Glycosphingolipids, ceramides and fibrosis in lung, muscle, liver and kidney – therapeutic opportunities and links with neurodegeneration

<table>
<thead>
<tr>
<th>Michael Spedding</th>
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<tbody>
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<td>(Spedding Research Solutions SAS)</td>
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</table>

1. Fibrosis, ageing, entropy, neuromuscular function.
2. Glycocalyx, glycans, glycosphingolipids, ceramides.
4. ALS and metabolomics: critical rôle of ceramides/GC, GM1.
5. Critical rôle of ceramides in lung, muscle, liver and kidney fibrosis.
6. Exploitation of this complexity by viruses – new directions for COVID-19
7. Rationale for use of ambroxol in ALS and COVID-19?

| Lipid rafts characterized by the presence of glycosylphosphatidylinositol (GPI)-anchored proteins, glycosphingolipids, and proteins for signal transduction. The 5–25 nm clusters of sphingolipid, cholesterol, and protein that make up a lipid raft are tightly packed and rapidly assemble and disassemble. Saturated ceramides and cholesterol create lipid rafts. GPCRs may be present in lipid rafts. Caveolae are 50–100 nm “cup-shaped” invaginations of the membrane associated with endocytosis, cell signaling, and also the entry of pathogens into the cell. Caveolae resemble lipid rafts but have the proteins caveolin-1, cavin. Caveolae are most present in endothelial cells. |
Quantitative super-resolution microscopy of the mammalian glycocalyx

Leonhard Möckl1,6, Kayvon Pedram1,6, Anish R. Roy1, Venkatesh Krishnan2, Anna-Karin Gustavsson1,3, Oliver Dorigo2, Carolyn R. Bertozzi1,4, W. E. Moerner1,5,7

A

Glycocalyx

Periodate

Aniline, Aminooxy-Fluor

GalNAz

Cu-click with Alkyne-Fluor

Cu-click with Alkyne-Fluor

N-glycans (O-glycans)

Lipid-Azide

B

C

Sia

GalNAz

Lipid-Azide

AF647

Percent of max

- Speding Research Solutions -
Immense Diversity in the sialic acids
- Perhaps the most diverse class of compounds
- Immense capacity in recognition

Ajit Varki:

Diversity in the sialic acids. The nine-carbon backbone common to all known Sias is shown, in the α configuration. The following variations can occur at the carbon positions indicated:

R1 = H (on dissociation at physiological pH, gives the negative charge of Sia); can form lactones with hydroxyl groups on the same molecule or on other glycans; can form lactams with a free amino group at C-5; tauryl group.

R2 = H; alpha linkage to Gal(3/4/6), GaINAc(6), GlcNAc(4/6), Sia (8/9), or 5-O-Neu5Gc; oxygen linked to C-7 in 2,7-anhydro molecule; anomic hydroxyl eliminated in Neu2en5Ac (double bond to C-3).

R4 = H; -acetyl; anhydro to C-8; Fuc; Gal.

R5 = Amino; N-acetyl; N-glycolyl; hydroxyl; N-acetimidoyl; N-glycolyl-O-acetyl; N-glycolyl-O-methyl; N-glycolyl-O-2-Neu5Gc.

R7 = H; -acetyl; anhydro to C-2; substituted by amino and N-acetyl in Leg.

R8 = H; -acetyl; anhydro to C-4; -methyl; -sulfate; Sia; Glc.

R9 = -H; -acetyl; -lactyl; -phosphate; -sulfate; Sia; OH substituted by H in Leg.

Around 2 million years ago humans lost Neu5Gc expression and had to adjust their CD33-related Siglec binding specificity to accommodate Neu5Ac.
Less than 60 genes for the sialome show more than 10 uniquely human genetic changes in comparison with our closest evolutionary relatives.
# SIGLECS

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Siglecs and their roles in the immune system

Paul R. Crocker*, James C. Paulson* and Ajit Varki*

Abstract | Cell surfaces in the immune system are richly equipped with a complex mixture of glycans, which can be recognized by diverse glycan-binding proteins. The Siglecs are a family of sialic-acid-binding immunoglobulin-like lectins that are thought to promote cell–cell interactions and regulate the functions of cells in the innate and adaptive immune systems through glycan recognition. In this Review, we describe recent studies on signalling mechanisms and discuss the potential role of Siglecs in triggering endocytosis and in pathogen recognition. Finally, we discuss the postulated functions of the recently discovered CD33-related Siglecs and consider the factors that seem to be driving their rapid evolution.

Figure 6 | Signalling mediated by CD22 and the CD33-related Siglecs. On phosphorylation of cytoplasmic tyrosine-based signalling motifs by SRC-family tyrosine kinases, sialic-acid-binding immunoglobulin-like lectins (Siglecs) recruit and activate SRC homology 2 (SH2)-domain-containing proteins, notably the tyrosine phosphatases SHP1 (SH2-domain-containing protein tyrosine phosphatase 1) and SHP2 or the SOCS3 (suppressor of cytokine signalling 3) protein. This initiates a range of functional activities that are indicated for the Siglecs listed. In the case of Siglecs without cytosolic signalling motifs, charge-dependent transmembrane region interactions with DAP12 can provide ITAM (immunoreceptor tyrosine-based activation motif)-based signalling functions that are typically initiated by the recruitment and activation of spleen tyrosine kinase (SYK). ↑, increased; ↓, decreased; IFN-α, interferon-α.

Figure 4 | Cis and trans interactions of Siglecs. a | Most sialic-acid-binding immunoglobulin-like lectins (Siglecs) are masked at the cell surface owing to cis interactions with abundantly expressed sialic acids. Following exposure of cells to sialidase, which cleaves the cis-interacting Siglec ligands, or in some cases following cellular activation, Siglecs become unmasked, which allows them to make interactions with ligands in trans. b | Even when Siglecs are masked by cis interactions, trans interactions might occur during an encounter with another cell or a pathogen expressing higher affinity ligands that can out-compete the cis interactions.
Skeletal muscle represents ~40% of human body mass in men, ~30% in women.

Critical in human evolution.
Muscle is also a secretory organ.
The extracellular matrix is critical to force transduction and encasing muscle cells, with complex responses to training and ageing. Crucial to passive load bearing.
The ECM is a support during both sarcopenia and ALS.

Fibrosis is related to ageing,
- repair (e.g. damage*), and
- neuromuscular diseases.

* 117,000 kms run?
Skeletal Muscle Fibrosis

Rat Tibialis, B – challenged with 2 injections of Botox
Lieber & Ward, 2013

Also measure by collagen mass, hydroxyproline etc.

**Myofibroblasts**
Stimulated by TGFβ – SMAD3-P pathway

**Fibroadipogenic progenitors (FAPs)**

**Other Key players:**
Myostatin
Angiotensin II
Collagen triple helix repeat-containing 1 protein
Wnt signalling (incl. GSK3β, b-catenin)
ADAM12 (perivascular cells to myofibroblasts)
**Fibrotic Scar in Neurodegenerative Diseases**

*Nadia D’Ambrosi* and *Savina Apolloni*

---

### Mediators and ECM Component

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<th>Disease</th>
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<th>Mediators</th>
<th>ECM component</th>
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<td>IL-6, CXCL1, CXCL10, CXCL12, TNFa, TGFβ, NGF, INFγ, PDG2, ADAMTS-4, CTGF, S100A4, MMP-9</td>
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<td>PDGFRβ, TGFβ</td>
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**The inhibition of CTGF/CCN2 activity improves muscle and locomotor function in a murine ALS model**

David Gonzalez\(^1,2\), Daniela L. Rebolledo\(^1,2\), Lina M. Correa\(^1,2\), Felipe A. Court\(^3\), Waldo Cerpa\(^1,2\), Kenneth E. Lipson\(^4\), Brigitte van Zundert\(^1,5\) and Enrique Brandan\(^1,2\,*\)

---

**Pamrevlumab, FG-3019**

phase III: IPF; COVID-19, DMD, Spedding Research Solutions**
1. Metabolic evolution to triple VO2 max in ~1 Myears ~3M years ago AND prolong lifespan.
2. Evolution of brain size and circuits
3. Very recent evolution (100K years) to occupy all planetary niches (SNPs, epigenetics, bacteriome and virome) which « hides » #1.
4. Modern lifestyle and modern diseases.

**ALS Program**

- **Mitochondria & Lipid Metabolism (Khaitovic)**
- Servier lipidomics 3000 lipids
- Superoxide dismutase (SOD1G86R) Tg model
- Metabolomic & transcriptomic analysis Ceramide/glucosylceramide ratio critical
- Human patient tissue.

**New enzymatic target (GCase)**

**Other Screens**
- CHMP2B
- C9orf72
- TDP43

**Powerful phenotypical screens**

**Ambroxol New (Old) Drug**
- EMA Orphan Drug Designation
- Phase II

**Other Screens**
- CHMP2B
- C9orf72
- TDP43

**New enzymatic target (GCase)**

**Powerful phenotypical screens**

- Spedding Research Solutions
The ALS preclinical consortium, thanks!

University of Oxford
Mylene HÜBECKER
David PRIESTMAN
Frances PLATT

University of Strasbourg
Alexandra Bouscary
Althéa Moshbach
Cyril Quessada
J-Philippe LOEFFLER

Spedding Research Solutions
Michael SPEDDING
Alexandre HENRIQUES

University of Tours
Hélène BLASCO
Philippe CORCIA
Christian ANDRES

IGBMC
Céline Keime

Servier
Vincent Croixmarie

Florey Institute, Melbourne
Brad TURNER

University of Queensland
Shyuan NGO

Spedding ALS
Ambroxol

2.6M€ raised in grants over 6 years
>0.5M€ spent by SRS

- Spedding Research solutions -
Massive reduction of triglycerides in late stage SOD1 mice

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Pre – presymptomatic
Par - end stage just before paralysed

Late generalized decrease of triglycerides corroborates the hypolipidemic trait of mutant SOD1 mice
Glucosyl ceramide is reduced in spinal cord presymptomatic, - GSLs increased in muscle

**Sphingolipid metabolism is early altered in a tissue-specific manner in mutant SOD1 mice**
Overview of ceramide and sphingolipid changes in all studies

Highly reproducible 50% reduction in all studies P<0.0000001

Direct enzyme study GCS no change in spinal cord but 5 fold upregulation in gastrocnemius

Elevated ceramides are a key player in cell death Ceramides downregulate nutrient transporters to kill cells. The link between nutrients, autophagy and cell death lag genes in yeast

Inhibition of β-Glucocerebrosidase Activity Preserves Motor Unit Integrity in a Mouse Model of Amyotrophic Lateral Sclerosis

Alexandre Henriques, Vincent Croixmarie, David A. Priestman, Angela Rosenholz, Sylvie Döring-Grosch, Elena D’Ambra, Mylène Huebecker, Giulia Hussain, Claire Bourrier-Neyret, Andoni Echazu-Laguna, Albert C. Ludolph, Frances M. Platt, Bernard Walther, Michael Spedding, Jean-Philippe Loeffler and José-Luis Gonzalez De Aguilar

Nearly all triglycerides lost in plasma, muscle, spinal cord at end stage!

Henriques et al. 2015, 2017, 2018

Fingolimod, weakly protective, acts as S1P agonist

Increased 77d, trend 70d
Meta-analysis identified GCS/GCase as relevant target for ALS

D’Angelo et al. 2013
GM1 Ganglioside Is A Key Factor in Maintaining the Mammalian Neuronal Functions Avoiding Neurodegeneration

Elena Chiricozzi, Giulia Lunghi, Erika Di Biase, Maria Fazzari, Sandro Sonnino and Laura Mauri

GM1 can be labelled with cholera toxin

One of the binding sites for influenza virus.
GM1 antibodies cause multifocal motor neuropathy (MMN)
There are two enzymes Glucocerebrosidase: GBA1 (lysosomal) and GBA2, non-lysosomal.
Loss of gangliosides on neuromuscular junctions of SOD1 mice

Proportion of NMJs with presynaptic gangliosides

ONLY GBA2 Increased in spinal cord

- Spedding Research Solutions -

Henriques et al. 2017 Scientific reports
Comparison of concentration-response of ambroxol on inhibition of GBA2 and increasing innervation of muscle cells by spinal explants in culture plates

(A) Number of functional explants per culture plate identified by the presence of contraction of muscle fibres (n=5/group).
(B) Total area of innervation of functional explants after differentiation (n=5/group).

Ambroxol inhibits non-lysosomal GBA2 And is a chaperone for GBA1, While not inhibiting GCS (Glucosylceramide Synthase). Increases autophagy, TFEB metabolism With an inverse relationship with α-synuclein.
Ambroxol hydrochloride delays disease onset in SOD1 mice

Ambroxol - Presymptomatic study (120mg/kg/d)
Preserved neuromuscular junction integrity
Delays disease onset (and delays decline after disease onset, grip strength)

In vivo – neuromuscular junctions

In vivo – Disease onset

Bouscary et al. 2000
Fibrosis
The distribution of ceramide versus sphingomyelin and sphingosine contributes to the delicate balance of anti-inflammatory function versus antimicrobial efficiency in the host response to infection.

Tracey Bonfield 2020

Myriocin, inhibitor of serine palmitoyl transferase (SPT) inhibits ceramide synthesis, used to show:
- upregulated ceramide synthesis in the alveoli is strictly related to alveolar infection and inflammation,
- alveolar ceramide (C16) can be specifically targeted by nanocarrier delivery of the ceramide synthesis inhibitor myriocin (Myr) and
- Myr is able to downmodulate pro-inflammation lyso-PC, favouring an increase in anti-inflammatory PCs.

Myr modulates alveolar lipids milieu, reducing hyperinflammation and favouring anti-microbial effective response in CF mouse model.
Acid ceramidase rescues cystic fibrosis mice from pulmonary infections
Katrin Anne Becker¹, Rabea Verhaegh¹, Hedda-Luise Verhasselt², Simone Keitsch¹, Matthias Soddemann¹, Barbara Wilker¹, Gregory C. Wilson³, Jan Buer², Syed A. Ahmad⁵, Michael J. Edwards⁵, Erich Gulbins¹,³

Previous studies have shown that sphingosine kills a variety of pathogenic bacteria, including Pseudomonas aeruginosa (P. aeruginosa) and Staphylococcus aureus. Sphingosine concentrations are decreased in airway epithelial cells of cystic fibrosis (CF) mice and this defect has been linked to the infection susceptibility of these mice. Here, we tested whether genetic overexpression of the acid ceramidase rescues cystic fibrosis mice from pulmonary infections with P. aeruginosa. We demonstrate that transgenic overexpression of the acid ceramidase in CF mice corresponds to an overexpression of the acid ceramidase in bronchial and tracheal epithelial cells and normalizes ceramide and sphingosine levels in bronchial and tracheal epithelial cells. In addition, expression of β1-integrin, which is ectopically expressed on the luminal surface of airway epithelial cells in cystic fibrosis mice - an alteration that is very important for mediating pulmonary P. aeruginosa infections of cystic fibrosis, is normalized in cystic fibrosis airways upon overexpression of acid ceramidase. Most importantly, overexpression of acid ceramidase protects cystic fibrosis mice from pulmonary P. aeruginosa infections. Infection of CF mice or CF mice that were inhaled with sphingosine with P. aeruginosa or a P. aeruginosa mutant that is resistant to sphingosine indicate that sphingosine and not a metabolite kills P. aeruginosa upon pulmonary infection. These studies further support the use of acid ceramidase and its metabolite sphingosine as a potential treatment of cystic fibrosis.

Acid Sphingomyelinase Deficiency Attenuates Bleomycin-Induced Lung Inflammation and Fibrosis in Mice
Rajwinder Dhami, Xingxuan He and Edward H. Schuchman

An intriguing feature of the bleomycin response in ASM− mice was the virtual lack of myofibroblasts in the pulmonary interstitium of these animals. Myofibroblasts are defined as SMA-expressing cells that are capable of collagen secretion. The presence of these activated cells at sites of tissue repair and subsequent tissue damage is believed to be imperative in fibrosis, and was abundantly seen in areas of excessive collagen deposition in wildtype mice treated by bleomycin (Fig. 5A). The origin of these cells is unknown, but one possible mechanism is the activation of fibroblasts by TGFbeta-mediated differentiation or via some other cellular mediators [34]. There is growing evidence showing that ceramide and S1P play important roles in cellular differentiation via TGFbeta mediation [39], and the specific role of this protein in the bleomycin-response of ASM− mice also requires further investigation. Of note, we have previously shown that TGFbeta levels are not elevated in these mice, despite marked inflammation [18].
Crosstalk Between Acid Sphingomyelinase and Inflammasome Signaling and Their Emerging Roles in Tissue Injury and Fibrosis

Cao Li¹, Shanshan Guo¹, Wenyuan Pang²,³ and Zhigang Zhao¹⁺

Inhibition of Sphingolipid Synthesis as a Phenotype-Modifying Therapy in Cystic Fibrosis

Alessandra Mingione² Michele Dei Cas² Fabiola Bonezzi² Anna Caretti² Marco Piccoli² Luigi Anastasia² Riccardo Ghidoni² Rita Paroni² Paola Signorelli²

FIGURE 1 | Acid sphingomyelinase/ceramide system in inflammasome signaling and tissue fibrosis. Upon PAMPs or DAMPs exposure, ASM is activated and ceramide enriched domains are formed. Ceramide enriched domains may either directly interact with the ASC-NLRP3-caspase-1 inflammasome complex, or, ASM-ceramide system may induce NADPH oxidase or mitochondrial ROS production or cathepsins released after lysosome rupture, leading to inflammasome activation. The subsequent cytokine production and TGF-β1 activation may contribute to cell apoptosis, tissue injury and fibrosis.
Sphingosine is a crucial apical membrane antimicrobial
Cell stress induces the membrane-based sphingomyelin pathway, whereas p53-dependent apoptosis occurs secondary to DNA damage.
GM1 as Adjuvant of Innovative Therapies for Cystic Fibrosis Disease

Giulia Mancini 1, Nicoletta Loberto 1, Debora Olioso 2, Maria Cristina Dechecchi 2, Giulio Cabrini 2, Laura Mauri 1, Rosaria Bassi 1, Domitilla Schiumarini 1, Elena Chiricozzi 1, Giuseppe Lippi 2,3, Emanuela Pesce 4, Sandro Sonnino 1, Nicoletta Pedemonte 4, Anna Tamanini 3,* and Massimo Aureli 1,*
Autophagy augmentation alleviates cigarette smoke-induced CFTR-dysfunction, ceramide-accumulation and COPD-empysema pathogenesis

Manish Boda\(^a\), Garrett Pehote\(^a\), David Silverberg\(^b\), Erich Gulbins\(^b\), Neeraj Viji\(^{c, d, g}\)

In this study, we aimed to investigate precise mechanism(s) of sphingolipid-imbalance and resulting ceramide-accumulation in COPD-empysema. Where, human and murine emphysema lung tissues or human bronchial epithelial cells (Beas2B) were used for experimental analysis. We found that lungs of smokers and COPD-subjects with increasing emphysema severity demonstrate sphingolipid-imbalance, resulting in significant ceramide-accumulation and increased ceramide/sphingosine ratio, as compared to non-empysema/non-smoker controls. Next, we found a substantial increase in emphysema chronicity-related ceramide-accumulation in murine (C57BL/6) lungs, while sphingosine levels only slightly increased. In accordance, the expression of the acid ceramidase decreased after CS-exposure. Moreover, CS-induced (sub-chronic) ceramide-accumulation was significantly (p < 0.05) reduced by treatment with TFEB/autophagy-inducing drug, gemfibrozil (GEM), suggesting that autophagy regulates CS-induced ceramide-accumulation. Next, we validated experimentally that autophagy/lysophagy-induction using an anti-oxidant, cysteamine, significantly (p < 0.05) reduces CS-extract (CSE)-mediated intracellular-ceramide-accumulation in p62 + aggresome-bodies. In addition to intracellular-accumulation, we found that CSE also induces membrane-ceramide-accumulation by ROS-dependent acid-sphingomyelinase (ASM) activation and plasma-membrane translocation, which was significantly controlled (p < 0.05) by cysteamine (an anti-oxidant) and amitriptyline (AMT, an inhibitor of ASM). Cysteamine-mediated and CSE-induced membrane-ceramide regulation was nullified by CFTR-inhibitor-172, demonstrating that CFTR controls reduced impaired autophagy dependent membrane-ceramide accumulation. In summary, our data shows that CSE-mediated autophagy/lysophagy-dysfunction results in intracellular-ceramide-accumulation, while acquired CFTR-dysfunction-induced ASM causes membrane-ceramide accumulation. Thus, CS-exposure alters the sphingolipid-rheostat leading to the increased membrane- and intracellular-ceramide-accumulation inducing COPD-empysema pathogenesis that is alleviated by treatment with cysteamine, a potent anti-oxidant with CFTR/autophagy-augmenting properties.
“When energy need exceeds the storage capacity in the liver, fatty acids are shunted into nonoxidative sphingolipid biosynthesis, which increases the level of cellular ceramides. Accumulation of ceramides alters substrate utilization from glucose to lipids, activates triglyceride storage, and results in the development of both insulin resistance and hepatosteatosis, increasing the likelihood of major metabolic diseases.”
Sphingolipid signaling in renal fibrosis

Andrea Huwiler\(^a\) and Josef Pfeilschifter\(^b\)

Table 1 Different roles and sites of action of ceramide.

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Role and site of action</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Neisseria gonorrhoeae</em></td>
<td>Formation of membrane platforms, internalization of pathogen</td>
</tr>
<tr>
<td><em>Neisseria meningitidis</em></td>
<td>Formation of membrane platforms, internalization of pathogen</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>Formation of membrane platforms, internalization of pathogen, clustering of CD95, Cfr and NADPH-oxidases, induction of cell death, control of cytokine release, oxidative burst</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>Induction of cell death</td>
</tr>
<tr>
<td><em>Listeria monocytogenes</em></td>
<td>Fusion of phagosomes with lysosomes, intracellular killing, systemic resistance</td>
</tr>
<tr>
<td><em>Salmonella typhimurium</em></td>
<td>Systemic resistance</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>Formation of membrane platforms, clustering of CD14, induction of cell death</td>
</tr>
<tr>
<td>Pathogenic mycobacteria</td>
<td>Actin nucleation, systemic resistance</td>
</tr>
</tbody>
</table>

Non-alcoholic fatty liver disease: Insights from sphingolipidomics

David J. Montefusco\(^a,1\), Jeremy C. Allegood\(^a,1\), Sarah Spiegel\(^a\), L. Ashley Cowart\(^a,2,3\)
Pharmacological inhibition of ceramide biosynthesis in obese mice, using myriocin (a selective inhibitor of SPT, the first rate-limiting enzyme in the ceramide synthetic pathway), induced profound changes in the adipose tissue, reducing steatosis.

Ceramides impair mitochondrial function and respiratory capacity by inhibiting oxidative phosphorylation and promoting mitochondrial fragmentation in numerous cell-types including adipocytes.

C16-ceramides species were highly enriched in adipose tissue, which was supported by the finding of CerS6, the enzyme essential for synthesizing C16-ceramides species, was elevated in various rodent models of obesity.

**CERS6 expression is dramatically increased in obese individuals (Bruning et al)**
Inhibiting Ceramide Synthesis Attenuates Hepatic Steatosis and Fibrosis in Rats With Non-alcoholic Fatty Liver Disease

Meng Jiang, Chun Li, Qiaoshu Liu, Aimin Wang and Minxiang Lei

Department of Endocrinology, Xiangya Hospital of Central South University, Changsha, China

Non-alcoholic fatty liver disease (NAFLD) is one of the most common metabolic disorder diseases, which include a histological spectrum of conditions ranging from simple steatosis to non-alcoholic steatohepatitis (NASH). Dysregulated metabolism of sphingomyelin in the liver plays a critical role in the pathogenesis of NAFLD. Ceramides are central molecules of sphingolipid biosynthesis and catabolism and play an important role in insulin resistance, apoptosis, and inflammation. In addition, apoptosis is a main contributor to the development of NAFLD. This study detected whether the inhibition of ceramide synthesis ameliorates hepatic steatosis and fibrosis in rats with NAFLD. Sprague-Dawley rats were used to establish the NAFLD model. Here, we showed that hepatic ceramide, steatosis, and fibrosis increased in liver tissue from rats with NAFLD. Chronic treatment with myriocin inhibited ceramide and lipid accumulation and improved fibrosis in liver tissue samples of high fat diet (HFD)-fed rats. In addition, hepatic inflammation and apoptosis were markedly ameliorated in HFD-fed rats treated with myriocin. Furthermore, myriocin treatment regulated the expression of pro-apoptosis and anti-apoptosis proteins by inactivating the c-Jun N-terminal kinase (JNK) signaling pathway in the liver of HFD-fed rats. Collectively, ceramide plays an important role in the pathogenesis of NASH and may represent a potential therapeutic strategy to prevent NAFLD.

Lipidomic biomarkers and mechanisms of lipotoxicity in non-alcoholic fatty liver disease

Gianluca Svegliati-Baroni, Irene Pierantonelli, Pierangelo Torquato, Rita Marinelli, Carla Ferreri, Chrysostomos Chatgilialoglu, Desiree Bartolini, Francesco Galli

FIBROSIS

Targeting acid ceramidase inhibits YAP/TAZ signaling to reduce fibrosis in mice


Hepatic stellate cells (HSCs) drive hepatic fibrosis. Therapies that inactivate HSCs have clinical potential as antifibrotic agents. We previously identified acid ceramidase (aCDase) as an antifibrotic target. We showed that tricyclic antidepressants (TCAs) reduce hepatic fibrosis by inhibiting aCDase and increasing the bioactive sphingolipid ceramide. We now demonstrate that targeting aCDase inhibits YAP/TAZ activity by potentiating its phosphorylation-mediated proteasomal degradation via the ubiquitin ligase adaptor protein β-TrCP. In mouse models of fibrosis, pharmacologic inhibition of aCDase or genetic knockout of aCDase in HSCs reduces fibrosis, stromal stiffness, and YAP/TAZ activity. In patients with advanced fibrosis, aCDase expression in HSCs is increased. Consistently, a signature of the genes most down-regulated by ceramide identifies patients with advanced fibrosis who could benefit from aCDase targeting. The findings implicate ceramide as a critical regulator of YAP/TAZ signaling and HSC activation and highlight aCDase as a therapeutic target for the treatment of fibrosis.
Viruses and sphingolipids
How an ancient microbial arms race remodeled human cells

**Battle at the cell surface**

Some pathogens use sialic acids, which sit on the outer edge of the cell membrane, to invade a cell. Pathogens sometimes coat themselves in humanlike sialic acids to trick signaling molecules called sialic-acid-binding immunoglobulin-type lectins (Siglec) into inhibiting immune responses. But other Siglec can instead turn on an innate immune response if they sense sialic acids on pathogens.

**VIEWPOINT: COVID-19**

How does SARS-CoV-2 cause COVID-19?

The viral receptor on human cells plays a critical role in disease progression

**Key phases of disease progression**

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) binds to angiotensin-converting enzyme 2 (ACE2). Initial infection of cells in the upper respiratory tract may be asymptomatic, but these patients can still transmit the virus. For those who develop symptoms, up to 90% will have pneumonia, caused by infection of cells in the lower respiratory tract. Some of these patients will progress to severe disease, with disruption of the epithelial-endothelial barrier, and multi-organ involvement.
25 patients recovered from SARS-CoV-1 had very poor health, only partially recovered lung function, and major changes in serum metabolomics due to changes in lipid metabolism: Lipid metabolomics are critical markers.
Dynamic remodeling of lipids coincides with dengue virus replication in the midgut of Aedes aegypti mosquitoes

Nunya Chotiwan1, Barbara G. Andre3, Irma Sanchez-Vargas1, M. Nurul Islam1, Jeffrey M. Grabowski2,3,6, Amber Hopf-Jannasch6, Erik Gough5, Ernesto Nakayasu4,6, Carol D. Blair1, John T. Bellisle1, Catherine A. Hill3,6, Richard J. Kuhn5,6, Rushika Perera1*
Review

Jürgen Schneider-Schaulies* and Sibyle Schneider-Schaulies

Sphingolipids in viral infection

- Spedding Research Solutions -
Time-dependent ‘chaos’ in the endoplasmic reticulum/Golgi after infection

SARS-CoV-1 – ER budding

Reconstruction of Viral particles budding from the ER
- Budding from lipid rafts.
- ‘the outer influenza virus leaflet should consist almost entirely of sphingolipids and cholesterol’
- Enrichment of sphingolipids (Lac-Cer) – from GM3.
- The presence of neuraminidase on envelope (to ‘deforest’ host cell glycocalyx) will also affect the envelope GSLs (eg GM3).
β-Coronaviruses Use Lysosomes for Egress Instead of the Biosynthetic Secretory Pathway

Graphical Abstract

β-coronavirus

Egress via lysosomal trafficking

Disrupted lysosomal functions

Normal biosynthetic pathway

Lysosome (acidified)

Lysosome (deacidified)

ER

Golgi/TGN

Highlights

- β-Coronaviruses do not use the biosynthetic secretory pathway to egress
- β-Coronaviruses traffic to lysosomes and egress by Arl8b-dependent lysosomal exocytosis
- Lysosomes are deacidified, and proteolytic enzymes are inactive in infected cells
- Antigen processing and presentation are perturbed in β-coronavirus infection

Authors

Sourish Ghosh, Teegan A. Delibovi-Ragheb, Adeline Kerriel, ..., John Kehrl, Grégoire Altan-Bonnet, Nihal Altan-Bonnet

Correspondence

gregoire.altan-bonnet@nih.gov (G.A.-B.),
nihal.altan-bonnet@nih.gov (N.A.-B.)

In Brief

Ghosh et al. provide evidence that β-coronaviruses do not use the biosynthetic secretory pathway typically used by enveloped viruses to leave infected cells. Instead, these viruses traffic to lysosomes for unconventional egress by Arl8b-dependent lysosomal exocytosis. Their non-lytic release results in lysosome deacidification, inactivation of lysosomal degradation enzymes, and disruption of antigen presentation.
Glucosylceramide synthase maintains influenza virus entry and infection

Kelly Drews¹, Michael P. Calgi², William Casey Harrison²ª³, Camille M. Drews¹, Pedro Costa-Pinheiro¹, Jeremy Joseph Porter Shaw¹, Kendra A. Jobe³ª⁴, John D. Han⁴, Todd E. Fox⁵, Judith M. White⁶,⁷, Mark Kester¹,²,⁵*

Influenza virus is an enveloped virus wrapped in a lipid bilayer derived from the host cell plasma membrane. Infection by influenza virus is dependent on these host cell lipids, which include sphingolipids. Here we examined the role of the sphingolipid, glucosylceramide, in influenza virus infection by knocking out the enzyme responsible for its synthesis, glucosylceramide synthase (UGCG). We observed diminished influenza virus infection in HEK 293 and A549 UGCG knockout cells and demonstrated that this is attributed to impaired viral entry. We also observed that entry mediated by the glycoproteins of other enveloped viruses that enter cells by endocytosis is also impaired in UGCG knockout cells, suggesting a broader role for UGCG in viral entry by endocytosis.

A

HEK 293 cells

B

A549 cells

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Glucosylceramidase Maintains Influenza Virus Infection by Regulating Endocytosis

Kelly Drews, Michael P. Calgi, William Casey Harrison, Camille M. Drews, Pedro Costa-Pinheiro, Jeremy Joseph Porter Shaw, Kendra A. Jobe, Elizabeth A. Nelson, John D. Han, Todd Fox, Judith M. White, Mark Kester

ABSTRACT  Influenza virus is an RNA virus encapsulated in a lipid bilayer derived from the host cell plasma membrane. Previous studies showed that influenza virus infection depends on cellular lipids, including the sphingolipids sphingomyelin and sphingosine. Here we examined the role of a third sphingolipid, glucosylceramide, in influenza virus infection following clustered regularly interspaced short palindromic repeats with Cas9 (CRISPR-Cas9)-mediated knockout (KO) of its metabolizing enzyme glucosylceramidase (GBA). After confirming GBA knockout of HEK 293 and A549 cells by both Western blotting and lipid mass spectrometry, we observed diminished infection in both KO cell lines by a PR8 (H1N1) green fluorescent protein (GFP) reporter virus. We further showed that the reduction in infection correlated with impaired influenza virus trafficking to late endosomes and hence with fusion and entry. To examine whether GBA is required for other enveloped viruses, we compared the results seen with entry mediated by the glycoproteins of Ebola virus, influenza virus, vesicular stomatitis virus (VSV), and measles virus in GBA knockout cells. Entry inhibition was relatively robust for Ebola virus and influenza virus, modest for VSV, and mild for measles virus, suggesting a greater role for viruses that enter cells by fusing with late endosomes. As the virus studies suggested a general role for GBA along the endocytic pathway, we tested that hypothesis and found that trafficking of epidermal growth factor (EGF) to late endosomes and degradation of its receptor were impaired in GBA knockout cells. Collectively, our findings suggest that GBA is critically important for endocytic trafficking of viruses as well as of cellular cargos, including growth factor receptors. Modulation of glucosylceramide levels may therefore represent a novel accompaniment to strategies to antagonize “late-penetrating” viruses, including influenza virus.

<table>
<thead>
<tr>
<th>A</th>
<th>HEK 293 cells (pmol/mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucosylceramide</td>
<td>WT</td>
</tr>
<tr>
<td>GBA KO</td>
<td>430.00</td>
</tr>
<tr>
<td>DihydrospHINGosine</td>
<td>0.70</td>
</tr>
<tr>
<td>DihydrospHINGosine-1-Phosphate</td>
<td>5.15</td>
</tr>
<tr>
<td>Ceramide</td>
<td>70.07</td>
</tr>
<tr>
<td>Sphingosine</td>
<td>113.22</td>
</tr>
<tr>
<td>Sphingomyelin</td>
<td>13011.30</td>
</tr>
<tr>
<td>Sphingosine-1-Phosphate</td>
<td>1.84</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>B</th>
<th>A549 cells (pmol/mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucosylceramide</td>
<td>WT</td>
</tr>
<tr>
<td>GBA KO</td>
<td>71.33</td>
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<td>DihydrospHINGosine</td>
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<td>12.19</td>
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<tr>
<td>Ceramide</td>
<td>87.46</td>
</tr>
<tr>
<td>Sphingosine</td>
<td>86.26</td>
</tr>
<tr>
<td>Sphingomyelin</td>
<td>8776.50</td>
</tr>
<tr>
<td>Sphingosine-1-Phosphate</td>
<td>0.51</td>
</tr>
</tbody>
</table>

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Ambroxol binds with high energy to TMPRSS2 protease in docking studies thus should reduce SARS-CoV-2 entry into cells

Identification of potential anti-TMPRSS2 natural products through homology modelling, virtual screening and molecular dynamics simulation studies

Rupesh V. Chikhale, Vivek K. Gupta, Gaber E. Eldesoky, Saikh M. Wabaidur, Shripad A. Patil and Md Ataul Islam

Table 1. Molecular docking score and Glide score obtained from docking studies. Prime-MM-GBSA and binding-free energy components for the protein–ligand complexes calculated by MM-GBSA and MM-PBSA analysis for the selected compounds (all energies are in Kcal/mol with standard deviation in parenthesis).

<table>
<thead>
<tr>
<th>Molecule name</th>
<th>Virtual screening workflow (VSW)</th>
<th>Molecular dynamics simulations (MDS)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GScore</td>
<td>DockScore</td>
</tr>
<tr>
<td>Natural products</td>
<td></td>
<td></td>
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<tr>
<td>Neohesperidin</td>
<td>−12.77</td>
<td>−12.77</td>
</tr>
<tr>
<td>Myricitrin</td>
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<tr>
<td>Quercitrin</td>
<td>−10.78</td>
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<td>Naringin</td>
<td>−10.73</td>
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<tr>
<td>Icacin</td>
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</tr>
<tr>
<td>Standard drugs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Camostat</td>
<td>−6.23</td>
<td>−4.62</td>
</tr>
<tr>
<td>Ambroxol</td>
<td>−7.21</td>
<td>−3.61</td>
</tr>
</tbody>
</table>

ΔGlocal—binding-free energy.
Ambroxol as a treatment for COVID-19?
As well as ALS and PD?

Inhibition of non-lysosomal GBA2; chaperone of lysosomal GBA1; increases GlcCer, GM1, GM3. Increases TFEB activation, Inhibits NaV1.7, 1.8.

Orphan Drug Designation from EMA for ALS.
Ambroxol is already registered as therapy as a safe mucolytic in 77 countries and may modulate:

1. Viral multiplication
2. Viral clearance from the lungs
3. Access of SARS-CoV-2 to ACE2/TMPRSS2 and lipid rafts, internalisation
4. Protection of the lungs
5. Protection from muscle loss, and recovery from intensive care.
Ambroxol for the treatment of fibromyalgia: science or fiction? Kern et al. 2017
Ambroxol inhibits interleukin 1 and tumor necrosis factor production in human mononuclear cells. Bianchi et al. 1990
Ambroxol suppresses influenza-virus proliferation in the mouse airway by increasing antiviral factor levels. Yang et al. 2002
Depressant effects of ambroxol and erdosteine on cytokine synthesis, granule enzyme release, and free radical production in rat alveolar macrophages activated by lipopolysaccharide. Jang et al. 2003
Depressant effects of ambroxol on lipopolysaccharide- or fMLP-stimulated free radical production and granule enzyme release by alveolar macrophages. Lee et al. 1999
Depressant effect of ambroxol on stimulated functional responses and cell death in rat alveolar macrophages exposed to silica in vitro. Kim et al. 2002
Inhibition of bleomycin-induced cell death in rat alveolar macrophages and human lung epithelial cells by ambroxol. Hong et al. 2003
Inhibition of inflammatory responses by ambroxol, a mucolytic agent, in a murine model of acute lung injury induced by lipopolysaccharide. Su et al. 2004
Effects of ambroxol combined with low-dose heparin on TNFα and IL-1β in rabbits with acute lung injury. Wang et al. 2011 (Chinese)
The experiment and clinical study of ambroxol against the airway inflammation of chronic hypoxic rat and patients with COPD. Jin and Zhang. 2002
The protective effects of ambroxol on radiation lung injury and influence on production of transforming growth factor β, and tumor necrosis factor α. Xia et al. 2010
Ambroxol suppresses influenza-virus proliferation in the mouse airway by increasing antiviral factor levels

B. Yang*, D.F. Yao*, M. Ohuchi†, M. Ide*, M. Yano*, Y. Okumura*, H. Kido*

ABSTRACT: The protective effect of ambroxol, a mucolytic agent which has antioxidant properties and stimulates the release of pulmonary surfactant, against influenza-virus proliferation in the airway was investigated in mice.

Ambroxol or the vehicle was administered intraperitoneally twice a day for 5–7 days to mice shortly after intranasal infection with a lethal dose of influenza A/Aichi/68 (H3N2) virus, and the survival rate, virus titre and levels of factors regulating virus proliferation in the airway fluid were analysed.

Ambroxol significantly suppressed virus multiplication and improved the survival rate of mice. The effect of ambroxol reached a peak at 10 mg·kg⁻¹·day⁻¹, higher doses being less effective. Ambroxol stimulated the release of suppressors of influenza-virus multiplication, such as pulmonary surfactant, mucus protease inhibitor, immunoglobulin (Ig)-A and IgG, although it stimulated the release of a trypsin-type protease that potentiates virus proliferation. In addition, ambroxol transiently suppressed release of the cytokines, tumour necrosis factor-α, interferon-γ and interleukin-12, into airway fluid.

Although ambroxol had several negative effects on the host defence system, overall it strikingly increased the concentrations of suppressors of influenza-virus multiplication in the airway.

Effect of bromhexine on clinical outcomes and mortality in COVID-19 patients: A randomized clinical trial

Khalil Ansarin1,2, Ramin Tolouian1, Mohammadreza Ardalan1,3, Ali Taghizadieh2,4, Mojtaba Varshochi5, Soheil Teimouri1, Tahere Vaezi1, Hamed Valizadeh1,2, Parviz Saleh1, Saeid Safiri5,6, Kenneth R. Chapman1

Abstract

Introduction: Bromhexine is a potential therapeutic option in COVID-19, but no data from a randomized clinical trial has been available. The present study aimed to evaluate the efficacy of bromhexine in intensive care unit (ICU) admission, mechanical ventilation, and mortality in patients with COVID-19.

Methods: An open-label randomized clinical trial study was performed in Tabriz, North-West of Iran. They were randomized to either the treatment with the bromhexine group or the control group, in a 1:1 ratio with 39 patients in each arm. Standard therapy was used in both groups and those patients in the treatment group received oral bromhexine 8 mg three times a day additionally. The primary outcome was a decrease in the rate of ICU admissions, intubation/mechanical ventilation, and mortality.

Results: A total of 78 patients with similar demographic and disease characteristics were enrolled. There was a significant reduction in ICU admissions (2 out of 39 vs. 11 out of 39, P=0.006), intubation (1 out of 39 vs. 9 out of 39, P=0.007) and death (0 vs. 5, P=0.027) in the bromhexine treated group compared to the standard group. No patients were withdrawn from the study because of adverse effects.

Conclusion: The early administration of oral bromhexine reduces the ICU transfer, intubation, and the mortality rate in patients with COVID-19. This affordable medication can easily be administered everywhere with a huge positive impact(s) on public health and the world economy. Altogether, the verification of our results on a larger scale and different medical centers is strongly recommended.

Trial Registration: IRCT202003117046797N4; https://irct.ir/trial/46969

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Investigator-driven trials in ALS and COVID-19