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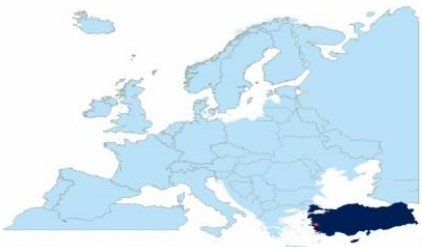

CESME / IZMIR, TURKEY

Meeting Booklet

INTERNATIONAL CERAMIDE CONFERENCE

SPHINGOLIPID CLUB

JOINT MEETING

MAY 6 - 10, 2015

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POSTER ABSTRACTS

INTERNATIONAL CERAMIDE CONFERENCE

and

SPHINGOLIPID CLUB

JOINT MEETING

SCIENTIFIC PROGRAM

Wednesday, May 6th

Time	Title	Speaker
3:00 – 7:00	Registration & Meeting Check-In	All
5:00 – 6:30 6:30 – 8:30	Reception Dinner	All
8:30 - 8:45	Welcoming Remarks	Besim Ogretmen, Medical University of South Carolina; Thierry Levade, Université Paul Sabatier; Yusuf Baran, Abdullah Gul University
KEYNOTE PRESENTATIONS		
8:45 – 9:15	Membrane Lipids Regulate Sphingolipid Catabolism, Their Enzymes And Lipid Binding Proteins	Konrad Sandhoff, Rheinische Friedrich-Wilhelms-Universitat Bonn
9:15 – 9:45	The Sphinx, Bioactive Sphingolipids, and Sphingomyelinases	Yusuf Hannun, Stony Brook University

Thursday, May 7th

Time	Title	Speaker
7:30 - 8:30	Registration & Breakfast	All
SESSION I – Sphingolipids and Membrane Biophysics Session Chairs: Richard Kolesnick - Memorial Sloan Kettering Cancer Center; Manuel Prieto - Universidade de Lisboa		
8:30 - 8:50	The New Sphingolipid Vascular Biology of Single Dose Radiotherapy	Richard Kolesnick, Memorial Sloan Kettering Cancer Center
8:50 - 9:05	Very Long Chain Ceramides Interfere with C16-Ceramide-Induced Channel Formation: A Plausible Mechanism for Regulating the Initiation of Intrinsic Apoptosis	Johnny Stiban, Birzeit University
9:05 - 9:20	Ceramide-Induced Biophysical Alterations in Membranes of Live Cells	Ana Ester Ventura, Universidade de Lisboa
9:20 - 9:35	Ceramide-Enriched Compartments in Neural Stem Cell Differentiation and Neurodegeneration	Erhard Bieberich, Georgia Regents University
9:35 - 9:55	Unravelling the Biophysical Properties of Sphingolipids: From Model to Cell Membranes	Manuel Prieto, Universidade de Lisboa
9:55 - 10:20	Coffee Break	All
SESSION II – Regulation of Trafficking, Autophagy, and Cancer Therapeutics by Sphingolipids Session Chairs: Mark Kester - University of Virginia; Kentaro Hanada - National Institute of Infectious Diseases		
10:20 - 10:40	Ceramide Nano-Liposomes: Road to the Clinic	Mark Kester, University of Virginia
10:40 - 10:55	Laptm4b Facilitates Late Endosomal Ceramide Export to Control Sphingolipid-Mediated Cell Death Pathways	Tomas Blom, University of Helsinki
10:55 - 11:10	Dihydroceramide Accumulation Mediates Cytotoxic Autophagy of Cancer Cells via Autophago-Lysosome Destabilization	Guillermo Velasco, Universidad Complutense
11:10 - 11:25	Lipid Oversupply to Cardiomyocytes Induces Sphingolipid-Dependent Oxidative Stress and Induction of Mitophagy Through Ceramide Synthase 2	Brittany Law, Medical University of South Carolina
11:25 - 11:40	Adjuvant Tamoxifen Improves Effectiveness of Ceramide-Centric Therapy in Acute Myelogenous Leukemia	Myles Cabot, East Carolina University
11:40 - 12:00	Host Sphingolipid-Transfer Proteins and Infectious Diseases	Kentaro Hanada, National Institute of Infectious Diseases
12:00 - 1:30	Lunch	All
SESSION III – Enzymology and Regulation of Sphingolipid Metabolism Session Chairs: Yasuyuki Igarashi - Hokkaido University; Gerhild van Echten-Deckert - University of Bonn		
1:30 - 1:50	Function of Ganglioside and Sphingolipid-Linked Exosome Secretion in Sequestering Alzheimer Amyloid-ß	Yasuyuki Igarashi, Hokkaido University
1:50 – 2:05	Molecular Mechanism of the Production of Acylceramide, the Key Lipid for Skin Barrier Formation	Akio Kihara, Hokkaido University

Time	Title	Speaker
2:05 - 2:20	Identification of a Novel Pathway for Acylceramide Generation in the Lipid Droplets by Ceramide Synthase, Fatty Acyl-CoA, and Diacyglycerol Acyltransferase Enzyme Complex	Can Senkal, Stony Brook University
2:20 - 2:35	Epigenetic Control of Glycosphingolipid Metabolic Switch	Giovanni D'Angelo, National Research Council of Italy
2:35 - 2:50	Elucidating Novel Metabolic Pathways of the Neurotoxic 1-Deoxysphingolipids	Irina Alecu, University Hospital Zurich
2:50 - 3:05	Production of 2-n-acyl-amino-14,16-dimethyloctadecan-3-ol (n-acyl-aod) in CHO-LY-B Cells	Brandon M. Kenwood, Georgia Institute of Technology
3:05 - 3:25	S1P Lyase Deficiency in the Brain	Gerhild van Echten-Deckert, University of Bonn
3:25 - 4:00	Coffee Break	All
SESSION IV – Sphingolipids in Health and Disease: I - Neurodegenerative Disorders Session Chairs: Anthony Futerman - Weizmann Institute of Science; Riccardo Ghidoni - University of Milan		
4:00 - 4:20	What Have We Learnt From the Study of Ceramide Synthase Knock-Out Mice?	Anthony Futerman, Weizmann Institute of Science
4:20 - 4:35	Lack of Ceramide Synthase 2 Suppresses the Development of Experimental Autoimmune Encephalomyelitis by Impairing the Migratory Capacity of Neutrophils	Sabine Groesch, Goethe University
4:35 - 4:50	Ceramide Transporters: The Link Between Lipid Metabolism, Inflammation and Amyloid-ß Aggregation In Alzheimer's Disease	Pilar Martinez, Maastricht University
4:50 - 5:05	The Pathomechanisms Underlying the Neurotoxicity of 1-Deoxysphingolipids	Saranya Suriyanarayanan, University Hospital Zürich
5:05 – 5:20	Development of Pheochromocytoma in Ceramide Synthase 2 Null Mice	Yael Pewzner-Jung, Weizmann Institute of Science
5:20 – 5:35	Alkaline Ceramidase 3 Deficiency Results in Purkinje Cell Degeneration	Kai Wang, Stony Brook University
5:35- 5:50	Administration of Phytoceramide and Glucosylceramide Ameliorated Memory Impairment in Mice	Seikwan Oh, Ewha Womnas University
5:50- 6:05	Structural and Functional Characterization of Serine Palmitoyl Transferase in Mammals	Museer Lone, University Hospital Zurich
6:05- 6:25	TBA	Riccardo Ghidoni University of Milan
7:00 - 9:00	Dinner	All

Friday, May 8th

Time	Title	Speaker
7:30 - 8:30	Breakfast	All
SESSION V – Sphingolipids in Health and Disease: II - Metabolic and Cardiovascular Disorders Session Chairs: Ashley Cowart - Medical University of South Carolina Scott Summers - Baker IDI Heart & Diabetes Institute		
8:30 - 8:50	Roles for Ceramide Synthase 5 in the Heart	Ashley Cowart, Medical University of South Carolina
8:50 - 9:05	Hepatic Up-Regulation of Serine Palmitoyltransferase Subunit 2 by Endoplasmic Reticulum Stress Modulates Glucose and Lipid Metabolism	Tae-Sik Park, Gachon University
9:05 - 9:20	Deoxysphingolipids, Novel Biomarkers for Diabetes, are Cytotoxic for Insulin-Producing Cells	Sabrina Sonda, University Hospital Zurich
9:20 - 9:35	Lysosomal Stress Drives Sphk1 Expression in Adipose Tissue Macrophages	Marco van Gijk, Amsterdam Medical Center
9:35 - 9:55	The Role of Adipose Ceramide in Metabolic Homeostasis	Scott Summers, Baker IDI Heart & Diabetes Institute
9:55 - 10:30	Coffee Break	All

SESSION VI – Sphingolipids in Health and Disease: III - Cancer Session Chairs: Sarah Spiegel - Virginia Commonwealth University; Nathalie Andrieu-Abadie - INSERM		
10:30 - 10:50	Active Phosphorylated FTY720/Fingolimod is a Potent Inhibitor of Class I Histone Deacetylases that Reactivates Estrogen Receptor Expression and Increases Hormonal Therapeutic Sensitivity of Breast Cancer	Sarah Spiegel, Virginia Commonwealth University
10:50 - 11:05	Cers4/Ceramide Metabolism in the Regulation of TGF-Beta Receptor Signaling and Tumor Metastasis	Salih Gencer, Medical University of South Carolina
11:05 - 11:20	C16-ceramide is a Natural Regulatory Ligand of p53	Natalia Krupenko, UNC Chapel Hill
11:20 - 11:35	Stimulating cAMP Overproduction Accelerates Development of Cutaneous Squamous Cell Carcinoma	Yoshikazu Uchida, University of California
11:35 - 11:55	Dysregulation of Sphingolipid Metabolism in Melanoma: Roles in Cell Survival and Tumor Progression	Nathalie Andrieu-Abadie, INSERM
12:00 - 1:00	Lunch	All
1:00 - 4:00	Free Time	All
SESSION VII – Sphingolipids in Health and Disease: III - General Health and Disease Session Chairs: Paola Bruni - Universita degli Studi di Firenze; Dagmar Meyer zu Heringdorf - Goethe University		
4:00 - 4:20	Key Role of S1P Receptors in the Action Mechanism of TGF Beta in Myoblasts	Paola Bruni, Universita degli Studi di Firenze
4:20 - 4:35	Thrombocytopenia Induced by Sphingomyelin Deficiency in Sms1-Knockout Mice	Toshiro Okazaki, Kanazawa Medical University
4:35 - 4:50	Ceramide Synthase 4 is Involved in the Regulation of Adult Hair Follicle Stem Cell Populations	Franziska Peters, University of Cologne
4:50 - 5:05	Apom+HDL Restrains Lymphopoiesis and Neuroinflammation Via Sphingosine 1-Phosphate Signaling	Victoria A. Blaho, Weill Cornell Medical College
5:05 - 5:20	Defining the Role of Acid Ceramidase in Ulcerative Colitis and the Inflammatory Response	Mel Pilar Espailla, Stony Brook University
5:20 - 5:35	Sphingolipid Metabolism and Signaling in the Regulation of Drug Resistance in CML	Yusuf Baran, Abdullah Gul University
5:35 - 5:55	Lipid Signaling in S1P-Lyase-deficient Fibroblasts	Dagmar Meyer zu Heringdorf, Goethe University
6:30 - 8:00	Dinner	All
8:00 - 8:30	Business Meeting (Joint iCC and SLC meeting)	All
8:30 - 10:30	Poster Session II: Even Numbered Abstracts	All

Saturday, May 9th

Time	Title	Speaker
7:30 - 8:30	Breakfast	All
SESSION VIII – Yeast and Plant Sphingolipid Biology Session Chairs: Howard Riezman - University of Geneva; Kutlu Ulgen - Bogazici University		
8:30 - 8:50	Crosstalk Between Sphingolipid and Glycerophospholipid Metabolism in Yeast	Howard Riezman, University of Geneva
8:50 - 9:05	Glycosylceramide Supply from Ethiopian Plants – Screening and Quantification Methods	Mathias Reisberg, Martin Luther University Halle-Wittenberg
9:05 - 9:20	Systematic Lipidomics of Mutants in the Early Secretory Pathway	Isabella Riezman, University of Geneva
9:20 - 9:35	Endosomal Sterol, Phosphoinositide and Sphingolipid Signaling Integrates Cell Cycle Control with Endosomal Membrane Trafficking	Vytas Bankaitis, Texas A&M University
9:35 - 9:55	Reconstruction and Analysis of Yeast Sphingolipid Protein Interaction Network	Kutlu Ulgen, Bogazici University
9:55 - 10:30	Coffee Break	All

SESSION IX – Sphingolipid Analogues and Inhibitors, Sphingolipid-based Therapeutics Session Chairs: Nigel Pyne - University of Strathclyde; Christoph Arenz - Humboldt University		
10:30 - 10:50	Sphingolipid Analogues and Inhibitors: Sphingolipid-Based Therapeutics	Nigel Pyne, University of Strathclyde
10:50 - 11:05	FTY720 Induces Necroptosis by Regulating Ceramide Signaling at the Plasma Membrane	Rose Ndeto, Medical University of South Carolina
11:05 - 11:20	Ceramide-1-Phosphate (C1P)-Stimulated Migration and Phospho-Ceramide Analogue-1 (PCERA-1)-Induced IL-10 Expression Are Mediated via Distinct Receptors in Macrophages	Tsaffrir Zor, Tel-Aviv University
11:20 - 11:35	Uracil and Benzoxazolone Carboxamides: Discovery of Potent Small-Molecule Inhibitors of Acid Ceramidase?	Daniela Pizzirani, Fondazione Istituto Italiano di Tecnologia
11:35 - 11:55	Sphingolipid Analogues as Inhibitors of Sphingolipid Metabolism and Trafficking	Christoph Arenz, Humboldt University
12:00 - 1:00	Lunch	All
1:00 - 4:00	Free Time	All
SESSION X – Sphingolipidomics **Session Chairs: Roger Sandhoff - University of Mannheim; Samar Hammad - Medical University of South Carolina**		
4:00 - 4:20	Targeted LC-Tandem MS Analysis and MS Imaging Reveal New Sphingolipid Structures and the Function of Ceramide Synthase 3 in vivo	Roger Sandhoff, University of Mannheim
4:20 - 4:35	Phenotypic Malignant Changes and Untargeted Lipidomic Analysis of Long-Term Exposed Prostate Cancer Cells to Endocrine Disruptors	Carmen Bedia, Institute of Environmental Assessment and Water Research
4:35 - 4:50	Ceramides in Anoxia Survival	J. Thomas Hannich, Geneva University
4:50 - 5:05	Elucidating the Double Bond Position of Endogenous 1-Deoxysphingosine	Regula Steiner, University Hospital Zurich
5:05 - 5:25	Increased Plasma Levels of Select Deoxy-Ceramide and Ceramide Species are Associated with Increased Odds of Diabetic Neuropathy in Type 1 Diabetes	Samar Hammad, Medical University of South Carolina
5:25 - 6:00	Group Photo	All
6:00 - 6:30	Awards and Closing Remarks	Besim Ogretmen and Thierry Levade
7:00 - 11:00	Closing Dinner	All

Sunday, May 10th

7:30 - 8:30	Breakfast & Departure	All
	Optional Tour to Ephesus and St. Mary's Chapel	

MEMBRANE LIPIDS REGULATE SPHINGOLIPID CATABOLISM, THEIR ENZYMES AND LIPID BINDING PROTEINS

Konrad SANDHOFF[1],

[1]Lymes Institut, University Bonn,

Cholesterol and sphingolipids (SLs) stabilize eukaryotic plasma membranes. Together with phospholipids (PLs) they reach luminal vesicles in the late endosomes as platforms for membrane degradation. A maturation process of the luminal vesicles removes lipids inhibiting lysosomal catabolism. Sphingomyelin (SM) is hydrolyzed by acid sphingomyelinase, facilitating cholesterol export to the cytosol by NPC2 and NPC1. SM and cholesterol poor luminal vesicles then serve as platforms for glycosphingolipid degradation employing soluble hydrolases, SAPs (sphingolipid activator proteins) and anionic PLs as stimulators. We reconstituted the catabolic proteins on liposomal surfaces, mimicking luminal vesicles of the lysosomes as platforms for SL degradation. Liposomes with no net surface charge generated only negligible and physiologically irrelevant catabolic rates even at lysosomal pH values. Incorporation of anionic PLs into the SL-carrying liposomes, however, stimulated the catabolic rate by up to more than an order of magnitude. However, the incorporation of cholesterol or SM into the SL carrying liposomal membranes generated a strong inhibition of SL hydrolysis and the transfer of membrane lipids between liposomal vesicles by SAPs, even in the presence of anionic phospholipids. Ongoing in vitro studies indicate that PM-stabilizing lipids, i.e. SM and cholesterol, inhibit several steps of lysosomal SL and glycosphingolipid catabolism, and also lipid solubilisation as studied by Plasmon Resonance (Biacore) and intervesicular lipid transfer activities of several SAPs and NPC2, even in the presence of activating anionic PLs.

THE SPHINX, BIOACTIVE SPHINGOLIPIDS, AND SPHINGOMYELINASES

Yusuf HANNUN[1],

[1]Stony Brook,

Sphingolipids were initially discovered by J. Thudicum, who identified them as unusual and perplexing lipidic substances from the brain. Their enigmatic nature led Thudicum to naming them after the riddle of the Greek Sphinx. Our studies initially resulted in identification of bioactivities for sphingosine, and intense research over 3 decades has now resulted in appreciation of sphingolipids as an important class of cell regulatory molecules that include sphingosine, sphingosine 1-phosphate, ceramide, ceramide 1-phosphate, and several others. The most recent studies on bioactive sphingolipids have been accelerated by the molecular identification of most/all known enzymes of sphingolipid metabolism, the deployment of model organisms (e.g. yeast and mouse), the development of mass spectrometry to analyze the complexity of lipid structure and levels, and the development of informatics approaches to study the 'sphingolipidome'. Several attributes are emerging from the study of bioactive sphingolipids. First, metabolism of bioactive sphingolipids constitutes a highly regulated network involving the operation of more than 30 distinct enzymes. Second, most enzymes of sphingolipid metabolism show very specific sub cellular localization, suggesting local metabolism and action of their substrates and products. Third, the study of ceramide in particular shows that this is indeed a family of closely related molecules that show structural specificity, are generated in a combinatorial fashion, and appear to participate in distinct functions. I will illustrate these concepts through the study of the neutral sphingomyelinase family.

ORAL ABSTRACTS

THE NEW SPHINGOLIPID VASCULAR BIOLOGY OF SINGLE DOSE RADIOTHERAPY

Richard KOLESNICK[1]

Sloan-Kettering Institute[1] - NYC, NY USA

Single dose radiotherapy (SDRT), facilitated by image guidance and intensity modulation technologies that improve precision in tumor targeting to reduce risk of normal tissue toxicity, has revolutionized cancer treatment with local control rates □90%, even in tumors resistant to conventional fractionation. While classic radiobiology focuses on response of tumor cells rather than non-tumor microenvironmental cells, initial pre-clinical studies in our lab found disruption of tumor vasculature obligate for SDRT cure. This endothelial cell dysfunction results from activation of acid sphingomyelinase (ASMase), converting sphingomyelin to the second messenger ceramide in endothelial plasma membranes, events inhibitable by the angiogenic factors bFGF, VEGF-121 or VEGF-165. Conversely, anti-angiogenic agents, such as anti-VEGFR2 Ab DC101 (Imclone), de-repress ASMase activity, synergistically increasing SDRT-induced ceramide elevation, enhancing endothelial dysfunction. That ceramide is critical for anti-angiogenic radiosensitization is evidenced by □-ceramide Ab inhibition of DC101-enhanced endothelial damage. These results translate in vivo, as anti-VEGFR2 DC101 or anti-VEGF G6-31 synergistically increase SDRT-induced endothelial injury in MCA/129 sarcomas, and enhance tumor response. Critically, anti-angiogenic Ab delivery must be administered immediately prior (0.5-2h) to SDRT, but not earlier or after, to de-repress ASMase effectively. At longer intervals between drug delivery and SDRT the system appears to counter-regulate, re-setting the ceramide-generating capability of ASMase at or near the original setting. In contrast, tumors in asmase-/- mice, which provide damage-resistant vasculature, are unaffected by either anti-angiogenic agent. This lecture will review fundamentals of this new biology and present unpublished data that define mechanism of coupling of endothelial dysfunction to DNA repair in tumor cells.

VERY LONG CHAIN CERAMIDES INTERFERE WITH C16-CERAMIDE-INDUCED CHANNEL FORMATION: A PLAUSIBLE MECHANISM FOR REGULATING THE INITIATION OF INTRINSIC APOPTOSIS

Johnny STIBAN[1], Meenu PERERA[2],

[1]Birzeit University, [2]University Of Maryland,

Objective: Mitochondria mediate both cell survival and death. The intrinsic apoptotic pathway is initiated by the permeabilization of the mitochondrial outer membrane to pro-apoptotic intermembrane space proteins. A number of pathways are known to cause the egress of IMS proteins. Of particular interest is the ability of ceramide to self-assemble into dynamic water-filled channels. The formation of ceramide channels is regulated extensively by Bcl-2 family proteins as well as by dihydroceramide, the immediate precursor in the de novo biosynthetic pathway. Here, we present evidence that the chain length of biologically active ceramides serve as an important regulatory factor. Ceramides are synthesized by a family of six mammalian ceramide synthases (CerS) each of which produces a subset of ceramides that differ in their fatty acyl chain length. Interestingly, the presence of very long chain ceramides reduces the potency of C16-mediated permeabilization of mitochondria indicating that the intercalation of the lipids in the dynamic channel is destabilizing. Moreover, cells overexpressing the ceramide synthase responsible for the production of C16-ceramide (CerS5) are more vulnerable to etoposide compared to cells over expressing CerS2 (very long chain fatty acyl ceramides). We also find that co-overexpression of CerS2 and CerS5 reduces the fraction of dead cells upon etoposide treatment, indicating that the product of CerS2 inhibits C16-channel formation in vivo. This interplay between different ceramide metabolic enzymes and their products adds a new dimension to the complexity of mitochondrial-mediated apoptosis, and emphasizes its importance as a key regulatory step that commits cells to life or death.

CERAMIDE-INDUCED BIOPHYSICAL ALTERATIONS IN MEMBRANES OF LIVE CELLS

Ana Ester VENTURA[1], Sandra PINTO[1], Ana Raquel VARELA[1], Anthony FUTERMAN[2], Manuel PRIETO[1], Liana SILVA[3],

[1]Instituto Superior Tecnico, [2]Weizmann Institute Of Science, [3]Faculdade De Farmácia,

Objective: Ceramides are involved in the regulation of several cellular events and implicated in a number of diseases. It has been hypothesized that their mechanism of action is related to the changes promoted by these lipids in the biophysical properties of cell membranes. The main goal of this study was to evaluate ceramide-induced alterations in membrane biophysical properties of live cells. Formation of ceramide upon treating HEK, HeLa or SW620 cells with TNF-α or bacterial sphingomyelinase (bSMase) led to a marked increase in the formation of intracellular vesicles compared to untreated cells. Analysis of membrane order through the variation of Laurdan generalized polarization (GP) showed that these vesicles are more ordered than the plasma membrane or intracellular membranes, displaying GP values typical of a gel phase. Both the extent and biophysical properties of the vesicles was cell-type dependent. Inhibition of neutral sphingomyelinase yielded a clear decrease in the number of vesicles and in the global order of the membrane. These results further suggest that alterations in membrane biophysical properties are driven by ceramide formation. To further evaluate the biological significance of ceramide-induced vesicles, studies were performed to address whether these vesicles would traffic along the endosomal-lysosomal pathway. A time-dependent increase in the colocalization of those vesicles with the endosomal and lysosomal markers, Rab5 and Lamp1, respectively, was observed. This was accompanied by a time-dependent increase in the order of the vesicles, suggesting that ceramide might be a modulator of the endo-lysosomal pathway by changing the biophysical properties of endocytic vesicles.

CERAMIDE-ENRICHED COMPARTMENTS IN NEURAL STEM CELL DIFFERENTIATION AND NEURODEGENERATION

Erhard BIEBERICH[1],

[1]Georgia Regents University,

Objective: Ceramide is a remarkable sphingolipid in that it organizes membrane microdomains and interacts with protein kinases and phosphatases, thereby eliciting cell signaling pathways regulating neural cell apoptosis, differentiation, cell polarity, and neurodegeneration. We hypothesized that these two functions of ceramide, structural and regulatory, combine the organization of structural cell signaling platforms by ceramide with its function as a specific cell signaling lipid. Using polyclonal antibodies, for the first time generated in our laboratory against specific ceramides, we found that C24:0/C24:1 ceramide was enriched in primary cilia of neural progenitors. Another ceramide species, C18:0 ceramide, was found to induce exosome formation and secretion in astrocytes exposed to Aβ1-42, a neurotoxic amyloid peptide in Alzheimer's disease. C24:1 as well as C18:0 ceramide bound to atypical PKCζ (aPKCζ), a protein kinase C isoform regulating cell polarity. This data suggests that the interaction of C24:0/C24:1 ceramide and C18:0 ceramide with aPKCζ (and other regulatory proteins in a ceramide-induced protein complex) is critical for the formation of cilia in neural stem cell differentiation and exosomes in neurodegeneration, respectively. Therefore, cilia and exosomes may constitute two compartments that are critically regulated by distinct ceramide species. Supported by NSF grant 112157 and NIH grant R01AG034389.

UNRAVELLING THE BIOPHYSICAL PROPERTIES OF SPHINGOLIPIDS: FROM MODEL TO CELL MEMBRANES

Ester VENTURA[1], Raquel VARELA[2], Sandra PINTO[3], Amelia GONCALVES DA SILVA[4], Anthony FUTERMAN[5], Liana SILVA[6], Manuel PRIETO[3],

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[2]IMed.UL - Research Institute For Medicines And Pharmaceutical Sciences, Faculdade De Farmácia, Universidade De Lisboa, Portugal; Weizmann Institute Of Sciences, Dept Of Biological Chemistry, Rehovot, Israel; Centro De Química-Física Molecular, Instituto Superior Tecnico, Universidade De Lisboa, Lisboa, Portugal,
[3]Centro De Química-Física Molecular, Instituto Superior Tecnico, Universidade De Lisboa, Lisboa, Portugal,
[4]Centro De Química Estrutural, Instituto Superior Tecnico, Universidade De Lisboa, Lisboa, Portugal,
[5] Weizmann Institute Of Science, Dept Of Biological Chemistry, Rehovot, Israel,
[6]IMed.UL - Research Institute For Medicines And Pharmaceutical Sciences, Faculdade De Farmácia Universidade De Lisboa, Lisboa, Portugal,

Objective: Sphingolipids (SLs) have emerged as an important class of lipids due to their bioactive role in several cellular events and in disease. The evidence that several SL species participate in the formation of lipid domains, and that this might underlie their biological mechanism of action has fostered research in the biophysical aspects of bioactive SLs. This work will focus on two important SLs – ceramide and glucosylceramide – and their interplay with other lipid components in simple and complex membrane models.

Methods: A combination of biophysical methodologies that include fluorescence spectroscopy, confocal and two-photon microscopy, surface pressure-area measurements, were used to elucidate the effects of these lipids on the biophysical properties of membranes with different lipidic components and displaying different phase properties.

Results: Our results showed that lipid-lipid interactions are modulated by alterations in the membrane environment, such as changes in pH. Moreover, small structural differences of these lipids influence their packing properties, membrane shaping and lateral organization. The importance of the headgroup, acyl chain length and unsaturation, on the modulation of membrane properties will be discussed in the framework of results obtained for cellular membranes.

Conclusion: Model membrane systems allow to predict the biophysical and biological implications of these lipids in cellular membranes. Supported by FCT (Portugal) grants PTDC/BBB-BQB/0506/2012 and RECI/CTM-POL/0342/2012, SFRH/BD/69982/2010 to ARV, Investigador FCT 2014 to LCS.

CERAMIDE NANO-LIPOSOMES: ROAD TO THE CLINIC

Mark KESTER[1]

Nanostar Institute, University Of Virginia[1]

The ceramide nanoliposome is a non-toxic, non-aggregating 80nm particle that selectively kills cancer cells in multiple solid and non-solid tumor models. Ceramide nanoliposomes are currently being developed for the clinic. Recent pharmacokinetic, pharmacodynamic and toxicology data will be reported from both rodent and canine models. These data are being assembled for a FDA IND submission to support a first in man human trial of the ceramide nanoliposome.

Disclosure: Keystone Nano, Inc. (PA, USA) has licensed ceramide nanotechnology from Penn State Research Foundation. MK is co-founder and CMO of Keystone Nano.

LAPTM4B FACILITATES LATE ENDOSOMAL CERAMIDE EXPORT TO CONTROL SPHINGOLIPID MEDIATED CELL DEATH PATHWAYS

Tomas BLOM[1], Shiqian LI[1], Andrea DICHLBERGER[1], Nils BÄCK[1], Young Ah KIM[2], Ursula LOIZIDES-MANGOLD[3], Howard RIEZMAN[3], Robert BITTMAN[2], Elina IKONEN[1]

[1]University Of Helsinki / Faculty Of Medicine / Anatomy, [2]City University Of New York / Queens College / Department Of Chemistry And Biochemistry, [3]University Of Geneva / Department Of Biochemistry,

Objectıve: The late endosomal organelles (LE) are a major site for sphingolipid catabolism in eukaryotic cells. The mechanisms by which sphingolipid degradation products exit LE are not well understood. In this study we set out to identify proteins that facilitate the removal of sphingomyelin (SM) degradation product(s) from LE, and to assess their potential role as modulators of cell death pathways.

Methods AND Results: By conducting an siRNA screen of membrane spanning LE proteins using [3H]-SM/LDL as a probe, we identified LAPTM4B as a regulator of endosomal ceramide removal. Using novel crosslinkable and fluorescent ceramide probes we found that LAPTM4B interacts with ceramide and facilitates its removal from LE. LAPTM4B lowers LE ceramide in parallel with, and independent of acid ceramidase-dependent catabolism. In LAPTM4B silenced cells, LE sphingolipid accumulation is accompanied by lysosomal membrane destabilization. However, these cells resist ceramide-driven caspase-3 activation and apoptosis induced by chemotherapeutic agents. Conversely, LAPTM4B overexpression reduces LE ceramide and stabilizes lysosomes but sensitizes to drug-induced caspase-3 activation.

Conclusıon: The data provide evidence for a novel ceramide export route from LE and identify LAPTM4B as its regulator. Moreover, LAPTM4B acts as a gatekeeper between intra- and extra-endosomal ceramide pools, modulating apoptosis sensitivity. By compartmentalizing ceramide, LAPTM4B controls key sphingolipid mediated cell death mechanisms and emerges as a candidate for sphingolipid targeting cancer therapies.

DIHYDROCERAMIDE ACCUMULATION MEDIATES CYTOTOXIC AUTOPHAGY OF CANCER CELLS VIA AUTOPHAGO-LYSOSOME DESTABILIZATION

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Objective: Autophagy is primarily a cell survival mechanism although depending on the cellular context and duration of the triggering stimuli, this cellular process can also lead to cell death. The molecular bases of this dual role of autophagy in cancer cell survival remains to be clarified. Previous work by our laboratory showed that Δ9-tetrahydrocannabinol (THC, the main active component of marijuana) triggers autophagy-mediated cancer cell death. In this study, we used THC and nutrient deprivation (an autophagic stimuli that triggers protective autophagy) to investigate the specific molecular mechanisms responsible for the activation of autophagy-mediated cancer cell death. Our results show that treatment with THC but not incubation with EBSS enhances the balance between dihydroceramides and ceramides in microsomes and in an autophagosome-enriched fraction. Biophysical experiments with model vesicles revealed that an increase in the dihydroceramide /ceramide ratio similar to that triggered by THC leads to the destabilization and subsequent release of the content of lipidic vesicles. Moreover, treatment with THC induced the release of cathepsins from lysosomes and the subsequent activation of apoptosis in a sphingolipid biosynthesis- and autophagy-dependent manner. Furthermore, pharmacological up-regulation of dihydroceramide levels by inhibiting dihydroceramide desaturase activates autophagy-mediated cancer cell death and exerts a similar anticancer action than THC. Taken together, our findings support that the mechanism of THC-induced autophagy-mediated cancer cell death relies on an increase in the dihydroceramide/ceramide ratio in the endoplasmic reticulum that is transmitted to autophagosomes and leads to autophago-lysosome destabilization, the release of cathepsins and the activation of apoptotic cell death.

LIPID OVERSUPPLY TO CARDIOMYOCYTES INDUCES SPHINGOLIPID-DEPENDENT OXIDATIVE STRESS AND INDUCTION OF MITOPHAGY THROUGH CERAMIDE SYNTHASE 2.

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Objective: Diabetic cardiomyopathy (DbCM) contributes to the high risk of heart failure (HF) in diabetics, but mechanisms underlying DbCM remain unclear. We previously showed that high saturated fat feeding in mice altered cardiomyocyte sphingolipid profiles leading to DbCM and that some of these maladaptations were dependent on autophagy and ceramide synthase 5 (CerS5). In the present study, we sought to further understand the cellular processes in which lipid overload leads to DbCM in in the context of sphingolipids. Mice fed an obesogenic diet and in vitro studies using H9c2 cardiomyocytes were utilized in this study. Increased oxidative stress and apoptosis were identified in the hearts of animals subjected to lipid overload, while animals treated with the sphingolipid synthesis inhibitor myriocin were protected. Similarly, cardiomyocytes treated with palmitate showed a sphingolipid-dependent increase of reactive oxygen species (ROS) and mitophagy. Treatment with mitochondria-targeted ceramide analogs revealed that very long chain ceramides, but not long-chain ceramides, induced cardiomyocyte cell death, which was exacerbated by inhibiting mitophagy. This suggested that lipotoxicity to cardiomyocytes occurs in part through oxidative stress in a sphingolipid-dependent manner, and mitophagy may occur to prevent further damage. In the observation that only very long chain species led to these outcomes suggested involvement of CerS2. Overexpression of CerS2 showed increased mitophagy in cardiomyocytes and knockdown of CerS2 by CRISPR-CAS9 technology decreased mitophagy. Taken together, our data suggest that lipid overload induces mitophagy as a protective measure in defense from CerS2-induced mitochondrial damage, oxidative stress, and cell death in DbCM.

ADJUVANT TAMOXIFEN IMPROVES EFFECTIVENESS OF CERAMIDE-CENTRIC THERAPY IN ACUTE MYELOGENOUS LEUKEMIA

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Objective: The breast cancer drug tamoxifen is an effective inhibitor of sphingolipid metabolism, blocking ceramide glycosylation as well as inhibiting acid ceramidase activity. These "off-target" actions make tamoxifen an interesting ancillary for improving the apoptosis-inducing potential of ceramide. The purpose of this study was to evaluate the therapeutic properties of single agent C6-ceramide versus a C6-ceramide-tamoxifen regimen in AML, an aggressive leukemia.

Methods: Studies were conducted in AML cells lines (KG-1, HL-60, HL-60/VCR), patients cells, and MLL-AF9 AML mice. Agents were administered in ethanol or as nanoliposomes. Cytotoxicity was assessed by MTS and propidium iodide, apoptosis by Annexin V and DNA fragmentation, therapeutic potential by spleen and marrow leukemia burden, mitochondrial function by respirometry, ATP levels by enzymatic end-point assays, glycolysis by Seahorse, and lipid analysis by mass spectroscopy.

Results: The therapeutic impact (viability, apoptosis) of the C6-ceramide-tamoxifen regimen was superior to single agents. Mitochondrial targeting was evidenced by sharp decreases in Complex I respiration, membrane potential, and ATP levels. In KG-1 cells, a 24 hr exposure reduced glycolytic capacity by 50% and downregulated survivin expression by 60%. Tamoxifen halted conversion of C6-ceramide to C6-glucosylceramide and also synergistically boosted effectiveness of the ceramide-generating retinoid, fenretinide (4-HPR) in drug resistant HL-60/VCR cells, which was accompanied by caspase-3 activation. Nanoliposomal administration of C6-ceramide-tamoxifen lowered leukemia stem cell burden in spleen and marrow in MLL-AF9 AML mice.

Conclusions: Our results demonstrate a multi-hit versatility of tamoxifen with ceramide-centric therapies for magnifying therapeutic potential in AML. Acknowledgment- NCI PO1CA171983.

HOST SPHINGOLIPID-TRANSFER PROTEINS AND INFECTIOUS DISEASES

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Objective: Intracellular trafficking of lipids are essential events for lipid metabolism and membrane biogenesis [Hanada & Voelker (2014) Traffic]. Ceramide is transported from the ER to the medial/trans Golgi region for the synthesis of sphingomyelin by the ceramide transfer protein CERT [Hanada et al (2003) Nature; Hanada (2014) BBA]. Ceramide is also transported to the cis Golgi compartment or a sub-region of the ER for the synthesis of glucosylceramide (GlcCer) by a CERT-independent pathway. GlcCer is then transported to the medial/trans Golgi region for the synthesis of lactosylceramide via the glycosphingolipid transfer protein FAPP2-dependent and –independent pathways [D'Angelo et al (2013) Nature]. Recent studies showed that several pathogens exploit the lipid-transfer proteins of host cells to steal host sphingolipids to their own purposes. The obligate intracellular bacterium Chlamydia spp forms the parasitophorous vacuole "inclusion", and proliferates in it, stealing various host metabolites. The inclusion protein IncD binds the PH domain of CERT, and recruits ceramide from the ER to the inclusion [Derre et al (2011) PLoS Pathog; Elwell et al (2011) ibid]. Although various viruses form membranous structures for their replication in host cells, the mechanisms underlying their formation are largely unknown. Intriguingly, human hepatitis virus type C (HCV) requires host FAPP2 for replication [Khan et al (2014) J Virol]. HCV might exploit FAPP2 to recruit GlcCer from the Golgi complex to the membranous viral factory, and such GlcCer-recruitment would be crucial for the HCV factory to acquire a detergent-resistant "raft-like" characteristics.

A POTENTIAL FUNCTION OF SPHINGOLIPID-DEPENDENT SECRETED EXOSOMES IN SEQUESTERING ALZHEIMER'S AMYLOID-ß.

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Objective: Increased levels of amyloid-ß peptide (Aß) in brain are linked to the pathogenesis of Alzheimer's disease (AD). Exosome, an extracellular lipid vesicle, contains several proteins related to neurodegenerative disorders, including Aß. However, the role of the exosomes in AD pathogenesis is largely unknown. In the resent study, we demonstrated that the neuronal exosomes were abundant in glycosphingolipids (GSLs) and coupled with Aß in a GSL-dependent manner. The exosome-bound Aß was then incorporated into brain-resident phagocytes, microglia, for degradation, suggesting that the exosomes contribute to Aß clearance. Moreover, we found that the exosome production was modulated by sphingolipid metabolism. Inhibition of sphingomyelin synthase activity enhanced exosome secretion and accelerated Aß clearance in a transwell study. Consistent with the notion of in vitro study, continuous administration of the exosomes into mouse brains resulted in marked reductions in Aß levels, amyloid depositions, and Aß-mediated synaptotoxicity in AD model mice. In addition, we showed that the densities of exosomes in mouse and monkey CSF declines during aging, inversely correlated with intracerebral Aß levels. Our data revealed that the exosomes act as potent scavengers for Aß by carrying it on exosome surface GSLs. Improving Aß clearance by exosome administration or upregulation of endogenous exosome secretion would be a novel strategy for AD therapy. References: Yuyama K., Igarashi Y. et al JBC 287:10977-10989(2012); Yuyama K, Igarashi Y et al. JBC 289: 24488-24498 (2014); Yuyama K, Igarashi Y. BBA 1841: 793-798 (2014); Yuyama K, Igarashi Y et al, FEBS Lett. 589:84-88 (2015)

MOLECULAR MECHANISM OF THE PRODUCTION OF ACYLCERAMIDE, THE KEY LIPID FOR SKIN BARRIER FORMATION

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Objective: The epidermal permeability barrier is essential for land-dwelling creatures. Lipid lamellae present in the stratum corneum, the uppermost layer of the epidermis, are responsible for barrier formation. Acylceramide is the most important of the epidermal lipids, both in physiological and pathological terms. Decreases in epidermal acylceramide levels are associated with cutaneous disorders such as ichthyosis and atopic dermatitis. However, the mechanism behind acylceramide production is poorly understood, leaving the broader picture of molecular mechanisms behind epidermal barrier formation still unclear. Here, we identified the cytochrome P450 CYP4F22 as the missing fatty acid ω-hydroxylase required for acylceramide production. Furthermore, we also revealed that PNPLA1 is required for the final step of acylceramide synthesis, ester bond formation between ω-hydroxyceramide and linoleic acid. Both CYP4F22 and PNPLA1 have been identified as causative genes of autosomal recessive congenital ichthyosis (ARCI). ARCI mutant proteins of CYP4F22 and PNPLA1 exhibited reduced enzyme activity. Furthermore, acylceramide levels were greatly reduced in CYP4F22 ARCI patient. Our findings clearly demonstrate a relationship between ARCI pathology, acylceramide levels, and enzyme activities of CYP4F22 and PNPLA1 and provide important new insights into the molecular mechanisms of acylceramide synthesis and skin barrier formation.

IDENTIFICATION OF A NOVEL PATHWAY FOR ACYLCERAMIDE GENERATION IN LIPID DROPLETS BY CERAMIDE SYNTHASE, FATTY ACYL-COA SYNTHASE, AND DIACYLGLYCEROL ACYLTRANSFERASE ENZYME COMPLEX

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Objective: Fatty acyl-CoA (FA-CoA) dependent ceramide generation is catalyzed by ceramide synthases (CerS) in the de novo pathway. Importantly, six CerS isoforms displaying different preferences to generate ceramides with distinct fatty acyl chains have been identified. Currently, there is little insight into the regulation of the distinct CerS enzymes. In an approach aimed at defining interacting partners of CerS' and shed light into substrate preference properties of CerS enzymes, we found that that fatty acyl CoA synthase ACSL5 interacts with CerS. Contrary to our initial hypothesis, ACSL5 generated FA-CoA was not a direct substrate utilized by CerS to generate ceramide as measured by LC/MS. Rather, ACSL5 generated FA-CoA was utilized together with de novo ceramide for the generation of acylceramides, poorly studied ceramide metabolites. Moreover, inhibition of ceramide channeling to acylceramide enhanced accumulation of de novo ceramide and resulted in augmentation of ceramide-mediated apoptosis. Mechanistically, we show that acylceramide generation is catalyzed by diacylglycerol acyltransferase 2 (DGAT2) and involves the formation of an ACSL5-CerS-DGAT2 complex on lipid droplets. In summary, this study identifies the molecular mechanism of a novel metabolic pathway of acylceramide generation and implicates the importance of this novel pathway in ceramide-mediated apoptosis.

EPIGENETIC CONTROL OF GLYCOSPHINGOLIPID METABOLIC SWITCH

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Objective: Glycosphingolipids (GSLs) are membrane lipid constituents characterized by a ceramide backbone linked to a glycan moiety. Hundreds of different glycan sequences can be incorporated into GSLs in a process, which does not depend on a pre-existing template nor is strictly genetically determined. Nevertheless GSLs expression is tightly regulated during embryogenesis and failure to synthesize specific GSLs leads to serious genetic diseases in humans. How individual cells decide which GSLs (among the many possible options) to synthesize under a given condition is not understood. Here we describe a self-contained control circuit regulating the metabolic direction of GSLs glycan elongation through the epigenetic control of GSL synthesizing enzymes expression. We found, indeed, that the globoside Gb3 represses the transcription of the first enzyme involved in the synthesis of gangliosides (i.e. GM3 synthase; GM3S). Gb3 exerts this function by negatively regulating the expression of the epigenetic modulator of neuronal differentiation AUTS2, which in turn activates GM3S promoter by fostering local histone acetylation. Our results provide a mechanistic explanation for the Globo-to-Ganglio GSL switch observed during neuronal differentiation, and ascribe a new role to GSLs in the epigenetic control of neuronogenesis.

ELUCIDATING NOVEL METABOLIC PATHWAYS OF THE NEUROTOXIC 1-DEOXYSPHINGOLIPIDS

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Objective: Serine palmitoyltransferase (SPT), which catalyzes the first step in the de novo synthesis of sphingolipids, typically condenses serine and palmitoyl-CoA. Under certain conditions SPT can also use alanine, resulting in the neurotoxic atypical 1-deoxysphingolipids (1-deoxySLs). Pathologically elevated 1-deoxySLs cause the inherited neuropathy HSAN1. Due to the missing C1-hydroxyl group, 1-deoxySLs are not metabolized to complex sphingolipids nor degraded by the canonical pathways. Here we investigated the metabolic pathways of 1-deoxySLs in order to understand and potentially control their buildup. We used high-resolution high accuracy mass spectrometry and metabolic profiling workflows to identify novel downstream 1-deoxySL-metabolites. The formation of these novel 1-deoxySL metabolites was modulated using specific chemical enzyme inhibitors and inducers. We identified five novel 1-deoxySL downstream metabolites. Blocking the conversion of 1-deoxysphinganine to 1-deoxysphingosine with Fumonisin B1 significantly decreased the levels of these metabolites. When directly treating cells with different synthetic 1-deoxysphingosine isomers, we observed corresponding variations in the newly identified metabolites. Furthermore, the formation of all five metabolites could be modulated by inhibiting or inducing a specific cytochrome P450 subfamily. While neurotoxic 1-deoxySLs are not metabolized by the canonical pathways, we showed for the first time that they are in fact metabolized downstream by a specific subfamily of cytochrome P450 enzymes. We have elucidated the structure of five novel 1-deoxySL metabolites and their metabolic order, which is downstream of 1-deoxysphingosine. This novel metabolic pathway may be further exploited as a novel therapeutic target to reduce 1-deoxySL levels in HSAN1 patients.

PRODUCTION OF 2-N-ACYL-AMINO-14,16-DIMETHYLOCTADECAN-3-OL (N-ACYL-AOD) BY CHO-LY-B CELLS IN CULTURE: IDENTIFICATION OF A NEW FAMILY OF NATURALLY OCCURRING N-ACYL-1-DEOXYSPHINGANINE ANALOGUES USING LIQUID-CHROMATOGRAPHY ELECTROSPRAY TANDEM MASS SPECTROSCOPY

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Objective: Fusarium avenaceum is a fungal contaminate of many starch plants including cereal grains. It produces 2-amino-14,16-dimethyloctadecan-3-ol (AOD), which has structural features of a sphingoid base--more specifically, a 1-deoxysphinganine. Many other categories of sphingoid bases are N-acylated by ceramide synthases, but it is not known if AOD undergoes acylation, or if so, which fatty acyl-chain-length metabolites would be made. To answer these questions, we have used CHO-LY-B cells, which cannot make endogenous sphingolipids due to a defective serine palmitoyltransferase, and culture them under conditions where they contain no detectable sphingoid bases. Thus, the cells can be incubated with AOD and the metabolites analyzed without interference from endogenous sphingoid bases nor ambiguity about the species that are found. When lipid extracts from CHO-LY-B cells incubated with sub-toxic levels of AOD were examined by liquid chromatography electrospray ionization-tandem mass spectrometry, it was evident that the cells produce N-acyl-derivatives of AOD with a wide range of chain lengths (C16 to C24), which suggests that AOD is a substrate for multiple ceramide synthases. The cellular effects and metabolism of N-acyl-derivatives of AOD warrant further investigation due to the prevalence of F. avenaceum in the world food supply. This work was supported by funds from NIH grant R01GM76217, the Smithgall Institute Chair in Molecular Cell Biology, and the Norwegian Veterinary Institute.

SPHINGOSINE-1-PHOSPHATE LYASE DEFICIENCY IN THE BRAIN

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The evolutionary conserved bioactive lipid sphingosine 1-phosphate (S1P) is essential for brain development but can exert detrimental effects in postmitotic neurons. Its content in the brain is regulated by specific kinases, phosphatases and by S1P-lyase (SPL).
The potential role of S1P in neurodegenerative disorders, especially in Alzheimer's disease (AD) is controversially discussed. On the one hand S1P was found to stimulate Abeta formation in neurons by directly interacting with β-secretase BACE1. On the other hand S1P was described as a neuroprotective factor that is lost early in AD pathogenesis. This raises intriguing questions on the pathophysiological role of S1P metabolism in AD etiology: Do age-dependent alterations in S1P/sphingosine metabolism trigger or reduce the accumulation of Abeta, hyperphosphorylated tau and ultimately neuronal death in AD? Recent studies in Alzheimer brains revealed that regions most heavily affected by Alzheimer pathology, exhibited the most pronounced decline of S1P levels. To increase S1P amounts in the brain we generated a mouse model with targeted deletion of SPL. Although no neuronal loss was detectable, these animals exhibit age-dependent deficits of motor coordination, spatial learning and memory. Preliminary studies indicate enhancement of sphingosine and S1P, decrease of phosphatidylethanolamine, impaired autophagy, altered processing of amyloid precursor protein (APP), increased proteasomal activity and decreased expression of presynaptic proteins. Potential molecular links between SPL deficiency and the observed biochemical and physiological changes will be presented.

WHAT HAVE WE LEARNT FROM THE STUDY OF CERAMIDE SYNTHASE KNOCK-OUT MICE?

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LACK OF CERAMIDE SYNTHASE 2 SUPPRESSES THE DEVELOPMENT OF EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS BY IMPAIRING THE MIGRATORY CAPACITY OF NEUTROPHILS

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Objective: Until now, the role of ceramides with specific chain lengths in the development of EAE/MS have not been elucidated. CerS2 null mice which lack very long chain ceramide and sphingolipids, but have increased C16-Cer and -sphingolipid level served as a good tool to study the chain length-specific role of ceramides in the development of EAE.

Methods: EAE was induced in female mice by subcutanously injected with MOG35-55 (myelin oligodendrocyte protein) emulsified in complete Freund`s adjuvant (CFA) and an i.p. injection of pertussis toxin (PTX). Ceramide levels were determined by LC-MS/MS, mRNA levels by quantitative PCR, protein levels of CerS2 by western blot and the expression of the chemokine receptor CXCR2 by FACS.

Results: In EAE mice we observed a significant elevation of CerS2 and its product, C24-ceramide, in CD11b+ cells (monocytes and neutrophils) isolated from blood. Specific genetic deletion of CerS2 significantly delayed the onset of clinical symptoms, due to a reduced infiltration of immune cells, in particular neutrophils, into the central nervous system. Neutrophils isolated from CerS2 null EAE mice, as opposed to WT EAE mice, were characterised by significantly lower CXCR2 receptor mRNA expression resulting in reduced migratory capacity towards CXCL2. Also G-CSF-induced CXCR2 expression was significantly reduced in CerS2 null neutrophils and their migratory capacity was significantly impaired.

Conclusion: Our data strongly indicate that G-CSF-induced CXCR2 expression is regulated in a CerS2-dependent manner and that CerS2 thereby promotes the migration of neutrophils, thus, contributing to inflammation and the development of EAE and MS.

CERAMIDE TRANSPORTERS : THE LINK BETWEEN LIPID METABOLISM, INFLAMMATION AND AMYLOID-ß AGGREGATION IN ALZHEIMER'S DISEASE

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Objective: Alzheimer's disease (AD) is the most common form of dementia, characterized by neuropathological hallmarks as synaptic loss, aggregates of amyloid-ß peptides (Aβ), neurofibrillary tangles, ceramide accumulation in the brain, gliosis and neuroinflammation. No treatment or intervention options are currently able to modify the pathophysiology of AD. Recently, ceramide transporters (CERTs) have been implicated as possible therapeutic candidates. CERT proteins have the unique function of transporting the lipid ceramide via its (StAR)-related lipid transfer (START) domain. CERTs are known to be responsible for the trafficking of ceramides inside and outside the cells. Interestingly, these ubiquitous proteins bind to proteins that are prone to misfolding and aggregation. And they are essential for the development and homeostasis of the central nervous system (CNS). The knockdown of CERT leads to loss of myelinated tracts and to extensive apoptosis. Recently, we identified CERTL as an Aβ-binding protein (binds Aβ1-42) associated to human and murine amyloid plaques. Our results show that CERTL reduces Aβ aggregation and has a protective effect against Aβ-induced toxicity in neuroblastoma cells. Additionally, we have identified this protein as a receptor for C1q in the surface of damaged cells, which can activate the complement system. This indicates that CERTL takes part in the cellular defensive response against Aβ- induced toxicity and is involved in the pathophysiological mechanisms associated with neurodegenerative processes.

THE PATHOMECHANISMS UNDERLYING THE NEUROTOXICITY OF 1-DEOXYSPHINGOLIPIDS

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Objective: Hereditary sensory and autonomic neuropathy type I (HSAN I) is a slowly progressing neurological disorder characterized by loss of pain and temperature sensation. It has been associated with several mutations in the enzyme serine palmitoyltransferase (SPT) which catalyzes the condensation of palmitoyl-CoA and serine - the first and rate-limiting step in the de novo biosynthesis of sphingolipids. The HSAN1 mutations shift the substrate specificity of SPT from L-serine to L-alanine which results in the formation of atypical and neurotoxic 1-deoxysphingolipids (1-deoxySLs) which are found to be pathologically elevated in HSAN1. However, the pathomechanisms underlying the 1-deoxySLs mediated neurotoxicity is unknown. This study aims to identify and characterize downstream pathways of 1-deoxySLs mediated neurotoxicity. Neurotoxicity of 1-deoxySLs was assessed using immunofluorescence, live cell imaging and by quantifying the neurite outgrowth in a neuronal cell models. Downstream signalling pathways involved in 1-deoxySLs mediated cytotoxicity and neurotoxicity are identified by screening a commercially available kinase inhibitor library using Hek293, SH-SY5Y cells as well as in primary DRG. 1-deoxySLs caused significant cytotoxic and neurotoxic effects in HEK293 and Neuronal cells. Neuronal cells showed changes in cell morphology and reduction in the length and number of neurites. On the basis of these results we developed a high throughput assay to screen a commercial kinase inhibitor library. First results indicate that some of the tested inhibitors are able to decrease 1-deoxySLs mediated toxicity. The 1-deoxySLs were shown to be toxic to neuronal cells. This toxicity appears to be related to specific cellular signalling pathways.

DEVELOPMENT OF PHEOCHROMOCYTOMA IN CERAMIDE SYNTHASE 2 NULL MICE

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Objective: Pheochromocytoma (PCC) and paraganglioma are rare neuroendocrine tumors of the adrenal medulla and sympathetic and parasympathetic paraganglia for which mutations in ~15 disease-associated genes have been identified. We now document the role of an additional gene in mice, the ceramide synthase 2 (CerS2) gene. CerS2 is one of six mammalian CerS, which synthesizes ceramide with very-long acyl (C22-C24) chains. The CerS2 null mouse has been well characterized, and displays lesions in several organs including the liver, lung and the brain. We now demonstrate that changes in the acyl chain profile of the adrenal gland leads to the generation of adrenal medullar tumors. Histological analyses revealed that about half of the CerS2 null mice developed PCC by ~13 months, and the rest showed signs of medullary hyperplasia. Norepinephrine and normetanephrine levels in the urine were elevated at 7 months of age consistent with the morphological abnormalities found at later ages. Accumulation of ceroid in the X-zone was observed as early as 2 months of age and as a consequence, older mice displayed elevated levels of lysosomal cathepsins, reduced proteasome activity and reduced activity of mitochondrial complex IV by six months of age. Together, these findings implicate an additional pathway that can lead to PCC formation, which involves alterations in the sphingolipid acyl chain length. Analysis of the role of sphingolipids in PCC may lead to further understanding of the mechanism by which PCC develops, and might implicate the sphingolipid pathway as a possible novel therapeutic target for this rare tumor.

ALKALINE CERAMIDASE 3 DEFICIENCY RESULTS IN PURKINJE CELL DEGENERATION AND CEREBELLAR ATAXIA DUE TO DYSHOMEOSTASIS OF CERAMIDES AND THEIR METABOLITES IN THE BRAIN

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Objective: Dysregulation of the metabolism of ceramides has been implicated in aging and age-related neurodegenerative disorders. However, much remains unknown about how the metabolism of ceramides is controlled in the central nervous system (CNS) during normal aging. Here we report that the mouse alkaline ceramidase 3 (Acer3) is critical for the homeostasis of ceramides in the aging brain and that its deficiency causes degeneration of Purkinje cells (PC) and thereby impairs mouse motor coordination in mice at around 8 months of age. Enzymatic activity assays revealed that, like human ACER3, the mouse Acer3 preferentially catalyzed the hydrolysis of unsaturated long-chain ceramides (ULCCs) such as C18:1-ceramide, a major ceramide species in the brain. Acer3 was found to be upregulated with age in the mouse brain, and its upregulation was associated with a decrease in the levels of C18:1-ceramide and a reciprocal increase in the levels of sphingosine (SPH) and sphingosine-1-phosphate (S1P). Acer3 knockout caused an age-dependent accumulation of various ceramides in the brain while abolishing the age-related increase in the levels of both SPH and S1P in this tissue. Acer3 knockout caused PC degeneration in the cerebellum and impaired the ability in motor coordination and balance in mice at around 8 months of age. Taken together, these results suggest that Acer3 plays critical roles in controlling the homeostasis of ceramides and their metabolites SPH and S1P in the brain as well as protecting PCs from premature degeneration.

ADMINISTRATION OF PHYROCERAMIDE AND GLUCOSYLCERAMIDE AMELIORATED THE MEMORY IMPAIRMENT IN MICE

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Objective: This study was aimed to investigate the possible roles of phyroceramide and glucosylceramide in memory function in mice.

Methods: Phytoceramide was orally administered to ICR mice for 7 days. Memory performances were assessed using the passive avoidance test and Y-maze task.Aged mice were given experimental diet pellets which contains glucosylceramide for 3months. Glucosylceramide (50mg/kg, p.o.) showed memory enhancing activity after 3-month treatment in the aged mice (C56BL/6, 18–20months old) through Y-maze, novel objective test, and Morris water maze test.

Results and Conclusion: Treatment of Pcer enhanced cognitive performances in the passive avoidance test and Y-maze task. Immunoblotting studies revealed that the phosphorylated CREB and BDNF were signifi cantly increased on hippocampus in the Pcer-treated mice.Long-term treatment of glucosylceramide decreased the expression of iNOS and COX-2 in the brain of aged mice. The LPS-induced mRNA level of iNOS, COX-2, IL-1β, and TNF-α was reduced by the acute treatment with glucosylceramide in adult mice. These results suggest that phytoceramide and glucosylceramide plays an important role in anti-inflammatory and memory enhancement, and it could be a potential new therapeutic agent for the treatment of neurodegenerative diseases such as Alzheimer's disease.

STRUCTURAL AND FUNCTIONAL CHARACTERIZATION OF SERINE PALMITOYL TRANSFERASE IN MAMMALS

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Objective: Regulatory and signaling mechanisms that govern SPT function have been the focus of several recent studies, primarily in yeast cells. However, initial findings from the cultured mammalian cells suggest that mammalian SPT is subject to differential and complex regulations. Moreover, mechanistic changes leading to an increased activity or the shift in substrate preference of the SPT enzyme have not been worked out yet. In particular, the factors which lead to the formation of neurotoxic 1-deoxySLs which are associated with peripheral neuropathies (HSAN1 or diabetic sensory poly neuropathy) is not understood. Here, we aim to identify the protein interactome of the functional SPT complex to determine the regulatory factors of the SPT holoenzyme and to characterize the biological relevance of the interactions in vivo.

Methods: We are using genetically modified KO-cell lines, in combination with fluorescent tagged proteins and FRET as well as co-immunoprecipitation studies followed by mass spectrometry to elucidate SPT subunit interactions. Furthermore, we challenge cells with pharmacologically and biological stimuli to modulate sphingolipid synthesis to investigate the effect on the molecular and enzymatic properties of the SPT complex.

Results: Methods to immuno-purify the SPT enzyme by targeting individual subunits have been established. Preliminary data show that specific mutations in certain SPT subunits influence regulation and alter the overall SPT activity and product spectrum.

Conclusions: SPT activity is subject to complex regulations which might be mediated by direct modifications of subunits and the association and dissociation with the inhibitory and activating proteins.

TARGETING SPHINGOLIPID SIGNALING IN THE VISUAL SYSTEM

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Current knowledge of mechanisms underlying photoreceptor death in retinal degenerative diseases has lead to the notion that cellular apoptosis is the predominant mechanism, even in genetically heterogeneous disorders such as Retinitis Pigmentosa (RP). We are performing follow up studies based upon pharmacological targeting of the biochemical pathway leading to the ceramide synthesis and sphingosine-1-phosphate (S1P) cleavage, in order to both counteract apoptosis and preserve survival.

Results obtained *in vivo* on rd10 mutant mice (a model of recessive RP) showed that ceramide levels in the retina increase at the peak of rod degeneration, but can be lowered by ocular administration of myriocin, an inhibitor of Serine Palmitoyl Transferase, the rate-limiting enzyme in ceramide biosynthesis. Noticeably, this treatment also decreases the number of dying photoreceptors. To achieve prolonged treatment (i.e. several weeks), we developed a non-invasive drug-delivery approach based on eye drops of a suspension of solid lipid nanoparticles (SLNs) loaded with myriocin. The nanoparticles function as vectors allowing topical drug administration. Daily supply of SLN-eye drops loaded with myriocin to rd10 mice lowered rod photoreceptor death and promotes survival, preserved their morphology and extended the ability of the retina to respond to light as assessed by ERG recordings. It is important to say that cone photoreceptors and cone-mediated vision were also considerably well preserved over time.

Investigation of the potential role of S1P in photoreceptor survival has been addressed by the use a cone-like 6661W cell line, previously treated with hydrogen peroxide to induce oxidative stress. We wished to increase S1P in cone photoreceptors aiming to enhance their survival and resistance mechanism to oxidative stress. We observed that: i) exogenous S1P, at low doses, recovers 661W cells viability from oxidative stress; b) administration of a S1P lyase inhibitor, THI, recovers 661W cells viability after oxidative stress; c) THI reduces apoptosis of H_2O_2-stressed 661W cells; d) THI reduces endogenous stress mediators in 661W cells; e) THI enhances anti-oxidant & survival response to H_2O_2 in 661W cells. Administration of THI to rd10 animals is currently addressed. These data pose promising premises to set up a combination therapy, with both Myriocin and THI, to delay photoreceptor degeneration in Retinitis Pigmentosa.

COMPLEXITIES OF CARDIAC SPHINGOLIPID METABOLISM

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In recent years, numerous studies have revealed important roles of sphingolipids including sphingosine-1-phosphate and ceramide in cell, tissue, and organ responses to nutrient surplus. Our lab has focused on how fatty acids differentially regulate sphingolipid metabolism, and how these bioactive lipids work to exacerbate and/or protect from metabolic insult. In the heart, ceramides serve key functions in various disease contexts. Moreover, data reveal specialized pathways of cardiomyocyte sphingolipid metabolism that make distinct contributions to pathophysiology during cardiac metabolic insult. Our laboratory has investigated two of these pathways: Sptlc3-derived sphingolipids, and CerS5 and CerS2-derived sphingolipids. Each of these pathways are stimulated under conditions of fatty acid surplus, and each mediates a distinct cell process. In addition to teasing out individual contributions of these pathways in cell culture systems, emerging data from mouse models has provided insight into how these pathways may converge to mediate diabetic cardiomyopathy; however, cell type-specific approaches will be vital to better understand cardiomyocyte-specific sphingolipid actions.

HEPATIC UPREGULATION OF SERINE PALMITOYLTRANSFERASE SUBUNIT 2 BY ENDOPLASMIC RETICULUM STRESS MODULATES GLUCOSE AND LIPID METABOLISM

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Objective: The endoplasmic reticulum (ER) is the principal organelle in the cell for protein folding and trafficking, lipid synthesis and cellular calcium homeostasis. Perturbation of ER function by accumulation of misfolded protein results in activation of the unfolded protein response (UPR). Chronic ER stress is reported to have an important role in abnormal lipid and glucose metabolism leading to development of insulin resistance. Here, we reports that transcription of serine palmitoyltransferase (SPT) is regulated by ER stress-mediated UPR pathways. Expression of Sptlc2, a major catalytic subunit of SPT in liver, was upregulated by high fat diet in liver of C57Bl6 mice. Treatment of tunicamycin, an ER stress inducer, elevated Sptlc2 and ceramide levels in primary mouse hepatocytes. Overexpression of the spliced form of X-box binding protein 1 (sXBP1) upregulated Sptlc2 expression, whereas the spliced form of activating transcription factor 4 (ATF4) had no effect on Sptlc2 expression as demonstrated by Sphk2 promoter assays and western blot analyses. Liver-specific overexpression of Sptlc2 (lSptlc2 Tg) elevated fasting glucose levels and inhibited phosphorylation of AKT by insulin. In addition, the response to insulin was diminished and hepatic lipoprotein secretion was increased in lSptlc2 Tg mice. These results demonstrated that Sptlc2 is regulated by ER stress-induced UPR pathways and involved in hepatic secretion of VLDL.

DEOXYSPHINGOLIPIDS, NOVEL BIOMARKERS FOR DIABETES, ARE CYTOTOXIC FOR INSULIN-PRODUCING CELLS

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Objective: Irreversible failure of pancreatic beta-cells is the main culprit in the pathophysiology of diabetes mellitus, a disease that is now a major global epidemic. Recently, elevated plasma levels of deoxysphingolipids, including 1-deoxysphinganine, have been identified as novel biomarkers for the disease. In this study, we analyzed whether deoxysphingolipids directly compromise the functionality of pancreatic beta-cells.

Methods: Effects of deoxysphingolipids were investigated in insulin-producing Ins-1 cells and primary islets. Cell functionality and signaling pathways were analyzed by biochemical methods and flow cytometry. Cytoskeletal alterations were imaged by confocal microscopy. Levels of mRNA and composition of sphingolipid species were quantified by real-time PCR and mass spectrometry, respectively.

Results: Treatment with 1-deoxysphinganine induced dose-dependent cytotoxicity with senescent, necrotic and apoptotic characteristics and compromised glucose-stimulated insulin secretion of beta-cells. In addition, 1-deoxysphinganine altered cytoskeletal dynamics, resulting in intracellular accumulation of filamentous actin and activation of the RhoGTPase Rac1. Moreover, 1-deoxysphinganine selectively up-regulated ceramide synthase 5 expression and was converted to 1-deoxy-dihydroceramides, without altering normal ceramide levels. Inhibition of intracellular 1-deoxysphinganine trafficking and ceramide synthesis improved the viability of the cells, indicating that the intracellular metabolites of 1-deoxysphinganine contribute to its cytotoxicity. Analyses of signaling pathways identified JNK and p38 MAPK as antagonistic effectors of cellular senescence.

Conclusion: Our results revealed that 1-deoxysphinganine is a cytotoxic lipid for insulin-producing cells, suggesting that the increased levels of this sphingolipid observed in diabetic patients may contribute to the reduced functionality of pancreatic beta-cells. Thus, targeting deoxy-sphingolipid synthesis may complement the currently available therapies of diabetes.

LYSOSOMAL STRESS DRIVES SPHK1 EXPRESSION IN ADIPOSE TISSUE MACROPHAGES

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Objective: During obesity adipose tissue macrophages (ATM) have to handle an increased lipid load. When adipocyte dysfunction occurs large amounts of lipids are spilled, which consequently impacts macrophages and their lysosomes. Recently it has been found that obesity induces a program of lysosome biogenesis in ATM. In addition, we found a novel obese ATM marker Gpnmb to be induced as a consequence of lipid-induced lysosomal stress. Previously we demonstrated that inhibition of GSL synthesis in models of obesity improved aspects of metabolic dysfunction including inflammation. It is not clear if GSL manipulation in adipose tissue impacts macrophages, adipocytes or both. This prompted us to analyze in detail in sorted ATM populations genes involved in (G)SL synthesis, degradation and transport. We observed a striking induction of Sphk1 in obese ATM. These findings are in agreement with literature. Interestingly, also Sphk1 activity and concomitantly formation of S1P was elevated in obese ATM. To get insight in the regulation of Sphk1 expression in ATM we made use of the macrophage cell line RAW 264.7. It was found that Sphk1 induction occurs as a consequence of overfeeding with palmitate,but also with lysosome stressors such as chloroquine. Additional evidence suggests that Sphk1 activity in obese ATM prevents induction of cell death upon lysosomal challenge. We postulate that high lipid load causes lysosomal stress, which triggers induction of sphk1. By controlling the overshoot of ceramide levels, and consequently cell death, it is ensured during obesity that enough ATM lipid buffering capacity persists.

THE ROLE OF ADIPOSE CERAMIDE IN METABOLIC HOMEOSTASIS

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The lipotoxicity posits that the accumulation of fat-derived metabolites in tissues not optimized for lipid storage induces the cellular dysfunction that underlies metabolic disorders. Of the metabolites that accumulate, sphingolipids such as ceramide appear particularly deleterious, as pharmacological inhibition or genetic ablation of enzymes requisite for sphingolipid production ameliorates insulin resistance, diabetes, and cardiovascular disease in rodents. To identify the tissues that produce the sphingolipids that impair metabolic homeostasis, we have studied knockout mice lacking the a requisite biosynthetic enzyme, serine palmitoyltransferase -2, in either liver, muscle, myeloid cells, or adipocytes. The speaker will share data on the metabolic phenotype of these conditional knockout animals, which reveal novel and important roles in adipose biology.

ACTIVE PHOSPHORYLATED FTY720/FINGOLIMOD IS A POTENT INHIBITOR OF CLASS I HISTONE DEACETYLASES THAT REACTIVATES ESTROGEN RECEPTOR EXPRESSION AND INCREASES HORMONAL THERAPEUTIC SENSITIVITY OF BREAST CANCER

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Objective: Hormonal therapies, including ovarian ablation, ER antagonists, and aromatase inhibitors, are the standards of care for treatment of ERα positive breast cancer. However, development of resistance to hormone therapies in advanced breast cancer is a major obstacle. Moreover, estrogen receptor-α (ERα)-negative breast cancer is clinically aggressive and does not respond to conventional estrogen targeted therapies. Strategies that lead to re-expression of ERα could sensitize breast cancers to selective ER modulators. FTY720/Fingolimod, a sphingosine analog, is an FDA-approved pro-drug for treatment of multiple sclerosis that also has anticancer actions that are not yet well understood. We have now found that FTY720 is phosphorylated in breast cancer cells by nuclear sphingosine kinase 2 and accumulates there. Nuclear FTY720-P in turn is a potent inhibitor of class I histone deacetylases (HDACs) that enhances histone acetylations and gene expression independently of its known effects on canonical signaling through sphingosine-1-phosphate receptors. In ERα negative human and murine breast cancer cells, FTY720 reactivated expression of silenced ERα and sensitized them to tamoxifen. Moreover, oral administration of clinically relevant doses of FTY720 to mice bearing ERα negative syngeneic breast tumors also re-expressed ERα and increased therapeutic sensitivity to tamoxifen in vivo more potently than a known HDAC inhibitor. Our work suggests that FTY720 is a promising strategy for effective treatment of conventional hormonal therapy-resistant breast cancer and triple-negative breast cancer. Supported by NIH grant RO1CA061774 and the Department of Defense BCRP program award W81XWH-14-1-0086 (S. Spiegel).

CERS4/CERAMIDE METABOLISM IN THE REGULATION OF TGF-BETA RECEPTOR SIGNALING AND TUMOR METASTASIS

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Objective: Recent studies indicate that ceramide species play diverse biological functions including, skin barrier function, liver homeostasis, cell death and cancer pathogenesis, highlighting the importance of ceramide synthases (CerS) in these processes. Migration, a part of these processes, also is effected by ceramide metabolism. However, the molecular mechanism of CerS/ceramide involved is unknown. Here, we investigated the effect of CerS on migration and its related signal pathways in situ and in vivo model. Interestingly, our data show that among CerS only CerS4 is related to cell migration. Here, we also have generated CerS4-/- mice, and these mice were viable with no lethal tissue. Interestingly, we observed that loss of CerS4 resulted in irreversible alopecia, which was associated with hyper-proliferation and migration of keratinocytes. Mechanistically, we show that knockout/knockdown of CerS4 enhances cell migration by which ligand-independent signaling of TGFβ receptors in various cell types, including keratinocytes, MEFs, and cancer cells. Additionally, low level of TGFβR1-Smad7 interaction was found in knockdown of CerS4 cells. Moreover, we found that ceramide interact with Smad7 and interaction was decreased by knockdown of CerS4. Thus, ceramide-Smad7 binding modulates plasma membrane association of TGFβR1, and inhibits its signaling through Sonic-Hedgehog (Shh) signaling for migration. In fact, inhibition of TGFβR/Shh signaling using molecular or pharmacologic inhibitors almost completely prevented cell migration in response to CerS4 knockdown. These data suggest that CerS4/ceramide signaling plays key roles in the regulation of cell migration via controlling the TGFβR/Shh axis.

C16-CERAMIDE IS A NATURAL REGULATORY LIGAND OF P53

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Objectives: We have previously observed that a transient elevation of one of the ceramide-generating enzymes, ceramide synthase 6 (CerS6), in cancer cells up-regulates p53 through an obscure mechanism. Since the immediate effect of CerS6 elevation was the raise of C16-ceramide, we hypothesized that the mechanism underlying the p53 elevation was the direct binding of this ceramide to the protein, which leads to the p53 release from its complex with MDM2.

Methods: NMR spectroscopy, membrane-binding assays, bimolecular fluorescence complementation, protein pulldown, titration of tyrosine fluorescence, and LC/MS-MS measurements of ceramides were applied to study the interaction between p53 and ceramide.

Results: We have demonstrated that p53 binds C16-ceramide with high affinity (Kd = 60 ± 20 nM) within its DNA binding domain and that the interaction between p53 and ceramide is highly selective towards the acyl chain length. NMR experiments have demonstrated chemical shifts perturbations upon C16-ceramide binding in the BOX-V sequence of p53, which interacts with the acidic domain of E3 ligase MDM2. In agreement with this finding, we have further demonstrated that the binding of ceramide disrupts the complex between p53 and MDM2, thus preventing the p53 ubiquitination and degradation.

Conclusions: Our study establishes C16-ceramide as the first natural small molecule regulating p53 tumor suppressor through the direct binding. This type of regulation is a novel physiological mechanism of p53 activation, which is fundamentally different from the canonical p53 activation through protein-protein interactions or post-translational modifications.

C STIMULATING CAMP OVERPRODUCTION ACCELERATE DEVELOPMENT OF CUTANEOUS SCC

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Objective: Cutaneous squamous cell carcinoma (cSCC) is a ccomon cancer, often initiated by oxidative stress, particularly ultraviolet irradiation. We demonstrated that oxidative stressors upregulate a key innate immune element, cathelicidin antimicrobial peptide (CAMP) via endoplasmic reticulum (ER)-mediated, sphingosine-1-phosphate signaling (Park K et al., Mol Cell Biol, 2011 & 2014). Prior studies showed that CAMP production also increases in certain cancers. Yet, why CAMP overproduction occurs and how CAMP could stimulate tumorigenesis remains unknown. We assessed whether and how CAMP contributes to the development of cSCC. CAMP mRNA/peptide and sphingosine-1-phosphate productions were significantly higher in cSCC cells than in normal human keratinocytes. Exogenous CAMP significantly stimulated the growth of cSCC (but not normal keratinocytes). We next showed that blockade of the CAMP receptor, formyl peptide receptor-like (FPRL) 1, by a specific receptor antagonist (WRW4) attenuated cSCC gowth. We then demonstrated cSCC invaded into dermis using cultured human skin model consisting with epidermis and dermis and exogenous CAMP further stimulated cSCC invasion into an in vitro dermal equivalent, but WRW4 suppressed cSCC invasion. Finally, elevated CAMP expression and cell growth in SCC were significantly suppressed by specific inhibitors of sphingosine kinase 1. These studies suggest that sphingosine-1-phosphate signaling stimulates CAMP overproduction resulting in a FPRL1-dependent enhancement of cSCC growth and invasion.

DYSREGULATION OF SPHINGOLIPID METABOLISM IN MELANOMA: ROLES IN CELL SURVIVAL AND TUMOR PROGRESSION

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Cutaneous melanoma is a complex disease that arises through the stepwise transformation of melanocytes within the basal epidermal layer of the skin and evolves due to a myriad of genetic aberrations correlated or not with the person's UV exposure behaviour. Although very promising with meaningful effects on progression-free survival, BRAF-directed therapies are usually short-lived due to the appearance of resistance, which leads to disease progression. This emphasizes the need to develop new therapeutic approaches that could overcome cancer relapse. Interestingly, ceramide metabolism is strongly altered in melanoma and represent an exploitable target for the development of novel therapies. Here, we show that the reduction of sphingosine 1-phosphate levels in resistant metastatic melanoma resulted in sensitization of these cells to apoptosis induced by a BRAF inhibitor. This phenomenon is associated with decreased expression of MITF, a major regulator of melanoma survival which controls the expression of Bcl-2 family members. Moreover, our findings reveal a key role for melanoma sphingosine kinase 1 in macrophage recruitment and polarization within the tumor microenvironment, thereby controling the aggressiveness of this cancer.

KEY ROLE OF S1P RECEPTORS IN THE ACTION MECHANISM OF TGFβ IN MYOBLASTS

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Solid experimental evidence is in favor of a key role of sphingosine 1-phosphate (S1P) signaling axis in the action mechanism of multiple extracellular agents, with a role in the onset of their final response.

In our laboratory we have studied the involvement of S1P signaling pathway in skeletal muscle precursor cells, named myoblasts, highlighting its requirement for the full accomplishment of specific biological actions by growth factors and cytokines. In this regard, we have addressed the possible implication of endogenous production of S1P in the action mechanism of TGFβ, a potent cytokine known to hamper skeletal muscle repair. Our study clearly shows that sphingosine kinase-1 is up-regulated by TGFβ. More importantly, the enhanced biosynthetic pathway of S1P is accompanied by a profound modification of S1P receptor expression profile. Overall, our results support the notion that by up-regulation of $S1P_3$ and $S1P_4$, TGFβ is able to readdress S1P pathway in myoblasts. Indeed, in unstimulated cells this signaling axis promotes myogenic differentiation and is physiologically involved in skeletal muscle regeneration. In contrast, via $S1P_3$ induction, TGFβ drives transdifferentiation of myoblasts into myofibroblasts while the induced up-regulation of $S1P_4$ accounts for its apoptotic action, making the S1P-directed signaling detrimental for skeletal muscle repair. Thus, it appears that the biological outcome of S1P signaling axis can be highly flexible depending on the pattern of S1P receptors, that can undergo rapid and critical changes.

THROMBOCYTOPENIA INDUCED BY SPHINGOMYELIN DEFICIENCY IN SMS1 KNOCK OUT MICE

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Objective: The degradation of platelets as well as the production is an important step to maintain the number of platelets. However, it is not fully understood whether sphingolipids and their metabolizing enzymes are involved in the degradation of platelets. Sphingomyelin synthase 1 (SMS1) and SMS2 are in charge of producing sphingomyelin (SM), which is a key sphingolipid consisting of microdomain and occupies around 10 % of all lipids on the plasma membrane. In the present study, we revealed that SMS1 is crucial for maintaining the number of platelets in mice. SMS1-knock out (KO) mice, but not SMS2, exhibit typical thrombocytopenia symptoms including low number of platelets, longer bleeding time and the increase in the number of reticulated platelets. In the complete blood counts the numbers of erythrocytes and leukocytes in SMS1-KO mice were similar to those of the control. SM levels on the plasma membrane of SMS1-KO mice , but not SMS2-KO mice, were massively decreased in platelets and megakaryocytes compared to other cell lineages. In addition, the significant increase of phosphatidylserine (PS) externalization was detected in the platelets and megakaryocytes of SMS1-KO mice as compared to SMS2-KO and wild mice, suggesting that SM deficiency facilitated PS externalization in the plasmamembrane. SMS1-KO mice showed the splenomegaly and exhibited the higher collocalization of CD41-positive platelets and CD-68-positive macrophages in the spleen than SMS2-KO and wild mice, suggesting up-regulation of the phagocytosis of platelets by macrophages in SMS1-KO mice. In the bone marrow SMS1-deficiency induced an increase of megakariocytes as a reactive synthesis to thrombocytopenia like the case of immune thrombocythemia (ITP). When the spelenectomy was performed in SMS1-KO mice the platelets increased after 4 days of operation. These results suggested that SM regulated by SMS1, but not SMS2, in the plasma membrane is involved in the controlling the degradation of platelets through PS externalization.

CERAMIDE SYNTHASE 4 IS INVOLVED IN THE REGULATION OF ADULT HAIR FOLLICLE STEM CELL POPULATIONS

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Objective: Ceramides are crucial for skin barrier function but little is known whether stem cell populations that control epidermal regeneration depend on specific ceramide species. Our study shows that ceramide synthase 4 (CerS4) is highly expressed in adult murine epidermis where it is localized in the interfollicular epidermis and specific compartments of the hair follicle where CerS4+ cells co-localize with bulge hair follicle stem cell markers. Inactivation of CerS4 led to precocious activation of hair follicle bulge stem cells. This was manifested in a loss of quiescent label retaining cells and in a continuous growth state of CerS4-/- hair follicles at a time point where CerS4+/+ hair follicles had entered the resting phase. This ultimately led to an almost complete depletion of bulge stem cells in one-year old mice. At the time point where CerS4+/+ hair follicles had entered the resting phase a reduction in BMP target gene mRNA expression was identified in CerS4-/- epidermis, indicating a decrease in BMP signaling. As BMP activity promotes entry into the resting phase and quiescence of bulge stem cells this may explain the inability of CerS4-/- hair follicle stem cells to properly enter the resting phase. Further the reduction in BMP activity likely promoted enhanced Wnt target gene mRNA expression in CerS4-deficient mice. Our data reveal an essential role of CerS4 in the regulation of hair follicle stem and progenitor cell activation and dynamics. In conclusion our data suggest a novel mechanism to hair follicle stem cell activation.

APOM+HDL RESTRAINS LYMPHOPOIESIS AND NEUROINFLAMATION VIA SPHINGOSINE 1-PHOSPHATE SIGNALING

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Objective: Lipid mediators influence immunity in myriad ways. For example, circulating sphingosine 1-phosphate (S1P) is a key regulator of lymphocyte egress. Although the majority of plasma S1P is bound to apolipoprotein M (ApoM) in the high-density lipoprotein (HDL) particle, how the ApoM-S1P complex regulates immunity is unknown. Here, we show that ApoM-S1P is dispensable for lymphocyte trafficking yet restrains lymphopoiesis by activating the receptor S1P1 on bone marrow (BM) lymphocyte progenitors. Mice that lacked ApoM (Apom-/-) had increased proliferation of Lin-Sca1+cKit+ hematopoietic stem and progenitor cells (LSK) and common lymphoid progenitors (CLP) in BM. Pharmacologic activation or genetic overexpression of S1P1 suppressed CLP proliferation in vivo. Activation of S1P1 on CLP in vivo could be visualized using novel S1P1 GFP signaling mice, and the lack of S1P1 stimulation in Apom-/- mice resulted in decreased ERK1/2 phosphorylation and subsequent increased STAT5 activation. ApoM was stably associated with CLPs in BM and specifically inhibited lymphopoiesis in vitro. Upon immune stimulation, Apom-/- mice developed more severe experimental autoimmune encephalomyelitis, characterized by increased lymphocytes in the central nervous system (CNS) and breakdown of the blood-brain barrier. Thus, the ApoM-S1P-S1P1 signaling axis restrains the lymphocyte compartment and adaptive immune responses. Since plasma HDL levels influence CNS and other inflammatory diseases, this regulatory pathway may represent a novel therapeutic

DEFINING THE ROLE OF ACID CERAMIDASE IN ULCERATIVE COLITIS AND THE INFLAMMATORY RESPONSE

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Objective: The sphingolipid enzyme sphingosine kinase-1 (SK1) and its metabolic product sphingosine-1-phosphate (S1P) are key regulators of disease pathology in inflammatory bowel disease (IBD). Ulcerative colitis, one of the major forms of IBD, is a debilitating condition characterized by chronic colonic inflammation and represents a significant risk factor for the development of colorectal cancer. Unfortunately, the molecular mechanisms that regulate the inflammatory response in ulcerative colitis remain incompletely understood. Our published work showed that SK1 deficient mice are protected from parameters of disease and inflammatory responses in the murine model of dextran sulfate sodium (DSS)-induced colitis. We aimed to investigate the upstream metabolic regulation of the bioactive sphingolipid S1P in ulcerative colitis by identifying the upstream ceramidase enzyme contributing to the inflammatory response. Ceramidases are sphingolipid metabolic enzymes that hydrolyze ceramide to generate sphingosine, the precursor of S1P. Using immunohistochemistry, we determined that lysosomal acid ceramidase is overexpressed in the inflammatory infiltrate of colonic tissue from patients with ulcerative colitis but not in normal tissue. Our preliminary data also shows that acid ceramidase activity is increased in the inflamed colon of DSS-treated mice. In vivo pharmacologic and genetic inhibition of acid ceramidase protects mice from disease pathology and inflammation in the DSS-induced colitis model. Furthermore, ex vivo studies demonstrate acid ceramidase-mediated regulation of macrophage inflammatory responses, including production of inflammatory cytokines. These results begin to implicate a novel and specific role for AC in the regulation of disease pathology in DSS-induced colitis and myeloid inflammatory responses.

SPHINGOLIPID METABOLISM AND SIGNALING IN THE REGULATION OF DRUG RESISTANCE IN CML

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Cellular resistance mechanisms developed by cancer cells and tissues in the beginning or proceeding times to applied anticancer agents is a significant problem preventing succesfull therapy. Trysine Kinase Inhibitors (TKIs) are very effective drugs used for the treatment of chronic myeloid leukemia (CML). TKIs bind to the amino acids of the BCR-ABL tyrosine kinase ATP-binding site and stabilize the non-ATP-binding form of BCR-ABL, thereby preventing phosphorylation of its substrates. Although imatinib has high rates of hematologic and cytogenetic response, after exposure of drug, resistance to TKIs has been recognized as a major problem.Various cellular mechanisms may be involved in the nature of cellular resistance. Increased amount of BCR/ABL, alteration in BCR/ABL structure, inhibition of apoptotic mechanisms, decreased imatinib uptake and increased detoxification are well-known mechanisms of resistance.

Aberrant ceramide metabolism is another one of these inherent or acquired mechanisms that contribute to cellular drug resistance. Stress increases *de novo* ceramide synthesis by Ceramide Synthase gene family or activate sphingomyelinases and ceramidase and elevate levels of ceramide leading to apoptosis. Many other stimuli, particularly growth and survival factors, convert apoptotic ceramide to antiapoptotic sphingosine-1-phosphate and glucosyleceramide (GlcCer) by Sphingosine Kinase-1 and glucosyle ceramide synthase (GCS).

In this talk, the roles and mechanisms of action of ceramide metabolism, besides all other possible resistance mechanisms, in the regulation of TKIs-induced cell death and resistance in sensitive and imatinib resistant cell lines and CML patient samples will be reviewed. This study was supported by TUBITAK with the Project number 111S391 to Y.B.

LIPID SIGNALING IN S1P-LYASE-DEFICIENT FIBROBLASTS

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Sphingosine-1-phosphate (S1P) lyase catalyzes the ultimate step in sphingolipid degradation, i.e., the irreversible cleavage of S1P. Mice lacking S1P lyase have a strongly reduced life span and suffer from multiple organ defects, lymphopenia, and generalized inflammation[1,3,4,6]. S1P and sphingosine accumulate in cells and tissues of these mice, which furthermore have a hypercholesterolemia and hypertriglyceridemia, despite their strongly reduced body fat[4]. Similar to the whole organism, both S1P and sphingosine accumulate in embryonic fibroblasts from S1P lyase-deficient mice (*Sgpl1*$^{-/-}$-MEFs). Therefore, these cells might be used as a model system to study intracellular effects of S1P and sphingosine, and they might help to elucidate the phenotype of the knockout mice. Several research groups have analyzed the properties of *Sgpl1*$^{-/-}$-MEFs[2,5,8,9,10]. It has been shown that the cells grow well and are protected from apoptosis[2,10]. They are furthermore characterized by a disturbed Ca^{2+} homeostasis, reduced HDAC activity and reduced expression of several HDAC isoforms[5,8]. S1P lyase deficiency has also been linked to neurodegeneration[7], and interestingly, even *Sgpl1*$^{-/-}$-MEFs have a defect in the processing of the amyloid precursor protein[9]. Our new data show that not only sphingolipid metabolism but also cholesterol homeostasis is considerably altered in these cells.

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CROSSTALK BETWEEN SPHINGOLIPID AND GLYCEROPHOSPHOLIPID METABOLISM IN YEAST

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Objective: Systematic lipidomics is a new, rich source of biological information that allows grouping of genes according to function via their lipid profile (1). We have now measured the lipidome of over 600 yeast mutants, including a collection of protein kinases and phosphatases as well as proteins found in the early secretory pathway (2). These studies have revealed new steps in the regulation of ceramide and sphingolipid homeostasis and have also revealed a correlation between a group of mutants with a particular type of defect in sphingolipid homeostasis with a misregulation of glycerophospholipid metabolism. We are currently working on the identification of a potential sphingolipid metabolite that is responsible for this misregulation as well as its mechanism of action. 1. Santos, AXS et al., (2014) Systematic lipidomic analysis of yeast protein kinase and phosphatase mutants reveals novel insights into regulation of lipid homeostasis. Mol. Biol. Cell 25, 3234-46. 2. Schuldiner, M et al., (2005) Exploration of the function and organization of the yeast early secretory pathway through an epistatic miniarray profile. Cell 123, 507-19.

GLYCOSYLCERAMIDE SUPPLY FROM ETHIOPIAN PLANTS – SCREENING AND QUANTIFICATION METHODS

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Objective: Ceramides are widely distributed in nature. They also provide a major lipid class in the outermost layer of mammalian skin – the stratum corneum. A ceramide balance disorder is related to skin diseases as psoriasis or neurodermatitis. A ceramide skin supplementation with plant glycosylceramide resources is therefore economically of high interest. Glycosylceramides occur in Fabaceae species, e.g. Albizia julibrissin. Five Fabaceae species, in part endogenous in Ethiopia, were collected in April 2013 in the Oromia Region. An easy sample preparation was developed. Total lipids were extracted from ground air-dried seeds using a mixture of isopropanol - n-hexane - water 55:20:22 (V/V/V) as solvents [Markham J and Jaworski JG, 2007. Rapid Commun Mass Sp. 21: 1304-1314] and were further partitioned by liquid-liquid-extraction. Qualitative analysis was carried out by TLC on silica gel 60 F254 plates (Merck), quantitative screening by densitometric analysis after an 18-step solvent gradient (Automated Multiple Development-AMD 2 (CAMAG)) was performed on HPTLC silica gel 60 F254 plates (Merck). Structures were identified by LC-MS using glycosylceramides previously isolated as reference compounds by means of intensive HR-FT-ICR-MS, NMR and MS/MS analyses. We demonstrate by using our developed methods and work flow, an intensive screening regarding the occurrence of ceramides in plants and other natural resources is feasible. Our results give first hints on glycosylceramide content in a broader range of plant species. Total amounts ranged from 3 to 13 mg per 100 g dry weight with highest contents in different Albizia species. The plants may serve as ceramide resources.

SYSTEMATIC LIPIDOMICS OF MUTANTS IN THE EARLY SECRETORY PATHWAY

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Objective: Systematic lipidomics is a new, rich source of biological information that allows grouping of genes according to function via their lipid profile (1). We have now measured the lipidome of over 450 yeast mutants representing proteins found in the early secretory pathway (2). We have used a statistical analysis of the results to cluster mutants with similar phenotypes in sphingolipid homeostasis. We have also used rankings to determine the mutants with the largest and smallest amount of particular sphingolipids as well as ergosterol, which has a particular relation to sphingolipids (3). Interestingly, apart from mutants in ergosterol biosynthesis, the mutant with the least amount of free ergosterol is sfb3/lst1. This COPII coat protein is specifically involved in the transport of GPI-anchored proteins (4), whose transport also requires ceramide, suggesting a possible coregulation or transport of ceramides and ergosterol. 1. Santos, AXS et al., (2014) Systematic lipidomic analysis of yeast protein kinase and phosphatase mutants reveals novel insights into regulation of lipid homeostasis. Mol. Biol. Cell 25, 3234-46. 2. Schuldiner, M et al., (2005) Exploration of the function and organization of the yeast early secretory pathway through an epistatic miniarray profile. Cell 123, 507-19. 3. Guan, XL et al., (2009) Functional interactions between sphingolipids and sterols in biological membranes regulating cell physiology. Mol. Biol. Cell 20, 2083-95. 4. Manzano-Lopez, J et al., (2014) COPII coat composition is actively regulated by luminal cargo maturation. Curr. Biol. 25, 152-62

ENDOSOMAL STEROL, PHOSPHOINOSITIDE AND SPHINGOLIPID SIGNALING INTEGRATES CELL CYCLE CONTROL WITH ENDOSOMAL MEMBRANE TRAFFICKING

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RECONSTRUCTION AND ANALYSIS OF YEAST SPHINGOLIPID PROTEIN INTERACTION NETWORK

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Objective: Sphingolipids are both structural and regulatory components of the cell, where they control processes decisive in cell's fate. The first effort of constructing the protein-protein interaction network of sphingolipids in Saccharomyces cerevisiae enabled us to understand the details of the topological properties of the newly constructed network as well as to assign functions to some of the uncharacterized proteins involving in the network of sphingolipids. The topological analysis of sphingolipid related proteins, especially those under clinical trials for cancer therapy, yielded novel potential drug targets. Novel interactions are predicted using a newly developed integrated methodology employing sequence and structure based computational interaction prediction tools, orthology, expression profiles, co-localization information and Gene Ontology (GO) terms. The sphingolipid network shows topological properties of a scale-free, small-world, and modular structure, as it is the case for biological networks. The function annotation of uncharacterized proteins of the network is performed using a multi-dimensional hybrid method which combines the results from modules and neighbors, and examines them by information gathered from genetic interactions, expression profiles, and sequence similarity. The here constructed sphingolipid network coupled with the newly developed hybrid function annotation method constitutes an efficient platform for function annotation and drug target identification.

SPHINGOLIPID ANALOGUES AND INHIBITORS: SPHINGOLIPID-BASED THERAPEUTICS

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Objective: Sphingolipids function as recognition molecules of cellular stress that can result in endoplasmic reticulum stress, unfolded protein responses, autophagy, apoptosis and senescence in cells. Sphingolipids can also represent danger signals to promote host-defense inflammatory reactivity to invading pathogens. Sphingolipid analogues are designed to mimic endogenous sphingolipid metabolites to modulate these cellular stress and inflammatory reactions. For example, sphingolipid analogues can modulate sphingolipid metabolizing enzymes to control the steady state levels of endogenous sphingolipids and thereby induce the death of cancer cells, such as T-cell acute lymphoblastic leukemia cells. In the case of sphingosine kinase inhibitors, there is a close relationship between the targeting these enzymes, sphingolipid metabolism, oxidative stress and the proteasome that when perturbed can result is catastrophic collapse of cell survival signaling networks in solid cancers. Another example is the use of sphingadienes that increase sphingosine 1-phosphate lyase expression to reduce intracellular sphingosine 1-phosphate levels and limit inflammatory driven down-regulation of tumour suppressor genes in colon cancer cells. Sphingosine mimetics also promote activation of the NLRP3 inflammasome to stimulate formation of the pro-inflammatory mediator, IL-1β, which could be exploited to produce antagonists that diminish exaggerated inflammation in disease. Alternatively, sphingosine mimetics could be used to promote host-defense against invading pathogens; thereby representing alternative therapeutic strategies to the development of antibiotics. The translation of sphingolipid analogues into therapeutics is challenging because of solubility, bioavailability and ‘low-affinity’ binding to targets. This presentation will highlight these issues and the potential for sphingolipid based therapeutics.

FTY720 INDUCES NECROPTOSIS BY REGULATING CERAMIDE SIGNALING AT THE PLASMA MEMBRANE

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Objective: Sphingolipids are important signaling molecules in cells and have recently been explored as cancer therapy targets. FTY720 (Fingolimod, Gilenya) is an FDA approved sphingosine analogue drug used for the treatment of multiple sclerosis (MS). FTY720 is phosphorylated by sphingosine kinase 2 (SK2), to generate P- FTY720 to exert its immunosuppressive properties through binding to sphingosine -1 phosphate receptors (S1PRs). FTY720 also exhibits anti-cancer properties. Our previous studies indicated that one of the mechanism by which FTY720 induces cell death is through necroptosis. FTY720 directly binds to I2PP2A/SET (Inhibitor 2 of PP2A), consequently activating the tumor suppressor protein phosphatase 2A (PP2A). The activated PP2A then induces cell death by stimulating the activity of Receptor-Interacting Protein kinase-1 (RIPK1), involved in necroptosis signaling. Previous studies have also shown that ceramide also binds I2PP2A and activates PP2A. However, little is known about the roles of FTY720 in ceramide signaling and regulation of necroptosis. We hereby seek to investigate the mechanisms of FTY720 in inducing necroptosis with regard to ceramide signaling. Preliminary data indicate that inhibitors of ceramide generation partially protect cells against FTY720-induced cell death. FTY720 and non-phosphorylated FTY720 analogues do not affect ceramide generation, but lead to the formation of specific ceramide-multi-protein complexes at the plasma membrane involved in plasma membrane disruption for necroptosis. Future mechanistic studies will help us understand the details of how these complexes are formed at the plasma membrane and how they regulate necroptosis in response to cellular stress invoked by FTY720 and other therapeutic agents.

CERAMIDE-1-PHOSPHATE (C1P)-STIMULATED MIGRATION AND PHOSPHO-CERAMIDE ANALOGUE-1 (PCERA-1)-INDUCED IL-10 EXPRESSION ARE MEDIATED VIA DISTINCT RECEPTORS IN MACROPHAGES

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Objective: Inflammation is an ensemble of tightly regulated steps, in which macrophages play an essential role. The endogenous phospholipid ceramide 1-phosphate (C1P) stimulates macrophages migration and suppresses LPS-stimulated secretion of the key pro-inflammatory cytokine TNF alpha, while the synthetic C1P mimic, phospho-ceramide analogue-1 (PCERA-1), suppresses TNF alpha secretion and amplifies production of the key anti-inflammatory cytokine IL-10, in LPS-stimulated macrophages. Previous reports suggested that the two compounds act via one or more G-protein coupled receptors. We show that C1P stimulated RAW264.7 macrophages migration via the NFκB pathway and MCP-1 induction, while PCERA-1 neither mimicked nor antagonized these activities. Conversely, PCERA-1 synergistically elevated LPS-dependent IL-10 expression in RAW264.7 macrophages via CREB transcriptional activity, while C1P neither mimicked nor antagonized these activities. Both PCERA-1 and C1P inhibited TNFα secretion, but while PCERA-1 suppressed TNF alpha transcription, C1P blocked the secretion step itself by inhibiting TNF alpha converting enzyme (TACE). Finally, PCERA-1 failed to interfere with a C1P binding assay. These results thus indicate that the natural sphingolipid C1P and its synthetic analog PCERA-1 bind and activate distinct receptors expressed in RAW264.7 macrophages. Identification of these receptors will be instrumental for elucidation of novel activities of extra-cellular sphingolipids, and may pave the way for the design of new sphingolipid mimics for the treatment of inflammatory diseases, and pathologies which depend on cell migration, as in metastatic tumors.

URACIL AND BENZOXAZOLONE CARBOXAMIDES: DISCOVERY OF POTENT SMALL-MOLECULE INHIBITORS OF ACID CERAMIDASE?

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Objectıve: Herein, we present an overview of our recent work on the discovery and development of the first two classes of potent small-molecule inhibitors of acid ceramidase (AC). As the pharmacology of sphingolipid signaling is still nascent, novel tools are needed to further drive understanding of the roles of ceramide in physiology and pathology.

Results: We identified promising small-molecule hits against AC by screening compound collections towards this target. Medicinal chemistry work around selected hits led to the development of two chemical series of potent AC inhibitors, the uracil- and the benzoxazolone-carboxamide classes. Carmofur and other uracil derivatives were the first identified nanomolar inhibitors of AC. We found that selected uracil carboxamides act synergistically with standard anti-neoplastic drugs in certain types of cancer, suggesting a potential use as chemosensitizers. Prototype members of the benzoxazolone class were found to be metabolically stable and capable of engaging AC both in vitro and in vivo, thus representing the first potent and systemically active inhibitors of this enzyme. We also characterized these compounds in terms of mechanism of action, proving that they inhibit AC by covalent binding to its catalytic cysteine.

Conclusıons: We have developed different chemical classes of potent small-molecule inhibitors of AC which can serve as tools to study the functions of ceramide in health and disease, and may help to validate AC as drug target with potential therapeutic applications in cancer and inflammation. These compounds may also provide promising starting points for the development of novel therapeutic agents.

SPHINGOLIPID ANALOGUES AS INHIBITORS OF SPHINGOLIPID METABOLISM AND TRAFFICKING

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TARGETED LC-TANDEM-MS ANALYSIS AND MS IMAGING REVEAL NEW SPHINGOLIPID STRUCTURES AND THE FUNCTION OF CERAMIDE SYNTHASE 3 IN VIVO

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Objective: Tandem-mass spectrometry (coupled to liquid chromatography) enabled the discovery of tissue-specific sphingolipid structures and of the natural composition of 1-O-acylceramides in mammals. Metabolism and cellular location of the latter remains enigmatic (Sphinx-like), but analysis of knockout mouse models gives first insight. The discovery of germ cell specific sphingolipids led to investigations of ceramide synthase 3 (CerS3) function in vivo. Analyzing systemic and cell-specific knockout mouse models with targeted LC-tandem MS proved CerS3 incorporates in a non-redundant fashion ultra long-acyl chains into sphingolipids of differentiated keratinocytes and of adluminal germ cells. Hence, CerS3 is quintessential for skin barrier/life and for male fertility. Tissue location is a prerequisite to understand the biological function of a molecule, which now can be addressed by mass spectrometry imaging (MSI) to distinguish closely related lipid structures. MSI revealed a highly specific location of such CerS3-dependent sphingomyelins in two different adluminal regions of seminiferous tubules supporting the idea, that these structures are made for more than one purpose in germ cells.

PHENOTYPIC MALIGNANT CHANGES AND UNTARGETED LIPIDOMIC ANALYSIS OF LONG-TERM EXPOSED PROSTATE CANCER CELLS TO ENDOCRINE DISRUPTORS

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Objectıve: Numerous studies involve the endocrine disruptors (EDs) exposure to the initiation and development of cancers, including prostate cancer. Three different EDs (aldrin, aroclor 1254 and chlorpyrifos (CPF)) have been investigated as potential inducers of a malignant phenotype in DU145 prostate cancer cells after a chronic exposure. Then, an untargeted lipidomic analysis has been performed to decipher the lipids involved in the observed transformations.

Methods: Phenotypic malignant changes such as epithelial to mesenchymal transition (EMT) induction, proliferation, migration, colony formation and release of metalloproteinase 2 (MMP-2) have been analyzed in 50-day exposed cells to the selected EDs. Changes in specific sphingolipids and other lipids were assessed by the means of an untargeted lipidomic analysis using chemometric tools on LC-MS data of lipid cell extracts.

Results: Chronic exposure to aldrin and CPF resulted in EMT induction. CPF and aroclor 1254 also increased cell migration, colony formation and MMP-2 release. On one hand, the untargeted lipidomic analysis revealed a global decrease in phospholipids, ceramides glucosylceramides and lactosylceramides in cells treated with aldrin; on the other hand, CPF and aroclor 1254 treatment resulted in an increase of certain phospholipids, glycosphingolipids and a remarkable increase of some cardiolipin species. All treatments resulted in increased levels of triacylglycerides.

Conclusıons: The untargeted lipidomic approach used in this study enabled the identification of some lipid compounds and lipid metabolic pathways which could be involved in the acquisition of a malignant phenotype under ED exposure in prostate cancer cells.

CERAMIDES IN ANOXIA SURVIVAL

J. Thomas HANNICH[1], Augustinus GALIH[1],

[1]Geneva University,

Objective: Sphingolipids are major eukaryotic membrane lipids which have been shown to play important signaling roles during cellular stress. Using targeted and non-targeted lipidomics we found a connection between 1-deoxy sphingolipids and resistance to anoxia in the nematode Caenorhabditis elegans. Animals that produce more 1-deoxy sphingolipids show lower survival during anoxia and suppression of 1-deoxy sphingolipid production can extend survival without oxygen. Genetic screens in yeast and worms to identify the target of toxic 1-deoxysphingolipids might help to not only extend survival of cells during lack of oxygen like in stroke or heart attack but also to reduce lipotoxicity during metabolic syndrome and diabetes where 1-deoxysphingolipids have been found to increase.

ELUCIDATING THE DOUBLE BOND POSITION OF ENDOGENOUS 1-DEOXYSPHINGOSINE

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Objective: 1-Deoxysphinglipids are atypical sphingolipids which are devoid of the 1-OH-group present in canonical sphingolipids. 1-Deoxysphingosine (1-deoxySO) is a downstream metabolite of 1-deoxysphinganine and has been found in mammalian cell lines and human plasma. We observed recently that natural 1-deoxySO shows a deviation in LC retention time when compared to a synthetic 1-deoxySO standard bearing a Δ4-5 trans double bond (DB), as in the canonical sphingosine, albeit the m/z is identical for both compounds. This indicates that the DB positon in native 1-deoxySO is at a different position than in sphingosine. Our objective was therefore to elucidate the double bond position in endogenous 1-deoxySO, which is so far not known. HEK 293 cells were fed with deuterium labelled 1-deoxysphinganine. Whole sphingolipid extract was hydrolyzed and derivatized by the addition of dimethyl disulfide (DMDS) across the double bond in 1-deoxySO. The [M]+ ion of the DMDS adduct of 1-deoxySO was analyzed by direct injection on an MS2. After collision-induced disassociation, we could identify two ions specific for the fragmentation between the two SCH3 adducts at the site of the original double bond position. The double bond position of 1-deoxySO was identified to be Δ14-15. These results were confirmed by ozone-induced dissociation. The DB of native 1-deoxySO is located at Δ14-15 and is therefore distinct to that of canonical sphingosine (Δ4-5). This indicates that 1-deoxySO might not be a substrate of dihydroceramide desaturase 1 and that the DB at Δ14-15 may be introduced by a distinct desaturase.

INCREASED PLASMA LEVELS OF SELECT DEOXY-CERAMIDE AND CERAMIDE SPECIES ARE ASSOCIATED WITH INCREASED ODDS OF DIABETIC NEUROPATHY IN TYPE 1 DIABETES

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Objective: The clinical presentation of diabetic neuropathy is similar to neuropathy in hereditary sensory and autonomic neuropathy Type 1 (HSAN1) patients, who exhibit elevated plasma levels of deoxy-sphingolipids (DSL). Supplementation with L-serine reduces plasma DSL levels and decreases neuropathic symptoms in HSAN1 patients. Plasma DSL are elevated in Type-2 diabetes and metabolic syndrome patients but no studies reported in Type-1 diabetes.

Methods: Using mass spectroscopy, plasma levels of DSL and free amino acids in DCCT/EDIC Type-1 diabetes patients (n=80), with and without symptoms of neuropathy were investigated. Patient-determined neuropathy was based on 15-item self-administered questionnaire [Michigan Neuropathy Screening Instrument] developed to assess distal symmetrical peripheral neuropathy in Type-1 diabetes. Patients who scored $\geq$ 4, or were unable to sense their feet during walking or to distinguish hot from cold water while bathing were considered neuropathic. Levels of ceramide, sphingomyelin, hexosyl- and lactosylceramide species were also measured.

Results: Deoxy-C24-ceramide, C24- and C26-ceramide were higher in patients with neuropathy than those without neuropathy (12.3±3.7 vs 10.6±4.1, 3184.6±762.7 vs 2709.5±921.8, & 131.1±39.7 vs 104.6±35.6 nM, $p<0.05$). Cysteine was higher in patients with neuropathy (2.0±1.0 vs 1.4±0.6 μM, $p<0.01$). No differences in other sphingolipids or amino acids were detected. The Odds Ratio of positive neuropathy diagnosis was associated with increased levels of cysteine ($p<0.01$), deoxy-C22:1-, deoxy-C24-, and deoxy-C24:1-ceramide ($p<0.05$), C22-, C24-, and C26-ceramide ($p<0.05$). The Odds Ratio of negative neuropathy was associated with increased sphingosine ($p<0.05$).

Conclusion: high plasma levels of select deoxy-ceramide and ceramide species may be involved with neuropathy in Type-1 diabetes.

POSTERS ABSTRACTS

P1: SPHINGOSINE KINASE 1 MEDIATES TUMOUR MONOCYTE INTERACTION AND CANCER CHEMORESISTANCE.

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Objective: Tumour-associated macrophages promote solid tumour growth through secretion of cytokines and chemokines. Sphingosine kinase 1 (SK1) is a known mediator of inflammation and is critically implicated in cancer progression, resistance and poor prognosis. Here we have investigated the role of SK1 as signal transduction component during the tumour-monocyte cellular interaction.

Methods: Boyden chambers were used for macrophage-tumour cells co-culturing. Cytokine production was measured by proteome profiler and ELISA assays and gene expression was measured by qRT-PCR. Cell viability was measured using MTT assay. Human prostate tumours were established in Nude mice.

Results: During co-culture both monocytes and cancer cells showed a transient increase in SK1 activity and mRNA expression together with an increase in MCP-1 and IL-6 secretion. Silencing of SK1 in cancer cells abrogated co-culture-induced phosphorylation of AKT, ERK1/2, NF-kB and SK1 in monocytes and monocyte-induced cancer cell proliferation. Furthermore, monocytes induced cancer cell chemoprotection via a SK1/Akt/ERK1/2-dependent mechanism, reducing the inhibitory effect of docetaxel on cancer cell proliferation. Selective inhibition of SK1 in tumour cells significantly reduced the cytokine secretion from both cancer cells and monocytes affecting IL-6, MCP-1, GROα, IL-32 and ICAM-1. Our data show that STAT1 binds to SK1 promoter and may be involved in SK1 transcriptional regulation in cancer cells upon monocyte stimulation. Pharmacological targeting of SK1 in mouse prostate cancer model reduced tumour volume, TAM infiltration and cytokine secretion.

Conclusion: Our results suggest a novel SK1/ERK1/2/IL-6/MCP-1 - dependent mechanism of tumour inflammation and monocyte-induced cancer chemoresistance.

P2: INVESTIGATION OF EPIGENETIC MARKERS IN THE SPHINGOSINE KINASE LOCUS FOR A PROGNOSTIC BLOOD TEST TO PREDICT PROSTATE CANCER RISK.

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Objective: Sphingosine kinase 1 (SPHK1) is a proto-oncogene and has been reported to be elevated in many cancers including the prostate, but its regulation is poorly understood. Chromosome conformation patterns represent early changes in epigenetic regulation during tumourigenesis.

Methods: Chromosomal conformation studies were performed in the locus of SPHK1 gene using PCR.

Results: Epigenetic regulation of SPHK1 was investigated in 80 treatment naive patients, which were separated into low and high risk groups according to the NCCN criteria. Relevance vector machine based algorithm was used to predict the potential chromosomal conformations, which were then identified using PCR. 20 Chromosmal conformations were identified in the SPHK1 locus. Of these, an inhibitory conformation SPHK1-5/17 was consistently associated with prostate cancer patients in a low risk group. Conversely, SPHK1-1/15 conformation was shown to be associated with high risk prostate cancer. When united with other chromosomal conformation markers, in the loci of several PCa-related genes, including MYC, PSMA, PTEN, SMAD4, and SAM68, SPHK1 formed a part of a final epigenetic test that was trained on 64 samples and cross-validated on 16 independent samples. The final EpiSwitchTM test can accurately predict PCa aggressiveness status in both training and validation sets, with a combined accuracy of 98.6% (95% CI, 93% - 99%).

Conclusion: In Addition to stratification of PCa patients this prognostic, non-invasive test has the potential to be used for making treatment decisions, monitoring and predicating for PCa mortality. The EpiSwitchTM platform technology can be extended to address other clinical questions in diagnostics and theranostics.

P3: MEMBRANE LIPIDS REGULATE GAMGLIOSIDE GM2 CATABOLISM AND THE ACTIVITY OF LYSOSOMAL LIPID BINDING AND TRANSFER PROTEINS

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Objective: During endocytosis, luminal endolysosomal vesicles are formed which serve as platforms of lipid and membrane digestion. Ongoing in vitro studies on the catabolism of radiolabeled liposomal sphingolipids (SL) and glycosphingolipids (GSL) indicated that stabilizing lipids of the plasma membrane, i.e. sphingomyelin (SM) and cholesterol, inhibit several steps of lysosomal SL and GSL catabolism, and also the lipid mobilization and transfer activities of several sphingolipid activator proteins (SAPs) and NPC2. We reconstituted the catabolic proteins on liposomal surfaces, mimicking luminal vesicles of the lysosomes as platforms for SL degradation. Liposomes with no net surface charge generated only negligible catabolic rates. However, incorporation of various anionic lipids into the liposomal membranes had a strong stimulatory impact on GM2 catabolism and on mobilization and intervesicular transfer of membrane lipids by GM2AP, whereas cholesterol exerted a strong inhibitory effect. The intervesicular transfer of 2-NBD-labeled GM1 from donor to acceptor liposomes was stimulated by BMP, ionic strength and low pH values, whereas both, cholesterol and SM, were inhibitory. The current work demonstrates the importance of electrostatic interaction between anionic SL-carrying vesicles and cationic proteins for the stimulation of SL hydrolysis. It also raises major concerns about the usage of His-tagged GM2AP and other lipid binding proteins to investigate the properties of the natural GM2AP. His-tagged GM2AP binds more strongly to anionic SL-carrying liposomal surfaces due to its positively charged His6-tag at low pH values. Its presence increases GM2 hydrolysis, allows transfer of 2-NBD-GM1 even at low ionic strength but prevents mobilization of membrane lipids.

P4: CERAMIDE MEDIATED LETHAL MITOPHAGY: A NOVEL CELL DEATH MECHANISM IN FLT3 TARGETED THERAPY OF ACUTE MYELOID LEUKEMIA

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Objective: Mutations in FLT3 receptor tyrosine kinase are common in Acute Myeloid Leukemia (AML) and confer a worse prognosis. Ceramide, a bioactive sphingolipid, is synthesized de novo by Ceramide Synthases (CerS) and mediates cancer cell death in response to various chemotherapeutic agents. This study investigates the biological role of ceramide in FLT3-positive AML pathogenesis. We show that AML cell lines and patient samples expressing FLT3 have suppressed CerS1 expression and lower levels of its product C18-ceramide compared with FLT3 negative AML cells. Silenced FLT3 expression or its pharmacological inhibition increased CerS1 and C18-ceramide levels while FLT3 overexpression suppressed them. The increase in C18-ceramide after FLT3 inhibition is required for cell death as silencing CerS1 expression or inhibiting its enzymatic activity protected from FLT3 inhibitors-induced cell death. Targeting FLT3 resulted in C18-ceramide dependent mitophagy, as determined by increased LC3B-II levels and formation of autophagosomes around mitochondria. Mechanistically, C18-ceramide accumulated in the mitochondria to bind directly to LC3B-II recruiting autophagosomal membranes for the execution of mitophagy. This process was accompanied by mitochondrial depolarization, decreased ATP generation, and DRP-1 oligomerization. In summary, our novel study is the first to highlight the importance of ceramide metabolism in AML oncogenesis by showing that FLT3 suppresses CerS1 expression and ceramide generation while its inhibition reactivates CerS1/C18-ceramide axis leading to lethal mitophagy and AML cell death.

P5: ACID CERAMIDASE EXPRESSION IN NORMAL AND NEOPLASTIC MELANOCYTES AND ITS ROLE IN MELANOMA PROGRESSION.

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Objective: In the present study, we used a combination of lipidomics, morphology, pharmacology and genetics to investigate the expression and functions of acid ceramidase (AC) in normal melanocytes and melanoma cells. We then evaluated AC inhibition as a possible strategy for melanoma therapy.

Results: We first measured AC levels in various human cancer cell lines, discovering a greater AC expression in melanoma than in other cancers. To determine whether AC overexpression distinguishes melanoma cells from non-malignant melanocytes, we measured AC gene transcription, protein concentration and enzyme activity in melanocytes and melanoma cells. Unexpectedly, normal melanocytes and melanoma expressed AC at similar levels. However, confocal microscopy experiments revealed that melanocytes and melanoma cells differ in the intracellular localization of this enzyme. Based on their similar AC levels, we predicted that ceramide content should be comparable in normal and malignant cells. In striking contrast with that prediction, lipidomic analysis showed significantly lower ceramide levels in melanoma, due to a deregulation of enzymes involved in the novo ceramide biosynthesis. Lastly, we examined whether AC inhibition influences melanoma cell viability. Our new AC inhibitor ARN14988, with nanomolar potency and good stability, inhibits AC and elevates ceramide levels in a concentration and time dependent manner. This compound alters the balance between ceramide and sphingosine-1-P and synergizes with different antitumoral agents in proliferative melanomas.

Conclusıon: In conclusion, we demonstrated a major involvement of AC and ceramide metabolism in melanoma and normal melanocytes and we propose the use of AC inhibitors for melanomas with a proliferative phenotype.

P6: THE ROLE OF SPHINGOSINE KINASE-1 IN VHL MUTANT CLEAR CELL RENAL CELL CARCINOMA

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Objectıve: Sphingosine kinase 1 (SK1), the enzyme responsible for sphingosine 1-phosphate (S1P) production, is overexpressed in many human solid tumors. However, its role in clear cell renal cell carcinoma (ccRCC) has not previously been described.

Methods: VHL defective ccRCC 786-0 cells were used in this study. SK1 down-regulation was achieved by short-hairpin RNA (shRNA) to study the role of SK1 in ccRCC. Proliferation, invasion, and in vivo angiogenesis assays were conducted.

Results: We showed that SK1 is over-expressed in 786-0 cells lacking functional VHL with high S1P levels. By using TCGA RNA seq database, we showed that SK1 expression was higher in solid tumor tissue from ccRCC patients that was associated with less survival. Knocking-down of SK1 in ccRCC cells had no effect on cell proliferation; whereas, it was associated with less invasion, less phosphorylation of focal adhesion kinase (FAK), and less angiogenesis. Additionally, S1P treatment of SK1-kncock-down cells resulted in S1PR2-mediated phosphorylation of FAK and invasion.

Conclusıons: These results suggest that higher SK1 and S1P levels in VHL-defective ccRCC could induce invasion in an autocrine manner and angiogenesis in a paracrine manner. Accordingly, targeting SK1 could reduce both the invasion and angiogenesis of ccRCC and therefore improve the survival rate of patients.

P7: UNTARGETED LIPIDOMIC ANALYSIS OF ENDOTHELIAL TO MESENCHYMAL TRANSITION IN PROSTATE CANCER CELLS

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Objectıve: Epithelial to mesenchymal transition (EMT) is a biological process that plays a crucial role in cancer metastasis in several human malignancies, including prostate cancer. Little is known about the involvement of lipids in EMT. In this work, an untargeted lipidomic analysis has been performed to investigate which are the important lipids and sphingolipids involved in this process.

Methods: EMT was induced in DU145 prostate cancer cells under TNFα treatment. Sphingolipid and total lipid extracts of induced and non-induced EMT cells were analyzed through UPLC-TOF. Then, the chemometric tools Partial Least Squares-Discriminant Analysis (PLS-DA) and Multivariate Curve Resolution–Alternating Least Squares (MCR-ALS) were applied to data in order to decipher which were the lipids (sphingolipids and other lipids) that presented significant changes after EMT induction.

Results: A significant increase of 14 unsaturated triacylglycerides (TAGs) was observed under EMT induction. According to this, cell presented an accumulation of lipid droplets, as well as a concomitant up-regulation of fatty acid synthase (FASN), a gene that has been related to cancer progression and metastasis. Among other changes in sphingolipid levels, a decrease of C16:0 ceramide was observed under EMT induction.

Conclusıons: The application of chemometric tools for the study of EMT induction enabled the detection of increased levels of TAGs and changes in specific sphingolipid compounds. These results could be helpful for a better understanding of the process in the research of therapeutic targets to prevent metastasis initiation.

P8: OVERCOMING NILOTINIB RESISTANCE BY SPECIFIC INHIBITION OF SPHINGOSINE-1-PHOSPHATE RECEPTOR 2/GQ/PHOSPHOLIPASE C AXIS IN CHRONIC MYELOID LEUKEMIA

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Objective: The changes in sphingolipid metabolism are considered a significant BCR-ABL1 dependent resistance mechanism as well as BCR-ABL mutations. Sphingosine kinase-1 (SK-1)/sphingosine-1 phosphate (S1P)-mediated drug resistance is related to sphingosine-1 phosphate receptor 2 (S1P2) signaling through the inhibition of protein phosphatase 2A (PP2A), resulting in increased stability of BCR-ABL1. However, specific signaling cascade involved in this process remain unkown.

Methods: The antiproliferative effects of nilotinib, SK-1 inhibitor (PF-543), S1P2 inhibitor (JTE-013), phospholipase C inhibitor (U-73122) and nilotinib/PF-543 and nilotinib/JTE-013 combinations on 32D-p210Bcr-Abl(wt) and 32D-p210Bcr-Abl (T315I) cells were determined by MTT assay. Isobologram analysis was performed using CompuSyn program. The mRNA and protein levels of BCR-ABL1, SK-1 and S1P2 were checked by qRT-PCR and western blotting. Resistant cells were also transfected with Gq peptide.

Results: IC50 values of nilotinib were 8 and >500 nm for wt and resistant cells, respectively. IC50 values for JTE-013 were calculated as 20 and 40 μM while that for PF-543 were 8 and 30 μM, respectively. Combination treatments displayed strong synergistic effects on both cell types. Although there is no significant changes in BCR-ABL1 levels for both cells, SK-1 and S1P2 levels increased in resistant cells. Combination studies decreased BCR-ABL1 protein levels in resistant cells comparing to untreated control, PF-543 or JTE-013 treatments. Although U-73122 and Gq peptide treatments decreased BCR-ABL1 protein, their combination with okadaic acid restored BCR-ABL1 protein levels.

Conclusion: BCR-ABL1 levels decreased by activating PP2A via Gq and phopholipase C inhibition, a novel mechanism, which could be a potential therapeutic target to overcome nilotinib resistanc

P9: CORRELATIONS BETWEEN EXPRESSION LEVELS OF BIOACTIVE SPHINGOLIPID GENES AND DRUG-SENSITIVITY AND -RESISTANCE IN CHRONIC MYELOID LEUKEMIA (CML) PATIENTS

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Objective: CML is characterized by Philadelphia chromosome encoding BCR/ABL oncoprotein with tyrosine kinase function. Tyrosine kinase inhibitors (TKIs) are used for CML therapy. In this study, we aimed to examine the correlation between expression levels of bioactive sphingolipid genes and the response of newly diagnosed (ND), TKI-treated and shown hematological response (HR), or TKI-resistant CML patients against therapy.

Methods: Bone marrow samples of 66 CML patients were harvested, and mononuclear cells were isolated by lysis buffer. cDNAs were synthesized via reverse transcription following the RNA isolation. Expression levels of ceramide synthase (CERS1-6), sphingosine kinase-1 (SK1), glucosylceramide synthase (GCS), and BCR/ABL genes were determined by qRT-PCR.

Results: Patients treated with TKIs and shown HR expressed significantly higher levels of CERS1-6 genes than ND or TKI-resistant patients, whereas TKI-resistant patients expressed higher levels of GCS and SK1 genes compared to ND or TKI-treated and shown HR. Comparing the expression levels in different samples acquired once in six months of the same patient revealed that expression levels of CERS1-6 genes increased, whereas GCS and SK1 decreased in patients treated with TKIs and shown HR. While the patients were developing resistance to the therapy, their GCS and SK1 expressions increased.

Conclusion: We showed a correlation between disease progression and bioactive sphingolipid gene expression levels in CML patients. Particularly, the results revealed that besides being alternative targets for more efficient therapy, bioactive sphingolipids might be crucial markers for anticipating drug resistance. This study was supported by The Scientific and Technological Research Council of Turkey with 111S392 project number.

P10: CERS4 AND CERS5 TRANSCRIPTION IS GPER1-MEDIATED AND AP-1-DEPENDENT REGULATED IN HUMAN BREAST CANCER CELLS

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Objective: Ceramide synthases (CerS) catalyze the synthesis of ceramides, which are beside an element of membranes, important signal molecules in the cell. Only little is known about the transcriptional and posttranscriptional regulation of CerS. We wanted to investigate whether the transcriptional regulation of the CerS in human breast cancer cells is G-protein coupled estrogen receptor (GPER) 1-dependent and what role does the activator protein (AP) -1 play.

Methods: MCF-7 cells were transfected with the CerS2, -4, -5, or -6 promoter and CerS4 and -5 promoter AP-1 deletion constructs, cloned into luciferase reporter gene plasmid, and an GPER1 expression plasmid. Subsequently, cells were treated with estrogen and luciferase activity was determined. An AP-1 transcription factor activation ELISA was performed after GPER1 transfection. The expression of GPER1 was determined by quantitative Real-Time PCR and Western Blot analysis. Activation of GPER1 by different ligands was determined by a cAMP-Assay.

Results: Co-transfection of CerSx promoter and GPER1 results in an increased CerS2, -4 and 6 promoter and a reduced CerS5 promoter activity in MCF-7- cells. Deletion of an AP-1 binding site in the CerS4 and -5 promoter as well as an AP-1 transcription factor activation ELISA revealed that GPER1 mediates its effect on the CerS4 and -5 promoter via the AP-1 site. Endogenous GPER1 expression is decreased after estrogen treatment in a concentration dependent manner.

Conclusion: The GPER1-effect on CerS4 and -5 promoter in MCF-7 cells is AP-1 mediated. The complex consists of cFos and Fra-1 and can be activated by diverse ligands.

P11: THE LYSOSOMAL LIPID STORAGE DISEASE NIEMANN PICK TYPE A DISEASE SENSITIZES TO ACETAMINOPHEN HEPATOTOXICITY BY IMPAIRING MITOPHAGY

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Objective: The role of lysosomes in acetaminophen (APAP) hepatotoxicity is poorly understood. Niemann-Pick type A (NPA) disease caused by acid sphingomyelinase (ASMase) deficiency is a lysosomal storage disease (LSD) characterized by increased lysosomal sphingomyelin (SM) and cholesterol accumulation.

Methods and Results: We show that ASMase-/- mice exhibit higher liver damage and mortality after APAP overdose than ASMase+/+ mice. APAP metabolism and predominant toxic mechanisms, including mitochondrial GSH depletion and JNK activation, are independent of ASMase. ASMase-/- hepatocytes exhibit lower threshold for APAP-induced cell death and defective fusion of mitochondria-containing autophagosomes with lysosomes compared to ASMase+/+ hepatocytes, which translates in decreased mitochondrial quality control. Lysosomal cholesterol (LC) accumulation in ASMase+/+ hepatocytes by the cationic amphiphilic drug U18666A reproduces the susceptibility of ASMase-/- hepatocytes to APAP. LC extraction by 25-hydroxycholesterol (25-HC) protects ASMase-/- hepatocytes against APAP hepatotoxicity by improving formation of mitochondria-containing autolysosomes. 25-HC treatment rescues ASMase-/- mice against APAP-induced liver injury. The regulation of LC by U18666A or 25-HC does not affect total cellular SM content or lysosomal distribution. LC accumulation and subsequent sensitization to APAP was also observed in hepatocytes treated with amitriptyline, a widely used tricyclic antidepressant that inhibits ASMase or conduritol B-epoxide, a chemical inducer of Gaucher disease (GD), the most common LSD, due to the irreversible inhibition of glucocerebrosidase.

Conclusion: These findings indicate that LC accumulation determines APAP hepatotoxicity by impairing mitophagy, implying that patients with NPA or GD may be at risk of developing APAP-induced acute liver failure.

P12: STRUCTURE AND REGULATION OF HUMAN NEUTRAL SPHINGOMYELINASE-2

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Objective: Neutral sphingomyelinase 2 (nSMase2) is a magnesium-dependent phophodiesterase that generates the bioactive sphingolipid ceramide. Ceramide is a critically important secondary messenger that acts in a variety of cellular pathways such as apoptosis, inflammation, cell growth and differentiation. Accordingly, mutations in nSMase2 can affect ceramide production and have been implicated in cancers of the breast, urinary bladder and colon. Obtaining a detailed understanding of the structure and function of this enzyme is of significant interest and has the potential to uncover new avenues for cancer therapeutics. We have obtained the first high-resolution X-ray crystallographic structure of human nSMase2 and developed a yeast two-hybrid system to analyze its inter-domain interactions, which likely play a role in activation of the enzyme by anionic phospholipids (APLs). Previous work in our laboratory identified amino acid residues in the N-terminal hydrophobic domain of nSMase2 that interact with APLs in the inner membrane. Our current results suggest that these residues also interact with the cytoplasmic C-terminal domain of nSMase2 and may help regulate activation of the enzyme by APLs. Furthermore, we have identified additional amino acid residues in the juxtamembrane region of nSMase2 that may also contribute to regulation of enzyme activity. In sum, our results provide a model for activation of nSMase2, and reveal a novel structure that paves the way for rational drug design endeavors.

P13: CELLULAR AND MOLECULAR EFFECTS OF ACID CERAMIDASE DEFICIENCY IN MEFS DERIVED FROM FARBER MICE

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Background: Farber disease is a severe LSD that results from reduced activity of acid ceramidase (ACDase), a catabolic enzyme required for the lysosomal breakdown of ceramide into sphingosine and a free fatty acid. We recently developed the first viable mouse model of ACDase deficiency and Farber disease by introducing a clinically relevant single nucleotide mutation into the Asah1 gene. The resulting Asah1P361R/P361R Farber mice accumulate ceramides in all organs, suffer from multiple abnormalities, and die by 9-13 weeks of age.

Objective: To examine the effects of mutant ACDase on enzyme processing, cell growth and potential activation of compensatory mechanisms to deal with the ceramide load. Methods: Using mouse embryonic fibroblasts (MEFs) derived from WT and Farber mice, we analyzed ACDase expression and trafficking by Western blot and immunofluorescence. Transcript levels of sphingolipid metabolic enzymes were assessed by qPCR.

Results: We show herein differential processing of WT and mutant ACDase. We also show activation of compensatory mechanisms in Farber MEFs that address the ceramide accumulation: upregulation of genes encoding ceramide-utilizing enzymes, including alkaline ceramidases, sphingomyelin synthases, and ceramide kinase, along with downregulation of genes encoding ceramide-producing enzymes, including glucocerebrosidase and ceramide synthases. Lastly, Farber MEFs show reduced proliferation and increased susceptibility to apoptosis.

Conclusions: Work from our mouse model will pave the way for understanding potential alternative functions of ACDase, identifying the particular ceramide species responsible for affecting specific cell signaling pathways, and elucidating the mechanism(s) by which lysosomal sphingolipid accumulations elicit changes in gene expression.

P14: BIBLIOMETRIC EVALUATION OF THE EVOLUTION OF THE CERAMIDE RESEARCH LANDSCAPE

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Objective: Research in lipidomics has been rapidly evolving in the post genomic era with the realization that many cell and organismal functions are mediated by lipids. Several years ago, work on ceramides, sphingosine phosphate, and many other bioactive lipids, has focused on the study of basic roles of these molecules in living systems, for example, as key regulators of important cellular and intercellular functions such as cell signaling, growth, senescence, cell death, and inflammation. We have analyzed bibliometric data collected at the Medical University of South Carolina (MUSC) to assess the evolution of lipidomics research over the several phases of Centers of Biomedical Research Excellence funding by the National Institutes of Health. Relevant PubMed MeSH terms (e.g. Ceramides and Sphingolipids) were used to identify a cohort of publications and authors in the field. Analysis of the interrelationship of MeSH terms shows increased emphasis in recent years on the translational aspects of the field and more focus on human diseases than in previous years. Network analysis using research networking software data reveals a steady rise in the average number of co-authorship consistent with a trend towards increased team science. This transition into translational science signifies a relative maturation of the lipidomics field as a whole.

P15: INVOLVEMENT OF S1P SIGNALLING IN FGF-INDUCED NEUROGENESIS AND PROLIFERATION IN INNER EAR SENSORY CELLS

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Objective: According to the world health organization (WHO) 360 million people in the world is affected by debilitating hearing loss. Currently the only available treatment is the cochlear implant, but this device is not suitable in many cases. Thus, it is necessary to explore new molecular targets that could prevent and/or restore hearing loss. Sphingosine 1-phosphate (S1P) signalling was recently shown to be essential for the maintenance of the auditory epithelium in postnatal mice. Nonetheless, the information about S1P signalling pathway in the context of inner ear is still scarce. Our aim is to understand in more detail the role of S1P signalling during the development of the inner ear sensory cells. For this purpose we are currently using a mouse otocyst-derived cell line (VOT33) suitable to study neurogenesis in vitro. As reported previously, FGF2 induced the formation of neuroblasts in VOT33 cells. Moreover, FGF2 induced proliferation and prevented apoptosis induced by serum-starvation and staurosporine treatment. Interestingly, pharmacological inhibition or knockdown of both isoforms of S1P-generating enzyme sphingosine kinase (SK)-1 and -2, and the S1P receptors $S1P_1$ and $S1P_2$ affected FGF-induced proliferation, indicating that S1P signalling axis is necessary in FGF biological action. In addition to the up-regulation of the sensory neuron markers Islet1/2 and β-Tubulin-III, FGF2 treatment induced the up-regulation of $S1P_1$, SK1 and SK2, and down-regulation of S1P lyase, suggesting a positive involvement of S1P in FGF-induced differentiation. Currently, we are developing new strategies to improve neural maturation, as well as the establishment of new complementary in vitro models of inner ear development to gain a more comprehensive insight of S1P signalling in this biological system.

P16: THE IMPACT OF CERAMIDE-SYNTHASES ON COLITIS ULCEROSA

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Background: We could show, that ceramides of various chain length have different impacts on colon cancer cell proliferation. Therefore, we want to investigate how ceramides of different chain length influence the development of colorectal carcinoma in the colitis ulcerosa mouse model by using ceramide synthase (CerS) knockout mice.

Methods: Colitis Ulcerosa was induced by Azoxymethane/Dextrane Sodium Sulfate in CerS knockout and wild type mice. CerS expression in different mouse tissues was assessed by RT-PCR and ceramide concentrations were determined by LC-MS/MS. The impact of CerS expression on different immune cell populations were investigated by FACS analysis. The protein levels of CerS in lamina propria cells of colon tissue from wild type and knockout mice were compared by immunohistochemistry after chronic inflammation.

Results: Inflammatory symptoms as well as tumor development were increased in CerS 4 knockout mice and reduced in CerS 2 knockout mice. Significant alterations in CerS expression and ceramide levels were detected in colon cells of wild type and knockout mice. CerS 2 and CerS 4 deficiency impacted various immune cell populations, like regulatory T-cells, in Blood, Lymph nodes and Spleen.

Conclusion: Ceramides of different chain lengths influence the progression and outcome of colon cancer development induced by chronic inflammation in a colitis ulcerosa mouse model.

P17: SPHINGOSINE 1-PHOSPHATE METABOLISM: A NOVEL WAY TO SENSITIZE MELANOMA CELLS TO ANTICANCER TREATMENTS

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Objective: The treatment of metastatic melanoma has changed considerably with the development of targeted therapies against the frequently mutated serine-threonine kinase BRAF. The survival rate increases by 6-8 months but relapses occur in a median of 6 months. This resistance is partly due to changes in the expression of several pro- and anti-apoptotic members of the Bcl-2 family. Our group has documented significant changes in ceramide metabolism in melanoma cells compared to healthy melanocytes (Albinet et al, Oncogene 2014). In particular, S1P lyase (SPL), which degrades sphingosine 1-phosphate (S1P), is under-expressed (Colié et al, Cancer Res 2009), conversely to sphingosine kinase 1 (SK1) which produces S1P. These alterations lead to increased production of this oncometabolite. Here, we show that SPL overexpression in metastatic melanoma which carries mutated BRAF resulted in sensitization of these cells to apoptosis induced by dacarbazine (DTIC) or vemurafenib (PLX4032). This phenomenon was associated with decreased expression of MITF, a major regulator in melanocyte differentiation and survival of melanoma, and a strong decrease in Bcl-2/Bim ratio that promoted caspase activation. In addition, the pharmacological inhibition of SK1 by SKi-1 or FTY720 induced a synergistic cytotoxic effect with PLX4032 on mutated BRAF melanoma cells which were sensitive or resistant to this agent. While sphingosine and ceramide levels increased in sensitive cells treated with PLX4032, they did not change in resistant cells. Thus, by controlling the expression of key proteins in cell survival, S1P metabolism could represent a new therapeutic approach to enhance the effectiveness of targeted therapies.

P18: THE ONCOGENIC LIPID KINASE SK1 PROMOTES THE MIGRATION AND POLARIZATION OF MACROPHAGES IN MELANOMA TUMORS

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Objective: Tumor infiltration by tumor-associated macrophages (TAM) is often correlated with poor prognosis in melanoma. However, the mechanisms by which TAM mediate melanoma growth are still poorly understood. Recent studies suggest a role for Sphingosine Kinase 1 (SK1), the enzyme that catalyzes the formation of the oncogenic lipid sphingosine-1-phosphate (S1P), in melanoma progression. The aim of this study was to investigate the role of SK1 in the interaction between melanoma cells and TAM. In vitro migration assays of human or murine monocytes, treated with S1P or melanoma cell-conditioned media, demonstrated that exogenous S1P as well as overexpression of SK1 in melanoma cells are able to amplify monocyte migration. This migration was disrupted in the presence of S1P receptor antagonists. On the other hand, macrophage polarization towards M1 antitumor phenotype was enhanced upon incubation with the medium of SK1-silenced melanoma cells. Intradermal injections of murine melanoma cells (B16F10), either knocked-down or not for SK1, into wild-type or SK1-deficient mice showed that the inhibition of SK1 in the host and/or in the tumor reduces melanoma growth. It also decreased tumor infiltration by macrophages. Furthermore, SK1 inhibition in melanoma cells significantly increased the expression of antitumor cytokines in the tumor microenvironment. Accordingly, preliminary flow cytometry studies indicated that, after SK1 inhibition macrophage polarization was reoriented toward an M1 antitumor profile. These findings suggest a key role of melanoma SK1 in macrophage recruitment and polarization within the tumor microenvironment, thereby enhancing the aggressiveness of this cancer.

P19: DOWN-REGULATION OF CERAMIDE SYNTHASE-6 DURING EPITHELIAL-TO-MESENCHYMAL TRANSITION ENHANCES PLASMA MEMBRANE FLUIDITY AND BREAST CANCER CELL MOTILITY TRIGGERED BY FAS/CD95

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Objective: Epithelial-to-mesenchymal transition (EMT) promotes cell motility, which is important for the metastasis of malignant cells, and blocks CD95-mediated apoptotic signaling triggered by immune cells and chemotherapeutic regimens. CD95L, the cognate ligand of CD95, can be cleaved by metalloproteases and released as a soluble molecule (cl-CD95L). Unlike transmembrane CD95L, cl-CD95L does not induce apoptosis but triggers cell motility. Electron paramagnetic resonance was used to show that EMT and cl-CD95L treatment both led to augmentation of plasma membrane fluidity that was instrumental in inducing cell migration. Compaction of the plasma membrane is modulated, among other factors, by the ratio of certain lipids such as sphingolipids in the membrane. An integrative analysis of gene expression in NCI tumor cell lines revealed that expression of ceramide synthase-6 (CerS6) decreased during EMT. Furthermore, pharmacological and genetic approaches established that modulation of CerS6 expression/activity in breast cancer cells altered the level of C16- ceramide, which in turn influenced plasma membrane fluidity and cell motility. Therefore, this study identifies CerS6 as a novel EMT-regulated gene that plays a pivotal role in the regulation of cell migration.

P20: DISCOVERY OF NOVEL S1PL INHIBITORS AGAINST MULTIPLE SCLEROSIS DISEASE

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Background: Sphingosine 1-phosphate lyase, a significant polar sphingolipid metabolite, serves as a contributory component in the regulation of cell migration, vascular stability and differentiation [1]. It carries out communication within the cell as a second messenger [2]. Multiple sclerosis (MS), a progressive inflammatory disorder of central nervous system (CNS), is mediated by the decreased intracellular S1P concentrations which induces migration of pathogenic T cells to the blood stream and disrupts the CNS [3]. The microsomal enzyme sphingosine 1-phosphate lyase (S1PL) degrades the intracellular S1P concentration and thus, S1PL is a promising drug target against MS disease.

Results: Virtual screening of ZINC database of 500,000 compounds via ligand-based and structure-based pharmacophore models retrieved 10,000 hits. After multistep docking and substructure search of common scaffolds, the compounds based on high docking scores, fitness values to the hypothesis and binding efficiency index (BEI), low molecular weight and high percentage of human oral absorption (HOA) were selected and further analyzed via induced fit docking (IFD) and molecular dynamic (MD) simulations in order to gain an insight into the binding modes and the key residues in binding. As a final outcome, the molecules based on having more drug-like properties were proposed as potential inhibitors of S1PL and some of the proposed compounds have also been proven effective against specific cancer cells and malarial parasites (antiviral action).

Conclusion: These potential inhibitors arouse future medicinal chemistry efforts to find out new compounds against destructive actions of pathogenic T cells.

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P21: COMPUTER-AIDED DISCOVERY OF SPHINGOSINE KINASE 1 (SPHK1) INHIBITORS

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Objective: Sphingosine-1-phosphate (S1P) signaling pathway plays an important role in controlling cell survival, cell proliferation, lymphocyte trafficking, angiogenesis, and cell differentiation. Sphingosine kinases (SphKs) catalyze the phosphorylation of sphingosine that leads to the production of S1P. Elevated expression of SphK1 is observed in many different cancers, including breast, colon, and prostate [1,2]. Thus, SphK1 inhibitors have been considered as effective chemotherapeutic agents for the treatment of cancers. The recently revealed crystal structure of SphK1 has two binding pockets for ATP-binding and sphingosine-binding. Calculations based on the X-ray crystal structure to compare the druggability of two pockets suggested that sphingosine-binding pocket is druggable [3,4]. In this work, it is aimed to identify potential SphK1 inhibitors by means of virtual screening and docking simulations. Using Schrödinger's software, 500,000 compounds from the ZINC database were screened to find their ability to interact with the SphK1 sphingosine-binding pocket. Different ligand-based pharmacophore hypotheses were developed by QSAR studies from 28 heterocycle derivatives containing oxadiazole and 54 heterocycle derivatives containing thiazole using ChEMBL database. The validity of these hypotheses was tested by their ability to predict the known activity (pIC50) data reported in literature. The compounds that matched with pharmacophore hypotheses were docked and scored. The pharmacophore hypotheses were further analyzed according to ligand efficiency indices. After strain energy corrections, top hits will be proposed as potential inhibitors of SphK1 after final elimination based on pharmacokinetic and druglikeness properties. This work will provide a guide for future development of inhibitors targeting SphK1.

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P22: TARGETING CERAMIDE TRANSFER PROTEIN CERT TO MITOCHONDRIA TRIGGERS CERAMIDE-DEPENDENT APOPTOSIS

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Objective: Ceramides are central intermediates of sphingolipid metabolism with critical functions in cell organization and survival. They are synthesized on the cytosolic surface of the endoplasmic reticulum (ER) and then transported by ceramide transfer protein CERT to the Golgi for conversion into sphingomyelin (SM) by SM synthase SMS1. Our lab previously identified SMS-related protein SMSr (SAMD8), an ER-resident ceramide phosphoethanolamine (CPE) synthase, as suppressor of ceramide-mediated cell death. Disruption of SMSr catalytic activity in HeLa cells causes a rise in ER ceramides and their mislocalization to mitochondria, triggering a mitochondrial pathway of apoptosis. Targeting a bacterial ceramidase to mitochondria rescued SMSr-deficient cells from apoptosis, arguing that ER ceramides exert their apoptogenic activity in mitochondria (Tafesse et al., 2014). To verify this concept, we set out to investigate the consequences of redirecting the biosynthetic ceramide flow from the Golgi to mitochondria on cell viability. To this end, the Golgi-directed PH domain of CERT was swapped for an outer mitochondrial membrane-targeting signal, yielding MitoCERT. MitoCERT accumulates at ER-mitochondrial contact sites owing to its interactions with the VAP-A receptor in the ER. Photo-affinity labeling experiments with a bifunctional ceramide analogue revealed that the protein binds ceramide through its START domain. Heterologous expression of MitoCERT in HeLa cells triggers mitochondrial apoptosis. Removal of the START domain did not affect the ability of MitoCERT to accumulate at ER-mitochondrial contact sites but completely abrogated its apoptogenic activity. Moreover, MitoCERT-induced apoptosis is suppressed by targeting bacterial ceramidase to mitochondria or treatment with myriocin. Our results indicate that mistargeting ER ceramides to mitochondria suffices to trigger a mitochondrial pathway of apoptosis.

P23: A KINOME-WIDE SIRNA SCREENING COMBINED WITH TARGETED LIPIDOMIC ANALYSIS REVEALS POTENTIAL NEW REGULATORS OF SPHINGOLIPID METABOLISM IN HUMAN CELLS

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Objective: The control of membrane lipid homeostasis is an essential process that allows cells to maintain both their energetic balance and the structural integrity of their different membrane systems. Eukaryotic cells invest substantial resources in generating thousands of different lipids, so there must be evolutionary advantages that are dependent on a complex lipid repertoire. If most enzymes involved in lipid metabolism are now characterized, some aspects regarding their genetic control are still outstanding. The methodology used in this work allows the monitoring of lipid changes in cells using a large-scale RNAi screening of the human kinome combined with targeted lipidomic analysis by mass spectrometry. This strategy makes possible to observe precisely the function of many genes on a large range of lipids species and it is a very promising strategy to discover aspects of lipid metabolism that remain unknown. Statistical analysis of the screening highlights some genes whose knockdown induces changes in sphingolipid levels. Among them some members of the TRIM (tripartite motif), MAPK (mitogen-activated protein kinases) and BRD (bromodomain kinase) family proteins were found to induce changes in ceramide, glucosylceramide and/or sphingomyelin levels. Validations of these results are presented here, which would be the first step to understand the mechanism by which these proteins regulate sphingolipid metabolism.

P24: SPHINGOSINE-1-PHOSPHATE LYASE DEFICIENCY IN THE BRAIN PROMOTES COGNITIVE DEFICITS

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Objective: Sphingosine-1-phosphate (S1P), a bioactive signaling molecule, has been shown to modulate a wide range of cellular processes including proliferation, differentiation, motility, cytoskeleton rearrangements and calcium homeostasis. It is generated by the phosphorylation of sphingosine, a catabolic intermediate of all sphingolipids. S1P is a rather short-lived molecule that is either recycled back to sphingosine via dephosphorylation by S1P phosphatases (SPP1 and SPP2) or irreversibly cleaved by S1P lyase (S1PL). Systemic deletion of S1PL leads to a severe phenotype and early postnatal death. Since our main goal was to investigate the impact of S1PL in the central nervous system we generated a mouse model with neural-restricted deletion of S1PL. The phenotype of these mice is rather unremarkable despite a considerable increase of sphingosine and of S1P that persists throughout life and a decrease of phosphatidylethanolamine (PE), detectable only in older animals. These changes in lipid composition of the brain were accompanied by biochemical and physiological changes including impaired autophagy and altered processing of the amyloid precursor protein (APP), increased proteasomal activity and decreased expression of presynaptic proteins. Finally, ablation of S1PL in neural tissue resulted in profound deficits of motor coordination, spatial learning and memory of the respective animals.

P25: LIGATION OF GQ-COUPLED GPCRS ELICITS PHOSPHORYLATION INDEPENDENT MEMBRANE TRANSLOCATION OF SPHINGOSINE KINASE-1 IN MURINE MYOBLASTS

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Objective: Sphingosine 1-phosphate (S1P) is synthesized by the enzyme sphingosine kinase (SphK)-1 and -2 isoforms. Regulation of the activity and intracellular localization of these enzymes is known to be crucial for all those biological processes which depend on the S1P signaling. It has been shown in HEK-293 cells that Gq-coupled receptors induce a rapid plasma membrane translocation of SphK1. Aim of the present study is to check if the translocation of SphK1 is a general mechanism related to Gq-coupled receptor activation, and if the phosphorylation of the enzyme is involved. We have performed studies in C2C12 myoblasts, a well recognized cellular model for studying physiological processes in vitro.

Methods: C2C12 cells were co-transfected with a vector encoding for SphK1 tagged with GFP (SphK1-GFP) or the phosphorylation-defective mutant of SphK1 (SphK1S225A -GFP), in the presence of different plasmids encoding for Gq-coupled GPCRs, separately. SphK1 translocation was analyzed using a confocal microscope (Zeiss LSM 510 Meta). Results: Activation of Gq-coupled GPCRs such as bradykinin B2, thromboxane TxA2, or histamine H1 induces a rapid translocation (translocation half life = 4-7s) of the SphK1 from cytosol to the plasma membrane. The translocation of the SphK1 following Gq-coupled receptor activation is an event independent from the phosphorylation of the enzyme, as proven by the observed translocation of the SphK1S225A -GFP following bradykinin treatment.

Conclusion: The Gq-mediated SphK1 translocation appears to be a general mechanism of rapid regulation of SphK1, as proven in HEK-293 and C2C12 cells; moreover, this event is independent from enzyme phosphorylation.

P26: DISCOVERY OF TWO POTENT AND SELECTIVE INHIBITORS OF THE NEUTRAL CERAMIDASE

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Objective: Although neutral ceramidase (NC) has been reported to be a therapeutic target in colon cancer, potent and selective inhibitors have not been identified. The aim of this study was to discover NC inhibitors by high throughput screening (HTS) of several libraries.

Experimental Procedures: Ceramidase activities were determined using a fluorogenic HTS method[1] in 96 well-plates using the appropriate buffer, different concentrations of test compound and pure human neutral ceramidase (rhNC). Ceramide analysis in lipid extracts was performed by LC/MS.

Results: Two compounds able to inhibit NC were identified in one of the screened libraries. Kinetic studies showed that both compounds are non-competitive inhibitors with the substrate with Ki values of 0.77 and 2 μM. No inhibitory activity occurred for acid and alkaline ceramidases. When the effect of detergents was examined, we found that NC inhibition was dependent on the presence of sodium cholate in the reaction mixture with no inhibition occurring when other detergents were used (i. e. Triton X100). Preliminary studies in cell culture indicate that the two compounds provoke an accumulation of ceramides.

Conclusions: Two potent and selective inhibitors of NC have been discovered by library screening. They probably act by impairing the activation of NC by bile acids. [1] Bedia, C. et al. ChemBioChem 2007, 8, 642–648.

P27: INHIBITION OF DIHYDROCERAMIDE DESATURASE AND AUTOPHAGY INDUCTION IN GLIOBLASTOMA CELL LINES.

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Objective: The occurrence of a relationship between dihydroceramides (dhCer) increase and autophagy has been reported. The aim of this study is to determine the implication of dhCer in autophagy induced by the dihydroceramide desaturase (Des1) inhibitors gamma-tocotrienol (gTE), celecoxib (CCX), phenoxodiol (PXD) and resveratrol (RV) in glioblastoma cell lines.

Experimental Procedures: U87 and T98 cell lines were used. Cell viability (MTT or Sulforhodamine B) was determined 24 h after treatments. Autophagy (WB analysis of LC3-II) and sphingolipid composition (LC/MS) were measured in cells treated with the maximum non-toxic concentrations of compounds for 24 h. Gene silencing of Beclin 1 and acid sphingomyelinase (ASMase, SMPD1 gene) was achieved by transfection with siRNA using lipofectamine (24 h). Myriocin (5 μM, 24 h) was used to inhibit serine palmitoyltransferase.

Results: All compounds, but not gamma-tocopherol (gT), affected cell viability. gTE, CCX, PXD and RV induced autophagy, while gT did not. gTE, CCX, PXD and RV, but not gT, produced an accumulation of dhCer. Induction of LC3-II by the test compounds was decreased by both myriocin-treatment and knocking down of SMPD1. Beclin 1 silencing impaired autophagy induction by the test compounds without modifying dhCer levels.

Conclusion: Autophagy induction by the Des1 inhibitors gTE, CCX, PXD and RV in T98 and U87 cells involves both the de novo ceramide synthesis pathway and ASMase activity. The lack of effect of Beclin 1 silencing on dhCer levels supports that, in T98 and U87 cells, dhCer increase occurs before autophagy activation by gTE, CCX, PXD and RV.

P28: MODULATION OF ACID SPHINGOMYELINASE IN MELANOMA REPROGRAMMES THE TUMOR IMMUNE MICROENVIRONMENT

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Objective: The inflammatory microenvironment induces tumors to acquire an aggressive and immunosuppressive behavior. Since acid sphingomyelinase (A-SMase) downregulation in melanoma was shown to determine a malignant phenotype, we aimed here to elucidate the role of A-SMase in the regulation of tumor immunogenic microenvironment.

Methods: We analysed two in vivo murine melanoma models in which A-SMase was either downregulated or maintained at constitutively high levels. In order to investigate the dependence of immune cell infiltration by A-SMase expression in melanoma we analysed by flow cytometry, the following cell populations: myeloid-derived suppressor cells (MDSCs), CD8+ and CD4+ T lymphocytes, regulatory T lymphocytes (Tregs) and dentritic cells (DCs).

Results: We found high levels of inflammatory factors in low A-SMase expressing tumors which also displayed an immunosuppressive/pro-tumoral microenvironment: high levels of MDSCs and Tregs, and low levels of DCs. In contrast, the restoration of A-SMase in melanoma cells not only reduced tumor growth and immunosuppression, but induced an high recruitment at tumor site of effector immune cells with an anti-tumoral function. Indeed, we observed a poor homing of MDSCs and Tregs and the increased recruitment of CD8+ and CD4+ T lymphocytes as well as the infiltration of DCs and CD8+/CD44high T lymphocytes.

Conclusions: This study demonstrates that changes of A-SMase expression in cancer cells is sufficient per se to tune in vivo melanoma growth and that A-SMase levels modulate immune cells at tumor site. This A-SMase-dependent modulation of microenvironment events may have a therapeutic outcome in terms of tumor growth.

P29: CERAMIDE IN CF LUNG INFECTION AND INFLAMMATION: COMPLEX ANALYSES FOR ENIGMATIC SPHINGOLIPIDS

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Objective: The sphingolipid ceramide is a known inflammatory mediator and its accumulation in lung inflammation has been reported in different models of emphysema, Chronic Obstructive Pulmonary disease and Cystic Fibrosis (CF). This latter is caused by mutation of chloride channel and associated with hyper-inflammation of respiratory airways and with high susceptibility to un-resolving infections. We previously demonstrated that ceramide de novo synthesis is enhanced in lung inflammation and sustains P.aeruginosa pulmonary infection in a CF murine model (Caretti et al. BBA 2014). Our present aim was to take advantage of quantitative and qualitative analyses to obtain further insight in the role of ceramide in CF. We concluded that the integrated use of Liquid Chromatography and MALDI Imaging coupled to Mass Spectrometry, Confocal Laser Scan Microscopy, histology analyses, is able to unmask information that is not decipherable by any single approach: i) ceramide up regulated synthesis in the alveoli is strictly related to alveolar infection and inflammation; ii) alveolar ceramide can be specifically targeted by nanocarriers delivery of the ceramide synthesis inhibitor Myr; iii) Myr is able to down-modulate pro-inflammatory Lyso-PC, favoring the increase of the anti-inflammatory PCs.

P30: CERAMIDE AND INFLAMMATION IN LABOR

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Objective: Gestation is a unique mechanism involving a pro-inflammatory phase that allows blastocyst implanting into the uterus, a second anti-inflammatory phase allowing fetus growth and a final acute inflammatory phase that triggers contraction of the uterus and parturition. The comprehension of the regulation of inflammatory switches represents an important acquisition for the identification of therapeutic targets in pregnancy defects. Sphingolipids are a broad class of structural membrane components endowed with important signaling activities. Among sphingolipids, ceramide is a well-known mediator of stress signals and pro-inflammatory responses. Ceramide is a known activator of NF-kB and this transcription factor controls the expression of many labor-associated genes. Placenta accumulation of ceramide was shown to be responsible for PGE2 induction in acute inflammation of labor and parturition. Our group recently demonstrated that de novo synthesis of ceramide is not only enhanced in acute lung inflammation but also supports it and that. pharmacological inhibition of this activity down regulates inflammatory response. In this project we evaluated the content of ceramide (by LC-MS analysis) and the expression of Serine Palmytoil Transferase (by Western blotting and RT-PCR techniques) in placenta from cesarean delivery versus spontaneous or induced parturition. We observed a significant co-relation between ceramide increased synthesis and inflammation. These observations are particularly interesting when considering that fast labor is desirable for both mother and fetus and that pre-term labor is initiated by inflammatory cascades. Thus ceramide metabolism can be implicated in controlling important process and may become a therapeutic target in parturition complications.

P31: THE ONCOGENE GOLPH3 AT THE CROSSROAD BETWEEN SPHINGOLIPID METABOLISM, MEMBRANE TRAFFICKING, AND DNA DAMAGE

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Objective: The GOLPH3 (Golgi phosphoprotein 3) gene is located in a human chromosome region (5p13), which is frequently amplified in several solid tumours. The precise molecular mechanism of GOLPH3 mediated oncogenesis is so far unknown, but its role in the positive control of cell proliferation via the Akt/mTOR pathway, is widely accepted. In mammalian cells GOLPH3 has been shown to contribute to vesicular trafficking and Golgi architecture, retention of Golgi enzymes and response to DNA Damage. The yeast homologue of GOLPH3, named VPS74, has essential roles in maintaining the localisation of some Golgi enzymes and in the regulation of sphingolipid homeostasis. Manipulating the expression levels of GOLPH3 in mammalian cells, we found that GOLPH3 positively regulates glycosphingolipid (GSL) synthesis by controlling the localization and stability of GSL synthesizing enzymes. Specifically we found that GOLPH3 interacts with a specific glycosphingolipid (GSL) metabolic enzyme and regulates its sub-cellular localization counteracting lysosomal degradation. The change in sphingolipid metabolism induced by the manipulation of GOLPH3 levels has an impact on p53 activation and DNA damage response following genotoxic insults. Our data suggest that GOLPH3 oncogenic activity involves the regulation of the key cancer modulator p53 through the control of sphingolipid metabolism.

P32: INHIBITION OF CERAMIDE SYNTHESIS AS POST-ISCHEMIC THERAPY FOR MYOCARDIAL REPERFUSION INJURY

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Objective: Therapeutical interventions aimed at reducing post-ischemic injury may have enormous potential to improve short and long-term morbidity and mortality. Besides an array of collateral effects, reperfusion after ischemia triggers a pathological inflammatory reaction caused by several mediators including lipotoxins such as the sphingolipid ceramide. Ischemia-reperfusion (I/R) injury was shown to increase myocardial ceramide content (Beresewicz A. 2002) and pharmacological inhibition of ceramide formation to ameliorate cardiac dysfunction (Gundewar S. 2008). We recently proved that pharmacological inhibition of ceramide synthesis significantly reduces acute inflammation in lung infection (Caretti A. BBA 2014). Our present aim was to evaluate the therapeutic potential of ceramide synthesis inhibition in I/R myocardial injury in mice. After 30 minutes of left anterior descending coronary artery ligation (LAD), we performed intra-myocardial injection of the ceramide synthesis inhibitor just at the beginning of 3 hours reperfusion. The treatment reduced infarct size (36% decrease), decreased ceramide content, expression of inflammatory cytokines, formation of hydroperoxides within the risk area. Finally, the treatment enhanced Nrf2 activated transcription of HO1. We concluded that inhibition of I/R induced accumulation of the stress lipid mediator ceramide during reperfusion of infarcted myocardium allows i) a decrease in tissue infarct, ii) a significant reduction in inflammatory and oxidative factors production, iii) enhanced myocardial pro-survival adaptive response.

P33: ACTIVITY OF NEUTRAL AND ALKALINE CERAMIDASES ON FLUOROGENIC N-ACYLATED COUMARINE-CONTAINING AMINODIOLS.

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Objectıve: In previous articles[1,2] we reported on the use of compounds RBM14, coumarinic analogues of ceramide with N-acyl chains of different length (RBM14-Cn), to determine acid ceramidase (AC) activity. Here we aim at determining the ability of other ceramidases to hydrolyze these substrates.

Experımental Procedures: Ceramidase activities were determined as reported[1,2] in 96 well-plates using the appropriate buffer for each ceramidase, different concentrations of test substrate and either recombinant bacterial or human neutral ceramidase (rhNC) or microsomes from cell lysates.

Results: In rhNC, the substrate affinity increases directly with increasing the N-acyl chain length; RBM14-C16 is the best substrate. All the RBM14 substrates, mainly RBM14-C14 and RBM14-C16, were hydrolyzed in both intact mouse embryonic fibroblasts defective in the ASAH2 gene and lysed cells at basic pH, suggesting that the compounds were hydrolyzed by at least one alkaline ceramidase (ACER). Lower fluorescence levels were released from RBM14-C12, RBM14-C14 and RBM14-C16 in microsomes from stable ACER3 knockdown HCT 116 cells than from their controls. However, microsomes from both HeLa-based cell lines ACER1-TET-ON and ACER2-TET-ON cultured in the presence of tetracycline had no activity on any RBM14 substrate.

Conclusıons: Compounds RBM14 are hydrolyzed by NC and ACER3, but not by ACER1 and ACER2. [1] Bedia, C. et al. ChemBioChem 2007, 8, 642–648. [2] Bedia, C. et al. J Lipid Res 2010, 51, 3542–3547.

P34: REGULATION OF VASCULAR CALCIFICATION BY BIOACTIVE SPHINGOLIPIDS

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Objective: Vascular calcification (VC) is associated with cardiovascular mortality and involves the osteogenic differentiation of vascular smooth muscle cells (VSMCs) and matrix mineralisation. It has similarities to bone formation, a process in which ceramide and sphingosine-1-phosphate (S1P) are implicated. Ceramide can be generated by lysosomal acid-sphingomyelinase (L-SMase) and be converted to sphingosine and S1P by acid ceramidase (ACDase) and sphingosine kinases (SK) respectively. We investigated whether ceramide and S1P regulate VC. VSMCs were cultured with β-glycerophosphate to induce differentiation and mineralisation. Controls were cultured without β-glycerophosphate. Changes in L-SMase and SK activity were determined using an in vitro assay and mRNA levels with Q-PCR. Ceramide, sphingosine and S1P were measured by mass spectroscopy. Inhibition of L-SMase and ACDase was achieved using desipramine (1-10 μM). When mineralisation was widespread; C18 and C20-ceramide increased 75%, sphingosine decreased 33%, S1P increased three-fold, L-SMase activity decreased 30% but mRNA levels were unchanged, SK activity increased 66%, SK2 mRNA increased but SK1 was unchanged; all compared to control VSMCs. Desipramine dose-dependently inhibited mineralisation, increased total ceramide two-fold, decreased sphingosine 33% and prevented the increase of S1P, when compared to VSMCs cultured with β-glycerophosphate alone. Exogenous C2-ceramide decreased, whereas S1P increased, mineralisation. All changes significant, n=3 ($p<0.05$). In conclusion, SK activity and S1P levels are increased during VSMC mineralisation. Inhibition of L-SMase/ACDase leads to ceramide accumulation, prevents S1P production and attenuates mineralisation; identifying ceramide as an inhibitor and S1P as a promoter of matrix mineralisation. These findings identify sphingolipids as potential therapeutic targets for the treatment of VC.

P35: EFFECTIVE SYNTHESIS AND TESTING OF CERAMIDE ANALOGUES WITH ANTICHLAMYDIAL ACTIVITY

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Objective: There is ample evidence for a role of sphingolipids in regulation of cellular signaling and for a therapeutic potential of sphingolipid inhibitors in the treatment of human disease. The quest for highly potent mediators of sphingolipid metabolism not only relies on the development of efficient synthetic routes but also on the discovery of homogenous high-throughput-screening approaches. In this study, we present two highly efficient and versatile approaches for the synthesis of ceramide analogues with anti-chlamydial activity. Parallely, we have developed robust, fast, and simple assays for assessment of biological activity in vivo and in vitro. In one project we have identified a number of ceramide analogues as a novel class of anti-Chlamydia compound that efficiently inhibits C. trachomatis growth without affecting the viability of the host cell. The potency of some compounds was higher than that of the sphingomyelin synthase inhibitor D609 or that of the antibiotic chloramphenicol. A notable result of our study is the identification of the first sub-micromolar inhibitors of chlamydial growth. Although the molecular targets of this compound class is yet unknown, the potency of the compounds, as well as the acquired knowledge about structure-activity relationship study of this compound class defines an important step for target identification soon .

P36: STRUCTURAL AND FUNCTIONAL STUDIES OF NEUTRAL SPHINGOMYELINASE 2 PROVIDE NEW INSIGHT INTO REGULATION AND INHIBITION

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Objective: Neutral sphingomyelinase 2 (nSMase2) hydrolyzes the relatively insert signaling lipid sphingomyelin to generate the bioactive lipid ceramide. NSMase2-derived ceramide has emerged as a critical regulator of exosome-mediated intercellular communication and has been proposed as a potential therapeutic target for breast cancer and Alzheimer's disease. To illustrate the mechanism of ceramide generation and aid in therapeutic development, we have conducted a detailed structural and functional analysis of nSMase2. We present the 2.0 Å crystal structure of the catalytic domain of human nSMase2 that provides insight into the critical residues involved in catalysis and sphingomyelin recognition. In addition, we delineate a key mechanistic step in modulating nSMase2 activity that mediates activation by anionic phospholipids and appears to be the target of the widely used, non-competitive nSMase2-specific inhibitor GW4869. Overall, this work provides fundamental insight into the action of this novel enzyme that may facilitate development of more potent nSMase2-specific inhibitors.

P37: REVERSE MODULATION OF GQ-COUPLED RECEPTOR-INDUCED [CA2+]I INCREASES AND SPHINGOSINE KINASE-1 TRANSLOCATION BY A WD REPEAT PROTEIN

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Objective: Sphingosine kinases (SphK), which catalyze the formation of sphingosine-1-phosphate, are activated by diverse signaling pathways according to the wide variety of their roles. We have shown before that Gq-coupled receptors induce a rapid plasma membrane translocation of SphK1, independently of phospholipase C (PLC) downstream signaling such as protein kinase C and [Ca2+]i. Recently, the WD repeat protein, WDR36, has been identified as a scaffold promoting the interaction of the thromboxane A2 receptor-β (TPβ), Gαq and PLCβ, thereby enhancing TPβ-induced PLC activation. Aim of the present study was to analyze the influence of WDR36 on Gq-mediated SphK1 translocation.

Methods: SphK1 translocation was analyzed by confocal laser scanning microscopy using a Zeiss LSM 510 Meta microscope. [Ca2+]i increases were analyzed in single cells using a Till Photonics Calcium Imaging system.

Results: [Ca2+]i increases induced by a maximal stimulation of the M3 muscarinic acetylcholine receptor stably expressed in HEK-293 cells were significantly enhanced in cells overexpressing GFP-WDR36 compared to vector-transfected cells. In contrast, M3 receptor-induced SphK1 translocation was significantly delayed by overexpression of WDR36. This was shown in three series of experiments with 1. non-tagged WDR36 and GFP-SphK1, 2. GFP-tagged WDR36 and CFP-SphK1 and 3. GFP-tagged WDR36 and mCherry-SphK1. Furthermore, bradykinin B2 receptor-induced CFP-SphK1 translocation was significantly delayed by GFP-WDR36 in C2C12 myoblasts.

Conclusion: We conclude that WDR36 may function as a molecular switch promoting the coupling of Gq to PLC-mediated [Ca2+]i increases while impeding Gq-mediated SphK1 targeting. The data support the hypothesis that SphK1 and PLC are independent effectors of Gαq.

P38: SPHINGOSINE INHIBITS NO-MEDIATED VASORELAXATION OF MOUSE THORACIC AORTA VIA BINDING TO CALMODULIN

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Objective: Calmodulin-dependent endothelial nitric oxide synthase (eNOS) is a main determinant of the vascular tone and permeability. The sphingolipid mediator sphingosine is suggested to have vasoactive properties. We investigated the effect and mechanism of action of sphingosine on the NO-dependent vasorelaxation.

Methods: To test the ability of sphingosine to alter eNOS activity in intact vessels, myography experiments were performed on mouse thoracic aorta ex vivo. We compared the acetylcholine-induced eNOS-mediated relaxation before and during incubation of the vessels with sphingosine. The binding of sphingosine to calmodulin and its effect on calmodulin's function in vitro were characterized by fluorescence spectroscopy, isothermal titration calorimetry, and crystallography.

Results: Treatment of the intact vessels with sphingosine induced significant rightward shift of the acetylcholine dose-response curve. Sphingosine exhibited no significant effect when vasodilation was elicited by the direct NO-donor sodium nitroprusside. We characterized in vitro the inhibition by sphingosine of calmodulin-dependent activity of eNOS, phosphodiesterase, and calcineurin. Calmodulin was found to bind sphingosine in vitro with low nanomolar affinity when the lipid was clustered. The crystal structure of their complex showed a few lipid molecules wrapped around by the protein in its inhibitory conformation.

Conclusions: No under physiologic conditions is a vasorelaxant and attenuates inflammation, while its increased production leads to the initiation and progression of inflammation. We have shown here, that the vascular effects of sphingosine in inflammation might be due to its influence on the activity of Ca2+-calmodulin-dependent eNOS enzyme via binding to calmodulin thereby preventing eNOS activation.

P39: SPHINGOLIPIDOMICS IN GANGLIOSIDOSES AND METACHROMATIC LEUKODYSTROPHY: IDENTIFICATION OF DIFFERENTIATED SPHINGOLIPIDS

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Objective: Sphingolipids are ubiquitous elements of the plasma membrane. They are synthesized in ER and Golgi. Their degradation are made by lysosomal enzymes in lysosome. Defective lysosomal enzymes cause lysosomal storage diseases. Accumulation of undegraded substrates in the nervous system leads to severe impairment of neurological function with a fatal outcome. The aim of this study is to identify sphingolipid/s for predicting clinical phenotype and rate of disease progression. In this study, sphingolipids were extracted from serums of patients with gangliosidoses and Metachromatic Leukodystrophy which are the most common sphingolipid storage diseases in Turkey. Their mass spectrums are taken by MALDI-ToF-MS. Sphingolipids that make difference between groups and clinical findings were determined by cluster analysis. Internal standards were used for quantitative analysis by LC-ESI-MS/MS. After evaluation by cluster analysis of the obtained sphingolipid profiles, sphingosine and sphinganine 1-P in gangliosidoses group were found as differentiated molecules. Results of quantitative analysis indicated increased ceramide and ceramide 1-P levels in gangliosidoses group. Our preliminary data supports sphingolipidomes of the serum may show difference between diseases, difference between diseases and the controls in sphingolipid storage diseases. MALDI-ToF-MS and LC-ESI-MS/MS are appropriate methods for detection of this differences.

P40: THE SPHINGOLIPID RHEOSTAT IS ASSOCIATED WITH FUNCTION AND VIABILITY OF DENDRITIC CELLS

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Objective: Regulation of the complex functions of the immune system has excitingly been proven to be sensitive to alterations in the sphingosine-1-phosphate (S1P) receptor-ligand interaction. Dendritic cells (DCs) mediate immune responses in inflammation, cancerogenesis and autoimmunity, however it is unclear whether DC-activation, function and life cycle are affected by the sphingolipid rheostat. Thus, here we investigated primary bone marrow-derived DCs upon toll-like-receptor (TLR) stimulation by RNA-, protein- and activity-measurements as well as LC-MS. Shortly upon stimulation of distinct TLRs, we found a rapid induction of IL12 secretion. In parallel we observed a transient increase in Sphingosine kinase (SK) 1 expression and total SK activity. However, upon long-term TLR-stimulation a much stronger up-regulation of SK1 mRNA levels was present in parallel to a down-regulation of SK2 levels. Interestingly, the absence of SK1 or SK2 in knockout DCs strongly augmented IL-12 induction but was dampened upon exogenous S1P supplementation. In addition, a pronounced decrease of mRNA, protein levels and enzyme activity of S1P-lyase was monitored in a time-dependent manner. Moreover, we discovered the induction of an intrinsic apoptotic pathway indicated by annexin V and caspase 3-activation and a time-dependent decrease of intracellular S1P and pronounced increase of sphingosine and ceramide levels in activated, not in resting DCs. Obviously, the ability of DCs to modulate immune responses by secreting specific cytokines but also determined by their balance between survival or cell death, is associated with a highly diverse regulation of S1P-enzyme expression and thus complex modulation of the sphingolipid rheostat.

P41: REGULATION OF CERAMIDE-MEDIATED MITOPHAGY BY HPV ONCOPROTEINS AND RB/E2F AXIS IN HEAD AND NECK CANCER

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Background: The five-year survival rate of Head and Neck Squamous Cell Carcinoma (HNSCC) is only 50% due, in part, to a lack of effective, targeted therapies. Human papillomavirus (HPV) is the causative agent of approximately 60% of HNSCC cases. Cellular transformation is achieved by the inhibitory actions of HPV E6 and E7 oncoproteins on p53 and Rb, respectively. Interestingly, HPV-associated HNSCC patients respond better to treatment, demonstrating a 54% higher survival rate compared to HPV-negative HNSCC patients. However, the underlying molecular mechanism for this has not been well-defined. We have identified a novel form of lethal mitophagy that is mediated by ceramide and dependent on DRP1 signaling as an important mechanism of cell death in HNSCC.

Objective: As no studies have examined sphingolipid signaling or mitophagy in HPV-associated HNSCC, we asked whether this form of mitophagy may be involved in the improved response to treatment. Methods: HPV-positive or HPV-negative HNSCC cells were treated with radiation or C18-pyridinium-ceramide. Overexpression or knockdown of relevant genes was performed then cell number, mitophagy, and binding assays performed.

Results: Radiation of HPV-positive cells resulted in increased levels of CerS1/ C18-ceramide. CerS1 overexpression or C18-pyridinium-ceramide treatment resulted in lethal mitophagy, dependent on DRP1 oligomerization. This response was attenuated by knockdown of E2F5 and enhanced by Rb knockdown. Additionally, E2F5/DRP1 association was detected in response to ceramide.

Conclusion: We have identified ceramide-mediated lethal mitophagy as an important cell death mechanism in HPV-associated HNSCC, regulated by the E7/Rb/E2F5 axis.

P42: INSIGHTS INTO THE EFFECTS OF THE MUTATIONS OF ARYLSULFATASE A ON ITS STRUCTURE USING MOLECULAR DYNAMIC SIMULATIONS

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Objective: Arylsulfatase A (ASA) is a lysosomal enzyme catalyzing the hydrolysis of sulfate ester bonds. Its major substrate is cerebroside-3 sulphate, and the product of the reaction, cerebroside, is the major constituent of myelin sheats. In case of the deficiency of this enzyme, demyelination of neurons occurs causing Metachromatic Leukodystrophy Disease (MLD). In this work, we focus on the effect of previously identified mutations on the activity and functionality of ASA [1]. We first checked for conservation of these residues, which are away from the active site, by comparison with their homologs and no significant conservation was found. Next, calculation of the free energies of the wild type and mutated types of ASA using FoldX showed that the mutant form has higher energy in the monomeric, dimeric and octameric forms. Then, the W318C mutant of ASA was generated using VMD and molecular dynamics (MD) simulations of 10 ns were performed for monomers at pH~7. The results of these MD simulations and the free energy calculations will be discussed. It is predicted that these mutations decrease the enzyme stability, which would result in loss of enzyme activity. In order to test this hypothesis, MD simulations for both pH values (5 and 7) of the wild type and mutants are performed. [1] Önder,E.,Sinici,I.,Sönmez,M.,Topçu,M.,Özkara,H.A.(2009) Identification of two noval arylsulfatase A mutations with a polymorphism as a cause of metachromatic leukodystrophy, in Neurological Research 31,pp 60-66

P43: INVESTIGATING THE ROLE OF ORMDL PROTEINS AS REGULATORS OF THE MAMMALIAN SERINE PALMITOYLTRANSFERASE

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Objective: Serine Palmitoyltransferase (SPT) typically catalyzes the first step in de novo sphingolipid synthesis. Studies in yeast demonstrated that SPT activity is tightly regulated by a metabolic feedback mechanism, mediated by Orm1 and Orm2 proteins. In contrast to yeast, mammalian cells express three Orm isoforms (ORMDL1-3). However, the role of ORMDL proteins in regulating SPT activity in mammalian cells is not yet understood. Several SNP variants in ORMDL3 were found to be closely associated with the risk for early childhood asthma. A role of SPT and de-novo sphingolipid metabolism in asthma is discussed. Methods. The role of ORMDL proteins on SPT activity was tested in HEK293 cells individually overexpressing ORMDL1, 2 or 3 and in MEF cells which are devoid of ORMDL3 expression. Furthermore we analyzed the association of ORMDL SNPs and plasma sphingoid base levels in a comprehensive population based cohort with 1200 individuals. Results. Neither the individual overexpression of ORMDL proteins nor the absence of ORMDL3 had a significant effect cellular SPT activity. None of the annotated SNPs in ORMDL genes including those that were reported to be associated with childhood asthma showed a significant association with plasma sphingoid base levels. However, increasing intracellular Ceramide levels by the addition of C6-Ceramide led to a significant suppression of SPT. Conclusion. Increasing levels of C6-Ceramides inhibit SPT activity, which indicates a regulatory mechanism of the mammalian SPT similar to yeast. However, a role of individual ORMDL isoforms including ORMDL3 in this regulatory process could not be confirmed.

P44: THE USE OF HAIR SAMPLES TO MONITOR 1-DEOXY-SPHINGOLIPIDS IN HSAN1

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Background: 1-Deoxysphingolipids (1-deoxySL) are formed by the enzyme serine palmitoyltransferase due to an alternative use of L-alanine over its canonical substrate L-serine. Pathologically elevated 1-deoxSL levels are formed in the context of the inherited neuropathy HSAN1 which is caused by missense mutations in SPT. HSAN1 is a progressive ulcerating axonopathy associated with a progressive peripheral sensory loss (pain, temperature, vibration), neuropathic pain and ulcers (slow healing wounds). Often, HSAN1 patients suffer also from anhydrosis, reduced hair growth and a fine and delicate skin structure. An oral L-serine supplementation was shown to suppress 1-deoxySL formation in transgenic HSAN1 mice and HSAN1 patients. Patients under L-serine therapy reported consistently improvements in skin structure, wound healing and a faster growth of hair and finger nails.

Method: The sphingoid base profile was analyzed in hair samples from healthy controls and HSAN1 patients before and during therapy. The extracted lipids were analyzed by high resolution mass spectrometry.

Results: We observed a variety of sphingoid bases including 1-deoxySL present in the analyzed hair sample. Canonical sphingoid bases were comparable between control and HSAN1 samples whereas 1-deoxySLs were significantly higher in HSAN1^samples. After extraction the HSAN1 hair appeared more brittle and showed structural abnormalities. Longitudinal, 1-deoxySL levels correlated with the history of L-serine supplementation in the patient.

Conclusion: Significant 1-deoxySL levels were present in hair of HSAN1 patients. The longitudinal profile reflected changes in 1-deoxySL production over time. Hair samples might therefore be a non-invasive alternative for the diagnosis and therapy monitoring in HSAN1.

P45: GLUCOSYLCERAMIDE AS A MODULATOR OF THE PROPERTIES OF LIPID RAFT-LIKE MEMBRANE DOMAINS

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Objective: Glucosylceramide (GlcCer) is a glycosphingolipid involved in different physiological and pathological events. It is widely accepted that GlcCer is an active participant in lipid domain formation, particularly in lipid rafts. However, the interplay between GlcCer and lipid raft components is still poorly studied. In the present study, we used fluorescence spectroscopy and microscopy to investigate the interaction between GlcCer and cholesterol (Chol) in models of biological membranes. To better understand the interplay between these lipids, the results were rationalized by a ternary phospholipid/sphingolipid/chol phase diagram. Our results showed that ternary POPC/GlcCer/Chol mixtures containing low GlcCer content display properties typical of raft-domains, i.e., a liquid-ordered (lo)/liquid-disordered (ld) phase coexistence. However, compared to the canonical POPC/Sphingomyelin/Chol raft-like mixtures, this phase coexistence region is smaller, mainly due to the higher ability of GlcCer to segregate into gel-enriched domains. This results in an extensive 3-phase coexistence region of ld(POPC-enriched)/ lo(Chol-enriched) and gel(GlcCer-enriched). Moreover, the effect of acidification in the properties of these mixtures, was also analyzed. Our data showed that upon acidification GlcCer solubility in the lo phase increases, leading to a larger raft-like coexistence region. Quantitative analyses of the data allowed determination of the phase's composition at neutral and acidic pH. These results enable to predict GlcCer impact in domain formation and membrane organization in complex biological membranes, and provide background to unravel the role of GlcCer-biophysical properties in its biological action. Acknowledgments: This work was supported by Fundação para a Ciência e Tecnologia (FCT), Portugal: PTDC/BBB-BQB/0506/2012, SFRH/BD/69982/2010 to ARV and Investigador FCT 2014 to L.C.S.

P46: FUNCIONAL LIPIDOMICS DURING ORIENTED MITOSIS IN ZEBRAFISH

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Objective: In zebrafish, dorsal epiblast cells are an excellent system to study oriented cell division in a developing tissue. Throughout gastrulation, these cells display divisions that are robustly oriented along the anterior-posterior axis. In these cells, division orientation is under the control of the Wnt/Planar Cell Polarity (PCP) pathway. How the PCP pathway specifies the division plane had been elusive until recently, when we found that the PCP pathway induces the formation of an asymmetrically polarized actin cap. This cortical actin cap triggers the spatial redistribution of the transmembrane protein Anthrax Toxin Receptor 2a (Antxr2a), which becomes enriched at the actin cap and is required to exert torque on the spindle to position it along the embryonic axis. Using a novel interdisciplinary approach combining system-level lipid analysis, high-resolution imaging, and quantitative cellular biology, we found that the PCP pathway controls oriented divisions by regulating lipid homoestasis. We have identified specific lipid species that are modified in Wnt/PCP mutants, where divisions are randomized. Indeed, interfering with the first step in de novo sphingolipid synthesis results in randomized divisions of epiblast cells. In these embryos, epiblast cells still exhibit a normal, asymmetrically polarized actin cap, whereas the asymmetric distribution of Antxr2a is lost. By imaging membrane-order sensing dyes in epiblast cells using fluorescence lifetime imaging microscopy (FLIM), we will evaluate whether epiblast cell membranes have a homogenous lipid order or are a mixture of different lipid-order microenvironments. We will investigate whether the existence and abundance of ordered lipid domains modulate Antxr2a behaviour.

P47: HSP70 BASED TREATMENT OF LYSOSOMAL STORAGE DISORDERS

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Objective: Lysosomal storage diseases (LSDs) often manifest with severe systemic and central nervous system (CNS) symptoms. The existing treatment options are sparse and none of them are effective against the devastating neurological manifestations. We have demonstrated proof-of-concept for heat shock protein 70 (Hsp70)-based strategies as potential pan-LSD therapies. Recombinant Hsp70 improves the binding of several sphingolipid-degrading enzymes to their essential co-factor, bis(monoacylglycero)phosphate, in vitro and reverts lysosomal pathology in primary fibroblasts from 14 patients with eight different LSDs. It penetrates effectively to murine tissues including CNS, inhibits glycosphingolipid accumulation in murine models of Fabry (GLA-/-), Sandhoff (HEXB-/-) and Niemann-Pick type C (NPC1-/-) diseases, and effectively alleviates a wide spectrum a disease-associated neurological symptoms in HEXB-/- and NPC1-/- mice. Importantly, oral administration of a clinically enabled small molecule co-inducer of Hsp70, recapitulates the pan-LSD and neurological potential of recombinant Hsp70 encouraging the development of Hsp70-based therapies for LSDs.

P48: ENDOTHELIAL-DERIVED SPHINGOLIPIDS PRESERVE SYSTEMIC VASCULAR FUNCTIONS

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Objective: Endothelial dysfunction is a critical event in many cardiovascular diseases including hypertension. Although lipid signaling is implicated in endothelial dysfunction and cardiovascular diseases, specific molecular mechanisms are poorly understood. The goal of our study was to define the role of locally produced sphingolipids on vascular tone regulation. To this aim, we employed genetic mouse models and assessed blood pressure in vivo by using tail-cuff system, in normotensive and angiotensin-II-hypertensive mice. Vascular reactivity of resistance arteries was evaluated ex-vivo by using the pressure myograph system. We identified endothelial-derived sphingosine 1-phosphate (S1P) as critical regulator of vascular tone and flow-mediated vasodilation through the activation S1P1-receptor-eNOS signaling axis. We discovered a novel regulation of endothelial sphingolipid de novo synthesis by Nogo-B, membrane protein of the endoplasmic reticulum that modulates local sphingolipid production with direct effects on vascular function and blood pressure. Nogo-B inhibits serine palmitoyltransferase, rate-limiting enzyme of the sphingolipid de novo synthesis, controlling endothelial S1P production and its autocrine G-protein-coupled receptor-dependent signaling actions. Mice lacking Nogo-B are hypotensive and resistant to Ang-II-induced hypertension (150.4±2.5 vs. 108.4±1.5 mmHg, compared to WT mice, 24 days after AngII infusion), and preserve endothelial function and nitric oxide release. Pharmacological inhibition of serine palmitoyltransferase with myriocin in mice that lack Nogo-B reinstated endothelial dysfunction and Ang-II-induced hypertension (143.9±1.5 vs. 90.1±1.6 mmHg, myriocin vs. vehicle treated Nogo-A/B-/- mice). Our study identifies Nogo-B as a key inhibitor of local sphingolipid synthesis and indicates that autocrine sphingolipids signaling within the endothelium are critical for vascular function and blood pressure homeostasis.

P49: EFFECT OF SPHINGOSINE ON THE BIOPHYSICAL PROPERTIES AND PERMEABILITY OF RAFT-MIMICKING MIXTURES

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Objective: In addition to its several roles as a bioactive lipid, when abnormally accumulated in the lysosomes and late endosomes, sphingosine (Sph) leads to Niemann Pick type C1 (NPC1), one of the most complex storage diseases in terms of molecular phenotype. At the moment little is known about the interaction of Sph with other membrane lipids. It is our aim to characterize the effect of Sph on the biophysical properties of model membranes composed of different lipids, so that new insights into its mode of action might arise. Using an established fluorescence spectroscopy approach we evaluated the effect of Sph in membrane organization and permeability of different 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC)/Sphingomyelin/Cholesterol raft-mimicking mixtures. Our results showed that Sph accumulation leads to the formation of Sph-enriched gel domains in raft mimicking mixtures. These domains are more easily formed at neutral pH mimicking the plasma membrane environment (PM) as compared to the acidic lysosomal membrane environment (LM), where higher Sph concentrations are required. The determination of ζ-potential further revealed that in PM-raft models Sph is mainly neutral, whereas in LM-raft models the positive charge of Sph leads to electrostatic repulsion, reducing Sph ability to form gel domains. It was also shown that the external addition of Sph affects the membrane permeability, being the effect more noticeable in acidic environment. These results support the hypothesis that Sph biological actions, including those related to NPC1, could be exerted through biophysical changes in cellular membranes.

P50: EFFECT OF CERAMIDE-ACYL CHAIN STRUCTURE ON THE BIOPHYSICAL PROPERTIES OF LIVING CELLS

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Objective: Ceramide (Cer) is involved in the regulation of several cellular processes by mechanisms that depend on Cer-induced changes on membrane biophysical properties. Accumulating evidence shows that ceramides with different N-acyl chain composition differentially impact cell physiology, which may in part be due to specific alterations in membrane biophysical properties. We now address how the sphingolipid (SL) N-acyl chain affects membrane properties in cultured human embryonic kidney cells by overexpressing different Cer synthases (CerS). Our results show an increase in the order of cellular membranes in CerS2-transfected cells caused by the enrichment in very long acyl chain SLs. Formation of Cer upon treatment of cells with bacterial sphingomyelinase promoted sequential changes in the properties of the membranes: after an initial increase in the order of the fluid plasma membrane, reorganization into domains with gel-like properties whose characteristics are dependent on the acyl chain structure of the Cer was observed. Moreover, the extent of alterations of membrane properties correlates with the amount of Cer formed. These data reinforce the significance of Cer-induced changes on membrane biophysical properties as a likely molecular mechanism by which different acyl chain ceramides exert their specific biological actions.

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P51: SPHINGOSINE 1-PHOSPHATE AND RENAL FIBROSIS.

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Objective: Fibrosis is a common end-point of nearly all forms of acute and chronic inflammatory kidney diseases. Here, we summarize our findings about the effect of sphingosine 1-phosphate (S1P) on the expression of connective tissue growth factor (CTGF) as an important mediator of renal fibrosis.

Methods: Using several pharmacological and genetic approaches, we investigated the effect of S1P on the expression of pro-fibrotic CTGF in glomerular cells (podocytes and mesangial cells) and in kidney glomeruli from sphingosine kinase (SK)-1 deficient mice (SK-1-/-) suffering from streptozotocin-induced diabetic nephropathy.

Results: The alteration of CTGF expression in renal cells strongly depends on the localization of S1P. In human mesangial cells endogenous S1P enhances CTGF expression by activation of S1P2, whereas S1P5 seems to be involved in the pro-fibrotic action of phospho-FTY720. Moreover, recent data show that S1P5 is directly involved in the induction CTGF expression by transforming growth factor beta 2 (TGF-b2). In contrast, activation of SK-1 by TGF-b2 reduces CTGF expression in renal glomerular cells. Our results using SK-1 siRNA, the SK inhibitor SKI II, and "caged" S1P demonstrate that the CTGF lowering effect of SK-1 activation is mediated by intracellularly formed S1P. Additionally, SK-1-/- mice develop a more severe diabetic nephropathy and express higher amounts of CTGF in glomeruli, further demonstrating the anti-fibrotic capacity of SK-1 activation and subsequent formation of intracellular S1P.

Conclusion: Overall, we suggest that the contribution of S1P to renal fibrosis is of Janus-faced nature as S1P exhibits both pro- and anti-fibrotic effects depending on its site of action.

P52: SYNOVIAL FLUID SPHINGOLIPIDS ACROSS SPECIES AND IN HUMAN OSTEOARTHRITIS

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Objectives: Sphingolipids within synovial fluid (SF) of articular joints can be involved in osteoarthritis (OA) and rheumatoid arthritis (RA). We quantified for the first time the composition of sphingolipids in the SF of knee joints from unaffected controls, patients with early (eOA) and late (lOA) stages of OA, and from patients with RA. Furthermore, sphingolipids of human, canine and equine SF were compared to identify a large animal particularly suited as a model of human lipid metabolism.

Methods: Lipids were extracted from cell and cellular debris free SF of 9 postmortem donors used as healthy controls, 17 eOA, 13 lOA, 18 RA patients as well as from 9 dogs and 14 horses with healthy joints. Sphingolipids were quantified using ESI-MS/MS directly or coupled with HILIC. This study was approved by the ethics committee of our university. The Kruskal-Wallis test, Benjamini-Hochberg adjustment and the Steel-Dwass statistical test were applied.

Results: We provide a novel overview of 35 sphingolipids present in human SF. Based on the fatty acids, 19 sphingomyelin, 6 ceramide, 5 hexosylceramide and 5 dihexosylceramide species were identified in SF. Compared to control SF, all sphingolipids were found to be elevated in OA and RA SF. Across species similar numbers of sphingolipids were observed whereas the ceramide levels appears to be species dependent

Conclusions: Compared to control SF the concentrations of sphingolipids are elevated in eOA, lOA and RA SF which may be used to develop biomarkers. Both large animal species partly reflect the normal human sphingolipid metabolism.

P53: UNRAVELLING THE BIOPHYSICAL PROPERTIES OF SPHINGOLIPIDS: FROM MODEL TO CELL MEMBRANES

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Objective: Sphingolipids (SLs) have emerged as an important class of lipids due to their bioactive role in several cellular events and in disease. The evidence that several SL species participate in the formation of lipid domains, and that this might underlie their biological mechanism of action has fostered research in the biophysical aspects of bioactive SLs. This work will focus on two important SLs – ceramide and glucosylceramide – and their interplay with other lipid components in simple and complex membrane models.

Methods: A combination of biophysical methodologies that include fluorescence spectroscopy, confocal and two-photon microscopy, surface pressure-area measurements, were used to elucidate the effects of these lipids on the biophysical properties of membranes with different lipidic components and displaying different phase properties.

Results: Our results showed that lipid-lipid interactions are modulated by alterations in the membrane environment, such as changes in pH. Moreover, small structural differences of these lipids influence their packing properties, membrane shaping and lateral organization. The importance of the headgroup, acyl chain length and unsaturation, on the modulation of membrane properties will be discussed in the framework of results obtained for cellular membranes.

Conclusion: Model membrane systems allow to predict the biophysical and biological implications of these lipids in cellular membranes. Supported by FCT (Portugal) grants PTDC/BBB-BQB/0506/2012 and RECI/CTM-POL/0342/2012, SFRH/BD/69982/2010 to ARV, Investigador FCT 2014 to LCS.

P54: DETECTION AND QUANTITATION OF NOVEL SPHINGOLIPIDS BY MASS SPECTROMETRY

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Objective: Serine Palmitoyl Transferase (SPT) is the enzyme that carries out the condensation reaction between Serine and Palmitoyl Co-A that is known as the committed step of sphingolipid biosynthesis. Dysregulation of this important pathway through its downstream sphingolipid metabolites has been implicated in a variety of human diseases, including diabetes, metabolic syndrome, neuropathy, and cancer, as well as several key regulatory pathways such as apoptosis, inflammatory responses, and autophagy. Recent discoveries from several laboratories have demonstrated SPT exhibits substrate promiscuity, both with other non-canonical amino acids as well as fatty acids, which has led to the uncovering of novel sphingolipid metabolite by-products of this promiscuity such as deoxysphinganine and deoxydemethylsphinganine. We have now developed novel mass spectral-based methods to allow for high sensitivity and accuracy detection and quantitation of this emerging new class of sphingolipids. Preliminary data suggest there may be a regulatory relationship between sphingolipids and amino acid availability that extends beyond the canonical Serine and Palmitoyl Co-A to other amino acids such as Alanine and Glycine, and other Fatty Acids including Stearate. We have employed Multiple Reaction Monitoring on a triple quad mass spectrometer in conjunction with high quality authentic SL standards to identify and measure relative levels of novel SL metabolites, in the absence of derivatization. Development of this quantitative methodology will serve to define novel SLs and their potential roles in signaling, and to define their biological functions in yeast.

P55: LIPID OVERSUPPLY TO CARDIOMYOCYTES INDUCES SPHINGOLIPID-DEPENDENT OXIDATIVE STRESS AND INDUCTION OF MITOPHAGY THROUGH CERAMIDE SYNTHASE 2.

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Objective: Diabetic cardiomyopathy (DbCM) contributes to the high risk of heart failure (HF) in diabetics, but mechanisms underlying DbCM remain unclear. We previously showed that high saturated fat feeding in mice altered cardiomyocyte sphingolipid profiles leading to DbCM and that some of these maladaptations were dependent on autophagy and ceramide synthase 5 (CerS5). In the present study, we sought to further understand the cellular processes in which lipid overload leads to DbCM in in the context of sphingolipids. Mice fed an obesogenic diet and in vitro studies using H9c2 cardiomyocytes were utilized in this study. Increased oxidative stress and apoptosis were identified in the hearts of animals subjected to lipid overload, while animals treated with the sphingolipid synthesis inhibitor myriocin were protected. Similarly, cardiomyocytes treated with palmitate showed a sphingolipid-dependent increase of reactive oxygen species (ROS) and mitophagy. Treatment with mitochondria-targeted ceramide analogs revealed that very long chain ceramides, but not long-chain ceramides, induced cardiomyocyte cell death, which was exacerbated by inhibiting mitophagy. This suggested that lipotoxicity to cardiomyocytes occurs in part through oxidative stress in a sphingolipid-dependent manner, and mitophagy may occur to prevent further damage. In the observation that only very long chain species led to these outcomes suggested involvement of CerS2. Overexpression of CerS2 showed increased mitophagy in cardiomyocytes and knockdown of CerS2 by CRISPR-CAS9 technology decreased mitophagy. Taken together, our data suggest that lipid overload induces mitophagy as a protective measure in defense from CerS2-induced mitochondrial damage, oxidative stress, and cell death in DbCM.

P56: CASPASE 2 IS REQUIRED FOR SPHINGOSINE KINASE 1 PROTEOLYSIS IN RESPONSE TO DOXORUBICIN IN BREAST CANCER CELLS: IMPLICATIONS TO THE CHK1-SUPRESSED PATHWAY

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Objective: Sphingosine Kinase 1 (SK1) is a lipid kinase whose activity produces the potent bioactive lipid sphingosine 1-phosphate. Sphingosine 1- phosphate is a pro-survival lipid associated with proliferation, angiogenesis and invasion; subsequently SK1 overexpression has been observed in numerous cancers. Recent studies have demonstrated SK1 proteolysis downstream of the tumor suppressor p53 in response to several DNA damaging agents. Moreover, loss of SK1 in p53 knockout mice resulted in complete protection from thymic lymphoma providing evidence that regulation of SK1 constitutes a major tumor suppresser function of p53. Given this profound phenotype, this study aims to investigate the mechanism by which wild type p53 regulates proteolysis of SK1 by doxorubicin in breast cancer cells. We find that p53-mediated activation of Caspase 2 was required for SK1 proteolysis and that Caspase 2 activity significantly alters the levels of endogenous sphingolipids. As p53 is mutated in 50% of all cancers, we extended our studies to investigate whether SK1 is deregulated in the context of triple negative breast cancer cells (TNBC) harboring a mutation in p53. Indeed Caspase 2 was not activated in these cells and SK1 was not degraded. Moreover, Caspase 2 activation was recently shown to be downstream of the CHK1-Suppressed pathway in mutant p53 cells, whereby inhibition of the cell cycle kinase CHK1 leads to Caspase 2 activation and apoptosis. Indeed knock-down and inhibition of CHK1 led to loss of SK1 in p53 mutant TNBC cells, providing evidence that SK1 maybe the first identified effector of the CHK1-Suppressed pathway.

P57: CERAMIDE LIMITS CELL MOTILITY IN OVARIAN CANCER: POTENTIAL OF CERAMIDE AS A METASTASIS SUPPRESSOR LIPID.

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Objective: Targeting cell motility, which is required for dissemination and metastasis, has a therapeutic potential for treating ovarian cancer metastasis, and regulatory mechanisms of cell motility needs to be uncovered for developing novel therapeutics. Invasive ovarian cancer cells spontaneously formed protrusions, such as lamellipodia, required for generating locomotive force in cell motility. Small interfering RNA screening identified class II phosphatidylinositol 3-kinase C2β (PI3KC2β) as the predominant isoform of PI3K involved in lamellipodia formation of ovarian cancer cells. Ceramide, one of bioactive sphingolipids, has been emerging as an antitumorigenic lipid, and treatment with short-chain C6-ceramide decreased the number of ovarian cancer cells with PI3KC2β-driven lamellipodia. Pharmacological analysis demonstrated that long-chain ceramide regenerated from C6-ceramide through the salvage/recycling pathway, at least in part, mediates the action of C6-ceramide. Mechanistically, ceramide was revealed to interact with PI3KC2β and affect its compartmentalization, thereby suppressing PI3KC2β activation and its-driven cell motility. Moreover, ceramide treatment suppressed cell motility promoted by epithelial growth factor known as a prometastatic factor. To examine role for ceramide in ovarian cancer metastasis, ceramide liposomes were employed and confirmed to suppress cell motility in vitro. Those ceramide liposomes displayed an inhibitory effect on peritoneal metastasis in a murine xenograft model of human ovarian cancer. Taken together, our studies identified ceramide as a bioactive lipid that limits PI3KC2β-governed cell motility, and ceramide is proposed to serve as a metastasis suppressor lipid in ovarian cancer. Those could be translated into developing ceramide-based therapy for metastatic diseases.

P58: SIGNALING PATHWAYS OF SPHINGOSINE-1-PHOSPHATE IN VASCULAR SMOOTH MUSCLE CONTRACTION

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Objectives: We aimed to examine the direct effect of sphingosine-1-phosphate (S1P) on vascular smooth muscle (VSM) contractility and to elucidate the underlying signaling pathways.

Methods: Isometric tension of endothelium-denuded thoracic aorta segments isolated from male wild type (WT) and knockout (KO) mice deficient in S1P2, S1P3 receptor or Gα12/13 was measured in myographs. Vasoactive effect of 10 μM S1P was detected at physiological (4 mM) and elevated (6-124 mM) extracellular K+-concentration [K+]e.

Results: At physiological [K+]e S1P had negligible vasoactive effect. Slightly increased [K+]e (6 mM) failed to influence the vascular tone by itself, but addition of S1P induced marked vasoconstriction that was further intensified if [K+]e was increased to 8 and 10 mM. At higher [K+]e (20-80 mM), K+ increased the vascular tone progressively and attenuated the additional vasoconstriction by S1P. Further experiments on the signaling pathways were performed with 8 mM [K+]e, which enhanced the action of S1P markedly without significantly influencing the resting vascular tone by itself. The vasoconstrictor effect of S1P disappeared in S1P2-KO and Gα12/13-KO vessels, whereas remained unchanged in S1P3-KO mice. S1P-induced vasoconstriction was also abolished by the Rho-kinase inhibitor Y-27632 (10 μM) in WT vessels.

Conclusion: S1P induces vasoconstriction in case of moderately increased [K+]e. The effect of S1P is mediated by S1P2-receptor, Gα12/13, and Rho-kinase. This phenomenon may lead to increased vascular tone under conditions of systemic or local elevation of [K+]e surrounding the VSM, like in hyperkalemia or tissue ischemia. Grant support: OTKA K-101775 and MedInProt Protein Science Research Synergy Program.

P59: REGULATION OF INFLAMMATORY CYTOKINE SIGNALING BY ACID SPHINGOMYELINASE

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Objective: Acid sphingomyelinase (ASM), which hydrolyzes sphingomyelin to produce ceramide, regulates a key step in the sphingolipid salvage pathway. Ceramides are biologically active lipids that have been linked to cellular stress responses and cytokine production in several model systems, but the mechanism by which ASM regulates cytokine production has not been established. In this work we define a mechanism by which ASM regulates IL-6 production in breast cancer, and demonstrate a role for ASM in tumor progression. We found that down regulation of ASM in two distinct genetic models, reduced IL-6 production in response to the tumor promoter, 4β-phorbol 12-myristate 13-acetate (PMA). Down-regulation of another ceramide producing enzyme, acid β-glucosidase (GBA), potentiated IL-6 production in response to PMA, whereas knock down of the sphingosine producing enzyme, acid ceramidase (ACD), had no effect. From these data we conclude that spatiotemporal regulation of ceramide production plays a role in its function as a second messenger within the cell, suggesting specific roles for GBA and for ASM derived ceramides. In addition, indirect pharmacologic inhibition of ASM with desipramine blocked both the PMA and TNF-α induced production of IL-6. Inhibition of ASM correlated with a reduction in p38 MAP Kinase phosphorylation, suggesting that ASM is regulating IL-6 production through modulation of MAP Kinase activity. Lastly, we found that inhibition of ASM or of p38 reduced tumor cell migration in matrigel transwell migration assays. Taken together, we have defined a role for ASM in pro-tumor signaling events, and identified a role for ASM in tumor progression.

P60: NEUTRAL SPHINGOMYELINASE 2 AND HEPATIC ACUTE PHASE RESPONSE

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Objective: Hepatic acute phase response (APR) plays a central role in host response to infection and it is exacerbated in aged animals and humans. In vitro studies indicate that neutral sphingomyelinase 2 (nSMase-2) is essential component of APR. Objectives: The goal of this study is to investigate the role of nSMase-2 in a rat model of inflammation.

Methods: Four- and 21- months old rats were administered with adenovirus encoding a GFP-tagged shRNAi against nSMase-2 or scrambled control and, 6 days later, with LPS (5.6 mg/kg) or saline. Hepatic samples were analyzed 4 hours later for efficiency of infection, nSMase activity, and levels of expression of nSMase-2 and several acute phase proteins.

Results: GFP fluorescence was observed in up to 30% of the cells in the liver but not in other tissues. Livers of LPS-treated animals exhibited 2-to 3-fold higher nSMase-2 mRNA levels than saline-treated animals without significant ageing-associated differences. The mRNA levels of IGFBP1 increased, while those of PEPCK1 decreased in response to LPS in an age-specific manner. These differences were attenuated (by approximately 20-25%) in animals treated with shRNA against nSMase-2.

Conclusions: (i) LPS stimulates hepatic expression of nSMase2 mRNA. (ii) Liver-specific silencing of nSMase-2 is achievable in vivo; (iii) in spite of a relatively moderate efficacy of silencing, a statistically significant reversal of LPS effects on IGFBP1 and PEPCK1 can be detected, confirming the role of NSMase2 in APR and indicating a possible therapeutic use of targeting nSMase-

P61: COMPARISON OF SERUM SPHINGOSINE-1-PHOSPHATE LEVELS AMONG EUTHYMIC, MANIC AND DEPRESSIVE PATIENTS WITH BIPOLAR DISORDER

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Objective: Bipolar disorder (BD) is a chronic and overwhelming psychiatric condition with manic-depressive episodes. Sphingolipids are biologically active lipids ubiquitously expressed in the CNS. Sphingosine-1-phosphate (S1P) is a bioactive sphingolipid acting through G-protein-coupled receptors. It has regulatory roles in endothelial injury, inflammation, and thrombosis in vascular biology. Increase of serum S1P levels was reported to have a role in stress-induced anxiety. In a recent study S1P receptor gene is reported as one of the susceptibility genes in BD. We hypothesized that serum levels of S1P might have been different among patients with manic, depressive or euthymic forms of BD.

Methods: There were three groups in the study involving euthymic (N=14), manic (N=10) and depressive (N=7) patients. Plasma samples (100 µl) were mixed with 500 µl of methanol containing an internal standard (IS) colchicine. Supernatants were separated after centrifugation. An Agilent 1260 Infinity UPLC system was used for chromatography. Total run time was 10 minutes; the flow rate was 0.5 ml/min with solvent A, water (10 mM ammonium acetate, 0.1% formic acid) and solvent B, methanol (0.1% formic acid). The eluent was directly introduced into a mass spectrometer by electrospray. An Agilent 6420 triple quadrupole mass spectrometer was used. S1P and IS were analyzed in positive ion mode and multiple reaction monitoring with ion pairs 380.2/264.3 and 400.2/358.2 respectively.

Results: There was no statistically significant difference between S1P levels among groups.

Conclusion: This is a preliminary study and S1P levels should be investigated in larger number of patients with BD.

P62: A DROSOPHILA NEURODEGENERATIVE AUTOPHAGY MUTANT PERTURBS SPHINGOLIPID METABOLISM, AUTOPHAGIC CLEARANCE, AND STRESS SIGNALLING

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Objective: We propose that imbalances in ceramide metabolism are a key factor in determining the neurodegenerative severity of a Drosophila autophagy mutant, blue cheese. Shotgun lipidomics on blue cheese mutant brains reveals increased ceramide levels, as well as expected sphingolipidomic changes in n-Ceramidase and n-Sphingomyelinase processing mutants that modify the degeneration in blue cheese. Mutant neurons show defects in autophagic trafficking and clearance of sphingolipids, an imbalance in de novo vs. recycled sources of sphingolipids, and alterations in growth vs. stress signaling by members of the MAPK and insulin cascades. Surprisingly, although total ceramides are higher in blue cheese mutant brains and RNAi-S2 cells, genetic backgrounds that increase the available ceramide pool rescue all phenotypes observed, including neuronal death, ceramide accumulation in autophagosomes, autophagic vesicular trafficking, MAPK signaling defects, and ER stress. Increasing autophagic flux by rapamycin treatment similarly rescues blue cheese degeneration, but also reverses the signaling defects in blue cheese RNAi cells.

P63: MOLECULAR MECHANISMS OF ATG7 AND P53 IN REGULATION OF SPHINGOLIPID-DEPENDENT AUTOPHAGY

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Objective: Diabetic cardiomyopathy (DbCM) is defined as significant changes in the structure and function of the heart in the absence of coronary artery disease and hypertension. Recently our lab found that cardiomyocyte hypertrophy and autophagy play a role in the development of DbCM in a mouse model and that inhibiting de novo sphingolipid synthesis attenuated this process. In vitro studies of fatty acid oversupply revealed that hypertrophy and autophagy are dependent on ceramide synthase 5 (CerS5) and we identified various autophagy-associated genes, including Atg7, that were upregulated in DbCM in a sphingolipid-dependent manner. Interestingly many of these genes are p53 targets. Further cells studies revealed that sphingolipids induced p53, and loss of p53 prevented sphingolipid-dependent autophagy. This indicated that p53, at least in part, mediates sphingolipid-dependent autophagy. Studies from other labs suggest that direct binding of Atg7 to p53 may regulate p53 function under some conditions. Therefore we tested whether Atg7-p53 association is sphingolipid-dependent and/or required for autophagy induction. Using proximity ligation assay technology and confocal imaging we found that Atg7 and p53 association is increased under autophagy-inducing conditions, and that this association is decreased with knockdown of CerS5. Furthermore, we found that nuclear levels of p53 are regulated by sphingolipids. From these results we conclude that Atg7 and p53 are important mediators of sphingolipid-induced autophagy and that their association may be regulated by specific sphingolipid species and in turn regulate sphingolipid-induced autophagy.

P64: DEFINING REGULATION OF THE SPHINGOLIPID NETWORK IN RESPONSE TO DOXORUBICIN

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Objective: Sphingolipids have been implicated in numerous cellular biologies, both pro-and anti-growth; however, the study of the enzymes responsible for their metabolism has been hindered by difficulties in detection. Furthermore, these enzymes represent a dynamic network in which a "single" signal results in diverse responses leading to multiple potential biologies, specifically in cancer progression. Our lab is exploring the effects of both lethal and sub-lethal doses of doxorubicin to mimic the decreasing clinical concentrations of chemotherapeutics over time. In MCF7 cells sub-lethal and lethal doses of doxorubicin result in biphasic ceramide generation paralleling two distinct biologies; growth arrest and apoptosis, respectively. While antibodies are available for some of the sphingolipid enzymes, there is a need for a systems approach to examine the role of sphingolipid enzymes, their metabolic products, and the resulting biologies in response to doxorubicin treatment. To this end, LC/MS/MS methods were developed to quantify 32 enzymes central to sphingolipid metabolism and monitor flux in sphingolipid content generated by this enzymatic regulation. The studies presented here will determine the role of sphingolipids and their metabolizing enzymes in response to chemotherapeutics.

P65: CHARACTERIZATION OF CHOLESTEROL HOMEOSTASIS IN SPHINGOSINE-1-PHOSPHATE LYASE-DEFICIENT FIBROBLASTS

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Objective: Mice lacking sphingosine-1-phosphate (S1P) lyase have a strongly reduced life span and suffer from multiple organ defects, immunosuppression, and accumulation of S1P and sphingosine in cells and tissues. Interestingly, these mice have enhanced plasma levels of cholesterol and triglycerides while suffering from strongly reduced body fat. Aim of the study was to further analyze the link between S1P lyase and cholesterol homeostasis using embryonic fibroblasts from S1P lyase-deficient mice (Sgpl1-/--MEFs).

Results and Conclusions: Since we had observed that ABCA1, which transports both S1P and cholesterol, was upregulated in Sgpl1-/--MEFs, we initially speculated that accumulation of S1P triggered reverse cholesterol transport. However, total cholesterol content was not altered in Sgpl1-/--MEFs grown in serum-free medium, and it was even enhanced in the presence of 10 % FCS. In agreement, the uptake of [3H]cholesterol was enhanced while [3H]cholesterol release was unaltered, and the low-density lipoprotein (LDL) receptor was upregulated in the knockout MEFs.These alterations were in line with an upregulation and enhanced proteolytic activation of the transcription factor, sterol regulatory element-binding protein (SREBP)-2. Interestingly, the expression of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase was decreased, leading to reduced formation of biosynthetic precursors of cholesterol in Sgpl1-/--MEFs. Expression and phosphorylation of AMP-activated protein kinase was not altered. We conclude that decreased HMG-CoA reductase expression and activity leads to activation of SREBP-2 which in turn induces ABCA1 and LDL receptor and increases cholesterol uptake in Sgpl1-/--MEFs.

P66: SPHINGOSINE KINASE 1 REGULATES ADIPONECTIN EXPRESSION

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Objective: Obesity affects more than 1/3 of the United States population and is associated with various pathophysiological conditions, which may arise in part from increased plasma lipids. Previous studies from our lab showed that palmitate induced sphingosine kinase 1 (SK1), leading to such outcomes as Il-6 production from skeletal muscle. Adipose tissue secretes cytokines and hormones and this is affected by obesity; thus we sought to test a role of SK1 in adipocyte function. We used a combination of methods including: in vitro models of primary adipose-derived stem cells (ADSCs) isolated from wild type and whole-body SK1-/- mice, in vivo models of adipose-specific SK1 deletion and obesogenic diets, quantification of lipids by LC/LC/MS, qRT-PCR, and western blotting. The adipocyte-specific cytokine adiponectin is a potent regulator of metabolic homeostasis, and we observed an increase in adiponectin mRNA expression in undifferentiated ADSCs isolated from SK1-/- mice compared to wild type, suggesting that SK1 may suppress adiponectin expression. Furthermore, we observed over the course of differentiation, S1P-mediated suppression of adiponectin mRNA compared to control. We then performed an S1P-dose response experiment, and found that S1P effectively suppresses adiponectin mRNA expression at a maximum concentration of 40 nM. This specificity indicated to us that the effects on adiponectin may be mediated through an S1P receptor. S1P receptor expression changed over the course of differentiation. These outcomes are under current investigation in mice with adipocyte-specific deletion of SK1. The involvement of the SK1/S1P axis in adiponectin regulation may present a novel target for obesity-related therapies.

P67: ROLES FOR SPHINGOLIPID METABOLISM IN NONALCOHOLIC FATTY LIVER DISEASE.

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Objective: Nonalcoholic fatty liver disease (NAFLD) is a progressive disease in which triacylglycerols first accumulate in the liver, and in 10-20% of cases, this progresses to an inflammatory state referred to as nonalcoholic steatohepatitis (NASH). Factors that promote this progression remain unknown. We developed a mouse model of progressive NAFLD by feeding a high saturated fat diet, which induced profound hepatic steatosis, inflammation, and pro-fibrotic signaling. Previous studies from our lab demonstrated that saturated fatty acids upregulated Sphingosine Kinase 1 (SphK1), and we found elevated SphK1 message in liver in this model and in human NASH samples. Consistent with a role for SphK1 in NAFLD progression, SphK1-/- mice fed a high fat diet were protected from inflammation. In addition to proinflammatory signaling, sphingosine 1-phophate induced ER stress in cultured hepatocytes, and ER stress is increasingly appreciated as a component of NASH. During the course of these studies we found that Ceramide Synthase 6 (CerS6) expression was also increased in the mouse model of NAFLD, yet remained unchanged in SphK1-/- mice. CerS6 expression was also increased following treatment of hepatocytes with sphingosine 1-phosphate, suggesting a possible cross-talk between different sphingolipid metabolic enzymes in NAFLD. We observed regulation of ER stress by manipulating CerS6. We speculate that this novel cross-talk between branches of sphingolipid metabolism may at least in part underlie the relationship between inflammation and ER stress in NAFLD, and regulation of this pathway by saturated fatty acids may promote progression of hepatic steatosis to NASH.

P68: INTRACELLULAR SK2-DERIVED S1P MEDIATES EGF-INDUCED ERM PHOSPHORYLATION AND CANCER INVASION

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Objective: ERM (ezrin, radixin, and moesin) proteins are a group of adaptor molecules linking the cortical actin cytoskeleton to the plasma membrane, and are emerging as critical regulators of cancer metastasis and progression via regulation of cell morphology and motility. Recently, our lab has identified S1P as an acute and potent ERM activator (via phosphorylation) through its action on its receptor S1PR2. We have also demonstrated that S1P-mediated filopodia formation, a first step in cell invasion, is through ERM activation. Growth factors are known activators of ERM proteins; however, it is not known if this involves the newly related S1P/S1PR2 axis as well as upstream metabolites of the sphingolipid pathway. Using pharmacological inhibitors, siRNA technology as well as genetic approaches, we have demonstrated that SK2 is not only essential but also sufficient in EGF-mediated ERM phosphorylation. Surprisingly, and for the first time, we proved that this event, although dependent on S1PR2 activation, does not require extracellular S1P secretion. Finally, we identified SK2 and S1PR2 as two novel and potent targets in the pathway of EGF-driven invasion. In fact, the inhibition of SK2 or S1PR2 eradicated EGF-mediated lamellipodia formation, and subsequent adhesion and extracellular matrix invasion. We also showed that SK2 overexpression increases EGF-mediated adhesion and invasion in an erin dependent manner. In conclusion, this body of work does not only uncover new mechanistic insights for EGF-mediated invasion, it also set the stage for two novel alternative therapeutic targets that could be of utmost importance especially in patients that become resistant to current EGFR-tyrosine kinase inhibitors.

P69: NEUTRAL SPHINGOMYELINASE-2 MEDIATES A PROTECTIVE ROLE BY MEDIATING S PHASE ARREST IN REPONSE TO DOXORUBICIN

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Objective: p53 is a tumor suppressor involved in mediating responses such as cell cycle arrest, DNA repair, and apoptosis in response to stress. While mutations in the p53 gene are associated with aggressive cancers, a shift in thought suggests that the presence of mutant p53 in cancer cells leads to better clinical outcomes after chemotherapy. This is based on the rationale that chemotherapeutics are DNA damaging agents and hence mutant p53 impairs endogenous defense mechanisms of cancer cells resulting in death without repair of the damage. However, synergistic inhibition of p53 with chemotherapy is not a viable option, as it would result in side effects. As such, targeting effectors of specific arms of p53 biology would be one avenue to explore. Neutral sphingomyelinase-2 (nSMase2) generates the bioactive lipid ceramide by hydrolyzing sphingomyelin. It has been implicated in biologies such as cell cycle arrest and apoptosis.

Methods: We use molecular biology techniques of siRNA silencing, flow cytometry, qRT PCR and immunoblotting Results: Here we show that, in MCF7 breast cancer cells, nSMase2 is activated in a dose-dependent manner by doxorubicin to generate ceramide. Moreover, this is concomitant with a p53-dependent S phase arrest that is abolished by knockdown of nSMase2. Finally, this inhibition of S arrest in nSMase2 knockdown cells results in failure to resume growth in these cancer cells following doxorubicin treatment.

Conclusion: Taken together, our data suggest that inhibition of nSMase2 could have synergistic benefits if combined with DNA damaging chemotherapeutics in the context of non-mutant p53 breast cancer.

P70: DEOXYSPHINGOLIPIDS AS NEUROTOXIC INTERMEDIATES OF SYSTEMIC TAXANE TREATMENT

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Objective: Taxanes are chemotherapy drugs widely used for treatment of variety of cancers, such as breast, ovarian, lung, prostate, bladder, Kaposi sarcoma. In some cases taxanes are used as the last line of treatment. A major dose limiting side effect of taxane treatment is peripheral neuropathy compromising its effectiveness. With our study we tested weather the levels of a neurotoxic class of lipids, the deoxysphingolipids, are elevated in the dorsal root ganglia of mice treated with a taxane. We also compared in vitro in neurons, the toxic effect of taxanes and deoxysphingolipids.

Methods: Lipids were extracted from ganglia isolated from mice intraperitoneally injected (three times with four weeks in between intervals) with a taxane (docetaxel) and subjected to quantitative mass spectrometry analyses of sphingolipids. We used immunocytochemistry analyses to compare the effect of taxanes and deoxysphingolipids in neurons.

Results: We observed significant elevation of deoxysphingolipid levels (i.e. deoxydihydroceramide, deoxyceramide, deoxysphinganine, and deoxysphingosine) in the mouse ganglia after the administration of the third taxane injection. Not all deoxysphingolipid species were affected the same way. Interestingly, deoxydihydroceramide and deoxyceramide species with unsaturated fatty acid moieties were not elevated. While ceramide levels were elevated, too, the levels of sphingosine and sphingosine-1-phosphate were not. Importantly, our in vitro data showed that only deoxysphingosine treatment resulted in morphological changes in the neurons; taxane and the sphingosine treatments did not.

Conclusion: taken together our data suggest that in the dorsal root ganglia, deoxysphingolipids are the likely toxic intermediates of the systemic taxane treatment.

P71: ASSOCIATIONS BETWEEN VITAMIN D AND LPA RECEPTOR EXPRESSIONS IN PREGNANT WOMEN AND THEIR INFANTS LIVING IN YOZGAT REGION, TURKEY

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Objective: Vitamin D (Vit D) induced phospholipase D (PLD) activity results in the production of diacylglycerol (DAG), which stimulates protein kinase C (PKC). Another consequence of PLD activation is the production of lysophosphatidic acid (LPA), a bioactive lysophospholipid that has recently been implicated in the regulation of bone and cartilage. These findings implicated LPA as a second messenger in Vit-D-directed signaling. In this study we aimed to study associations between lysophosphatidic acid(LPAR) expressions in pregnant women and their infants at delivery living in Yozgat region, Turkey. We studied 50 pregnant women within a prospective longitudinal study of maternal nutrition and lifestyle before and during pregnancy. Using high-resolution ultrasound, we measured fetal femur length along with head and neck circumference. Vitamin D, Vit D receptor, LPA and LPA receptors levels were measured in serum, placental fluid and tissues samples with ELISA. Pregnant women with serum vitamin D levels less than 5 ng/ml were classified as Vitamin D deficient group (n=14), the rest were controls (n=36). In placental tissue, the levels of Vit D, Vit D receptor, LPA and LPA receptors were all low in deficiency. In placental fluid, the levels of Vit D, Vit D receptor and LPA were all low in deficiency and in serum samples only LPAR levels were decreased in deficiency. In linear regression analysis serum and placental vitamin D and PLA levels were associated with femoral size. Correlation revealed the strength of LPA levels observed associations and their statistical significance in pregnant women. Our results demonstrated that levels of Vit D, LPA and their receptor were reduced in placental tissues in Vitamin D deficiency. Additionally, these reductions in placental tissues might also be associated with the rapid removal of the possible complexes formed by Lpa and autoantibodies by various cells. Further studies should be performed to investigate the role LPA acting in placenta during bone development.

P72: SELECTIVE SPHINGOSINE-1-PHOSPHATE RECEPTOR 5 AGONISTS CAN MODULATE LIPID CONTENT IN THE BRAIN AND THEREBY POTENTIALLY TREAT NEURODEGENERATIVE DISORDERS

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Objective: Sphingosine-1-phosphate (S1P) plays an important role as a regulator of signal transduction, cellular plasticity, cell proliferation, membrane stability/BBB integrity, and is proposed to modulate the ceramide-S1P and cholesterol homeostasis. S1P exerts these actions through G protein-coupled receptors, such as the brain-preferred S1P5 receptor. Given these actions, S1P5 agonism could potentially be a beneficial treatment for neurodegenerative disorders.

Methods: AbbVie has developed highly selective S1P5 receptor agonists, such as A-971432. A-971432 has an EC50 of 10 nM on the hS1P5 receptor with a large selectivity window. As the molecule shows excellent pharmacokinetic properties and good brain penentration, it was examined in models to test the hypothesis that selective S1P5 agonists will a) reduce CNS, but not peripheral lipid content, b) improve cognition, and c) change disease progression as assessed in a model mimicking key features of AD.

Results: Sub-acute treatment with A-971432 (≥7 days) in either T-maze (0.03 mg/kg – 3 mg/kg) or MWM/ORT (0.1 and 0.5 mg/kg) fully reversed the age-related cognitive deficits with a minimale effective dose of 0.1 mg/kg (40 ng/mL). Concomitantly, A-971432 normalizes the age-related CNS sphingolipid imbalance without affecting plasma levels. In a murine model of Niemann Pick C disease (NPC), A-971432 was able to improve the behavioral phenotype (dystonia, motor impairment), promote survival, normalize CNS sphingolipid content, and normalized Amyloid-β levels in the CSF.

Conclusion: These data indicate that S1P5 agonism provides an innovative mechanism for the potential treatment of neurodegenerative disorders such as AD and lysosomal storage disorders such as Niemann Pick C.

P73: CERANIB-2, A NOVEL CERAMIDASE INHIBITOR, INDUCES APOPTOSIS OF PROSTATE CANCER CELLS.

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Objective: Ceramide has been identified as an important second messenger that plays important roles in various aspects of inducing apoptosis. We questioned ceranib-2, a novel ceramidase inhibitor, affects the survival of prostate cancer cells (LnCaP and DU145) in vitro.

Methods: The cell viability was determined with MTT and apoptosis with flow cytometry. We examined structural changes both with confocal and transmission electron microscopy.

Results: Comparing to the control 0.1, 1, 5, 10, 25 and 50 μM ceranib-2 reduced the percentage of viable LNCaP cells to 84, 80, 64, 56, 40 and 15 after 24 hr and 81, 74, 60, 55, 27 and 11 % after 48 hr, respectively. Treatment of DU145 cells with the same six doses of ceranib-2 lowered numbers of living cell by 84, 82, 63, 50, 41 and 18 % after 24 hr; 64, 42, 30, 20, 8 and 5 % in 48 hr, respectively. Observed early apoptotic rate of LNCaP cells were 5 and 36 % after 24 hr and 15 and 60 % after 48 hr treatments with 25 and 50 μM ceranib-2, respectively. The signs of apoptosis were detected as fragmented nuclei, chromatin condensations and cytoskeleton laceration in the cells.

Conclusions: Ceranib-2 posseses a strong dose and time dependent survival decreasing effect on both prostate cancer cell lines.

P74: EVALUATION OF AN INHIBITOR OF HUMAN CERAMIDASES, CERANIB-2 INDUCED APOPTOSIS CYTOTOXICITY ON MCF7 CELLS

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Objective: The aim of the study was to investigate the potential cytotoxic effects of ceranib-2 on MCF7 cells and its effects on MCF7 cell structure.

Methods: Cytotoxic effects of ceranib-2 on MCF7 cells was detected via MTT test system. Dilutions prepared from the stock solution (in DMSO) of ceranib-2 was applied on MCF7 cells (1x104 cells/well) for 24 hours at 37 °C and 5% CO2 in air. The plates were read on ELISA reader (ELx808), at wavelength of 540 nm (n=3). For detecting the structural alterations IC50 concentration of ceranib-2was applied on MCF7 cells for 24 hours. Treated cells were stained with Alexa fluor-488 phalloidine and acridine orange and observed under confocal microscope.

Results: Viability percentages and IC50 (13µM) value were determined. Morphological alterations detected on our confocal micrographs were damaged cytoskeleton as hole formation, shrinked cells and fragmented and condensed nuclei as apoptotic hallmarks.

Conclusion: According to our results, ceranib-2 caused structural changes in MCF7 cells morphology. We can conclude that ceranib-2 showed high cytotoxicity on MCF7 cancer cells in low concentrations and may be encouraging in designing of pharmaceutical products helpful in cancer treatment.

P75: YEAST SPHINGOLIPID PROTEIN INTRACTION NETWORK: FUNCTION ANNOTATION FOR YDL222C

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Objective: Sphingolipids are both structural and regulatory components of the cell, where they control processes decisive in cell's fate. The first effort of constructing the protein-protein interaction network of sphingolipids in Saccharomyces cerevisiae enabled us to understand the details of the topological properties of the newly constructed network as well as to assign functions to some of the uncharacterized proteins involving in the network of sphingolipids. The topological analysis of sphingolipid related proteins, especially those under clinical trials for cancer therapy, yielded novel potential drug targets. Novel interactions are predicted using a newly developed integrated methodology employing sequence and structure based computational interaction prediction tools, orthology, expression profiles, co-localization information and Gene Ontology (GO) terms. The sphingolipid network shows topological properties of a scale-free, small-world, and modular structure, as it is the case for biological networks. The function annotation of uncharacterized proteins of the network is performed using a multi-dimensional hybrid method which combines the results from modules and neighbors, and examines them by information gathered from genetic interactions, expression profiles, and sequence similarity. The here constructed sphingolipid network coupled with the newly developed hybrid function annotation method constitutes an efficient platform for function annotation and drug target identification.

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