MI).
Male C57BL6/N mice were subjected to AngII-infusion for 6 weeks. Two-weeks of subcutaneous SphK2-inhibitor or vehicle treatment was initiated at 4-weeks of AngII-infusion. Thereafter, mice were subjected to experimental MI by permanent left anterior descending coronary artery ligation. SphK2-inhibition lowered elevated BP irrespective of vascular function and plasma S1P. Post-MI plasma S1P concentrations were lower compared to sham-operated controls in normotensive mice, and in hypertensive compared to normotensive MI mice. In both cohorts, stroke volume and end-diastolic volume inversely correlated with plasma S1P. Left ventricular ejection fraction was significantly lower in hypertensive compared to normotensive and SphK2-inhibitor treated mice at different time points post-MI. Hypertension affected post-MI left ventricular gene expression for natriuretic peptide b (*Nppb*), fatty acid binding protein 3 (*Fabp3*), smooth muscle actin (*Acta2*) and S1P receptor 1 (*S1pr1*). Therapeutic SphK2-inhibition mitigated the MI-associated *Nppb* and *S1pr1* augmentation but not *Fabp3* and *Acta2* expression. Neurohumoral compensation, evidenced by increases in heart rate and BP between 9-to-12-weeks post-MI, was absent in SphK2-inibitor treated mice.
In summary, SphK2-inhibition as antihypertensive therapy improves outcome after MI and may represent a promising target for harnessing cardiovascular risk in hypertension.

P28

Sphingosine inhibits calmodulin action: Implication for regulation of endothelial nitric oxide synthase-mediated vasorelaxation

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Calmodulin (CaM), the main modulator of intracellular calcium signaling, regulates the function of a great number of proteins. The signaling lipid sphingosine (Sph) has been reported to inhibit several CaM-dependent enzymes, and we hypothesized that Sph can directly bind to CaM to modulate its function. Here we show that Sph binds to both apo and Ca^{2+}-saturated CaM in a concentration and stoichiometry dependent manner. The crystal structure of the Sph-Ca^{2+}CaM complex gains insight into the structural basis of the interaction. We demonstrate that Sph was able to displace the model CaM-binding peptide melittin from CaM, and to inhibit the CaM-dependent activity of nitric oxide synthase (NOS) and myosin light chain kinase (MLCK) thereby regulating the vascular tone. We show that in *ex vivo* conditions, accumulation of Sph results in reduced NOS-dependent vasorelaxation, attributed to the inhibition of CaM by Sph. We conclude that the mediator lipid Sph and their analogues formed endogenously in eukaryotic cells might regulate CaM function and contribute to the control of the vascular tone *in vivo*.

P29

Sphingomyelin level in bovine meat from organic short supply chain

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The control of the supply chain, in terms of services, processes and products is relevant for maintaining quality of meat, essential for human health. The short supply chain is a valid alternative to the long supply chain. The short supply chain implies a great advantage because the meat is easily perishable and has a short shelf life. The aim of this work was to compare the phospholipid composition in the diaphragm of beef from organic farming and traditional farming. Furthermore, the effects of short supply chain and long supply chain were compared. Thu from organics, we analyzed meat samples coming from the traditional short supply chain, the organic short supply chain, the traditional long supply chain, and the organic long supply chain. The results showed that total phospholipid content was higher in both organic samples from short supply chain and from long supply chain than their respectively traditional samples. No changes were found between traditional short supply chain and traditional long supply chain or between organic short supply chain and organic long supply chain. Thus, we analyzed separately each phospholipid. Phospahtidylserine plus phosphatidylinositol, sphingomyelin and phosphatidylethanolamin content was higher in both organic samples than their respectively traditional samples. Moreover, the phosphatidylcholine was higher in organic short supply chain than traditional short supply chain. Intriguingly, the value of sphingomyelin reduced in long supply chain respect to short chain in both organic and traditional samples. Since sphingomyelin facilitates the brain development (Albi et al., 2022), regulates lipid metabolism and prevents metabolic syndrome (Li et al., 2024), the use of meat from short chain might be essential for human health.
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P30

Loss of Globotriaosylceramide in Kidney Protects Against Acute Cisplatin Injury but Worsens TGF-B1 mediated Epethilial-Mesenchymal Transition

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Background & Aim: In acute kidney injury inhibition of glycosphingolipid globotriaosylceramide (Gb3) by knockout (KO) of Gb3 synthase (Gb3S) was shown to protect proximal tubular cells (PTCs), potentially due to reduced uptake of toxins. However, the underlying molecular mechanisms remain poorly understood. In patients receiving chemotherapy with cisplatin, a platinum based drug, severe damage to the kidney occurs in one-third of the patients. In this study we aimed to investigate the therapeutic potential of targeting Gb3 against cisplatin mediated acute kidney injury.
Results: Upon cisplatin injury Gb3s KO cells had reduced apoptosis, while Gb3s over-expression (OE) cells had increased apoptosis compared to WT. Lipidomic analysis revealed that the apoptotic response was not due to accumulation of apoptotic ceramides as shown previously in cisplatin injury models in literature. Instead, we observed decreased mRNA expression of cisplatin uptake transporters on Gb3s KO and increased expression on Gb3s OE. Moreover, the Gb3s KO cells expressed more mesenchymal markers in baseline; whereas, Gb3s OE cells showed increased cell polarisation markers. We observed that globoside dowregulation lead to a ganglioside enriched lipidome in PTCs, which has been previously shown in other cell types to alter cell signalling. These observations suggested that loss of Gb3 in human PT cells resulted in reduced PT epithelial identity potentially due to altered signalling. Concurrently, we found the Gb3s KO cells had increased susceptibility to TGF-B1 signalling and resultant epethilial-mesenchymal transition. Lastly, while the Gb3s KO mice were identical to WT in baseline conditions, they still showed a protective effect upon acute cisplatin injury.
Conclusion and Outlook: Loss of Gb3 protected against acute damage in cisplatin kidney injury potentially by decreased expression of PT transporters and reduced uptake of the drug. However, the increased mesenchymal status upon Gb3 ablation could potentially make the kidneys more susceptible to TGF-B1 mediated induction of fibrosis. We are further investigating the underlying molecular mechanisms.

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