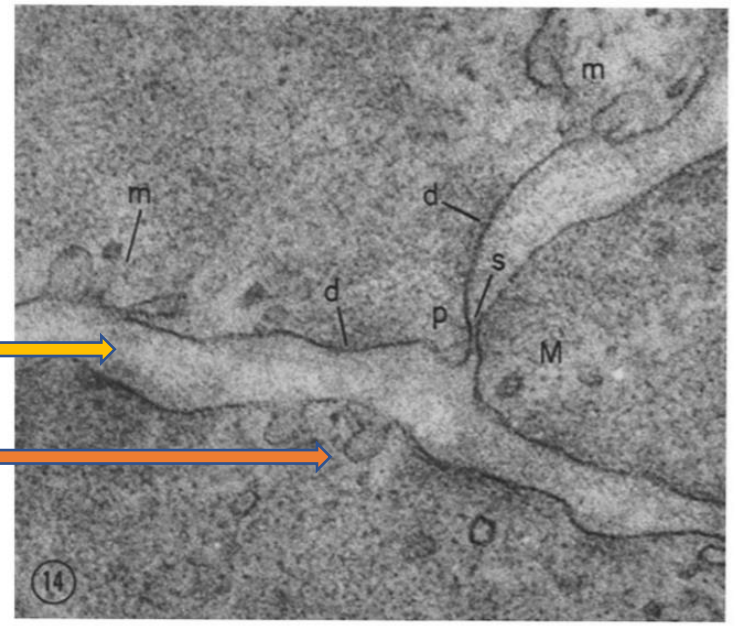
Glycosphingolipids, ceramides and fibrosis in lung, muscle, liver and kidney – therapeutic opportunities and links with neurodegeneration

Michael Spedding

(Spedding Research Solutions SAS)

1. Fibrosis, ageing, entropy, neuromuscular function.
2. Glycocalyx, glycans, glycosphingolipids, ceramides.
3. Sialic acids and human evolution, Sugar and lipid complexity.
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?



Glycocalyx

Caveolae

A STUDY OF THE INNERVATION OF THE TAENIA COLI

J Cell Biol 1967 33, 573

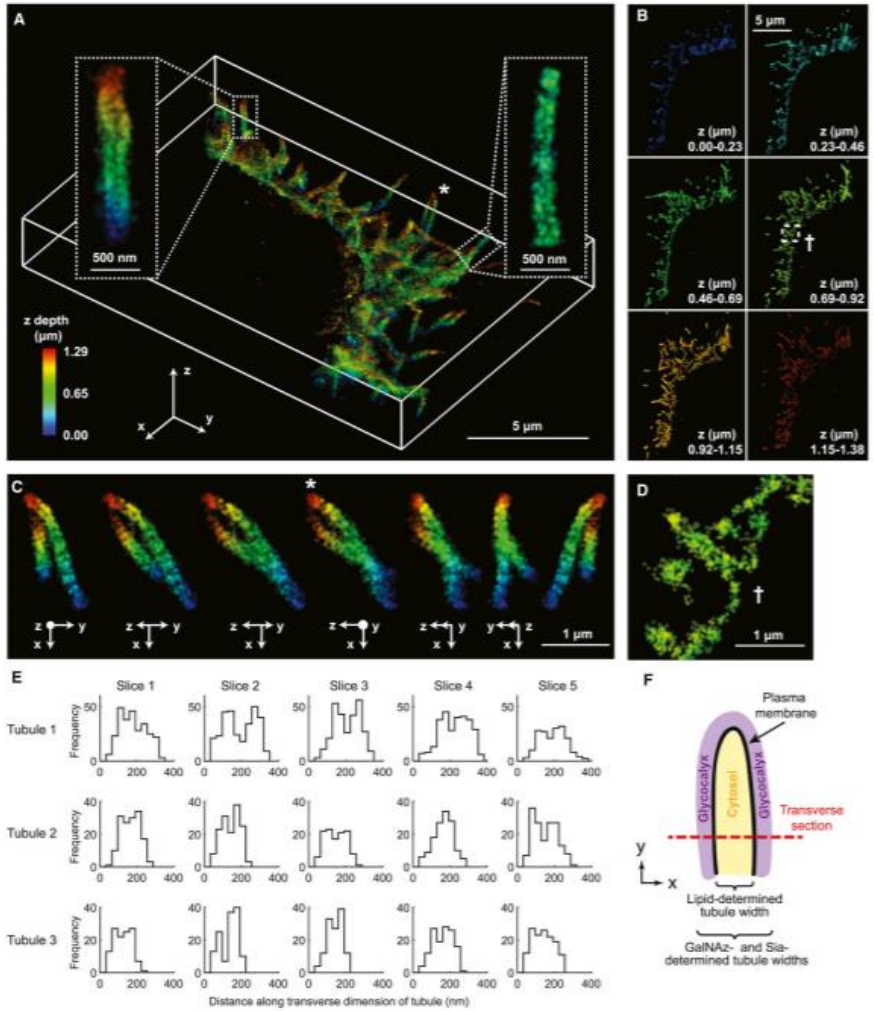
M. R. BENNETT and D. C. ROGERS

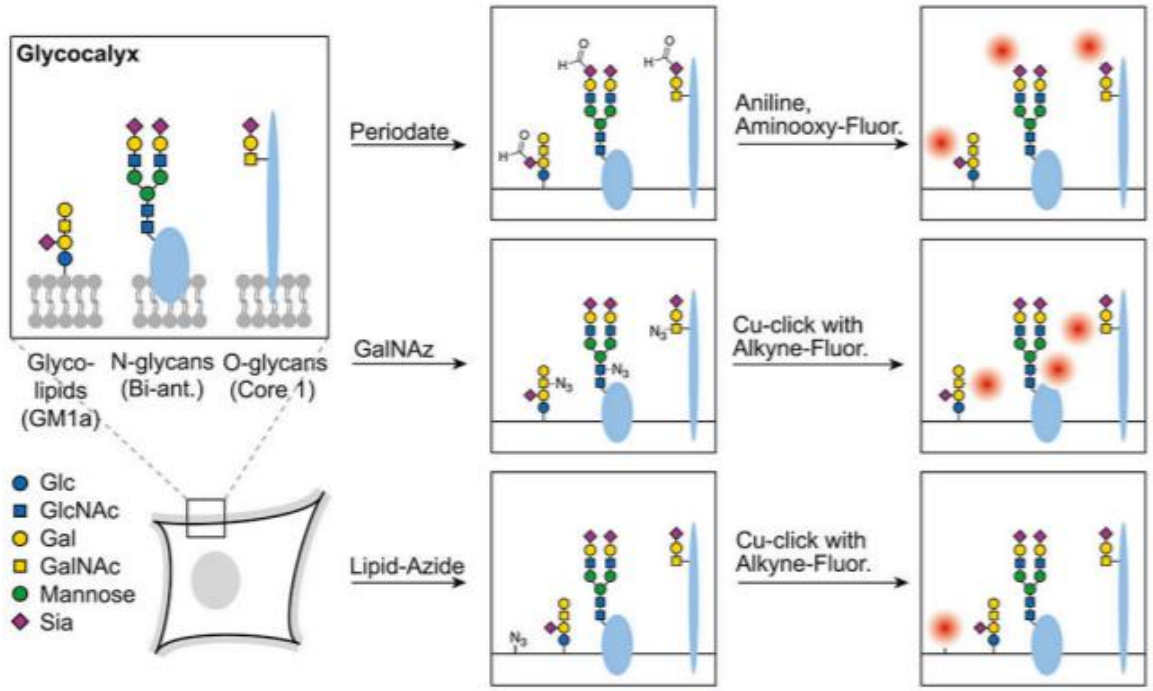
Lipid rafts characterized by the presence of glycosylphosphatidylinositol (GPI)-anchored proteins, glycosphingolipids, and proteins for signal transduction. The 5–25 nm clusters of sphingolipid, cholesterol, and protein that make up a lipid raft are tightly packed and rapidly assemble and disassemble.
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.

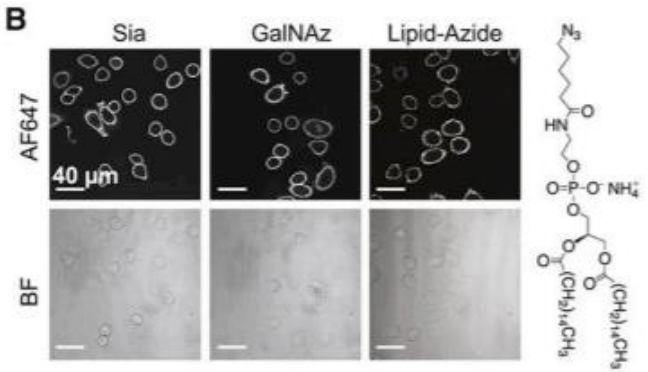
Dev Cell. 2019 July 01; 50(1): 57–72.e6. doi:10.1016/j.devcel.2019.04.035.

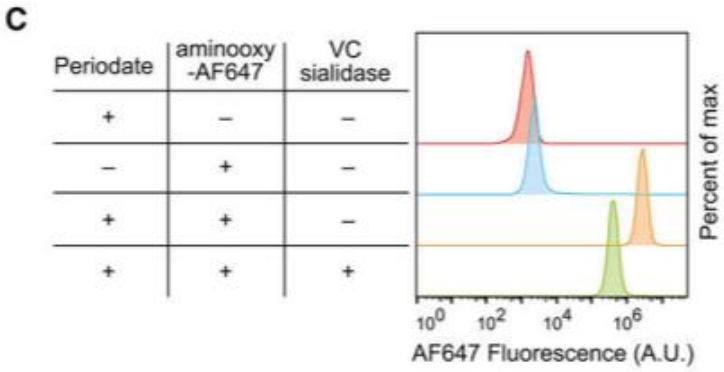
Quantitative super-resolution microscopy of the mammalian glycocalyx

Leonhard Möckl[1,6], Kayvon Pedram[1,6], Anish R. Roy[1], Venkatesh Krishnan[2], Anna-Karin Gustavsson[1,3], Oliver Dorigo[2], Carolyn R. Bertozzi[1,4,*], W. E. Moerner[1,5,*]



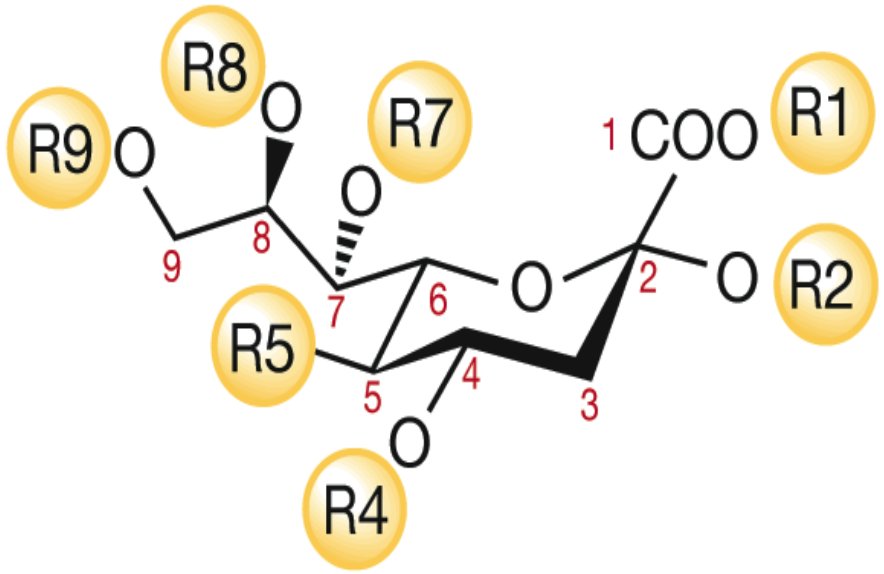






Immense Diversity in the sialic acids

- **Perhaps the most diverse class of compounds**
  - **Immense capacity in recognition**



Around 2 million years ago humans lost Neu5Gc expression and had to adjust their CD33-related Siglec binding specificity to accommodate Neu5Ac.

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), GalNAc(6), GlcNAc(4/6), Sia (8/9), or 5-O-Neu5Gc; oxygen linked to C-7 in 2,7-anhydro molecule; anomeric hydroxyl eliminated in Neu2en5Ac (double bond to C-3).

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

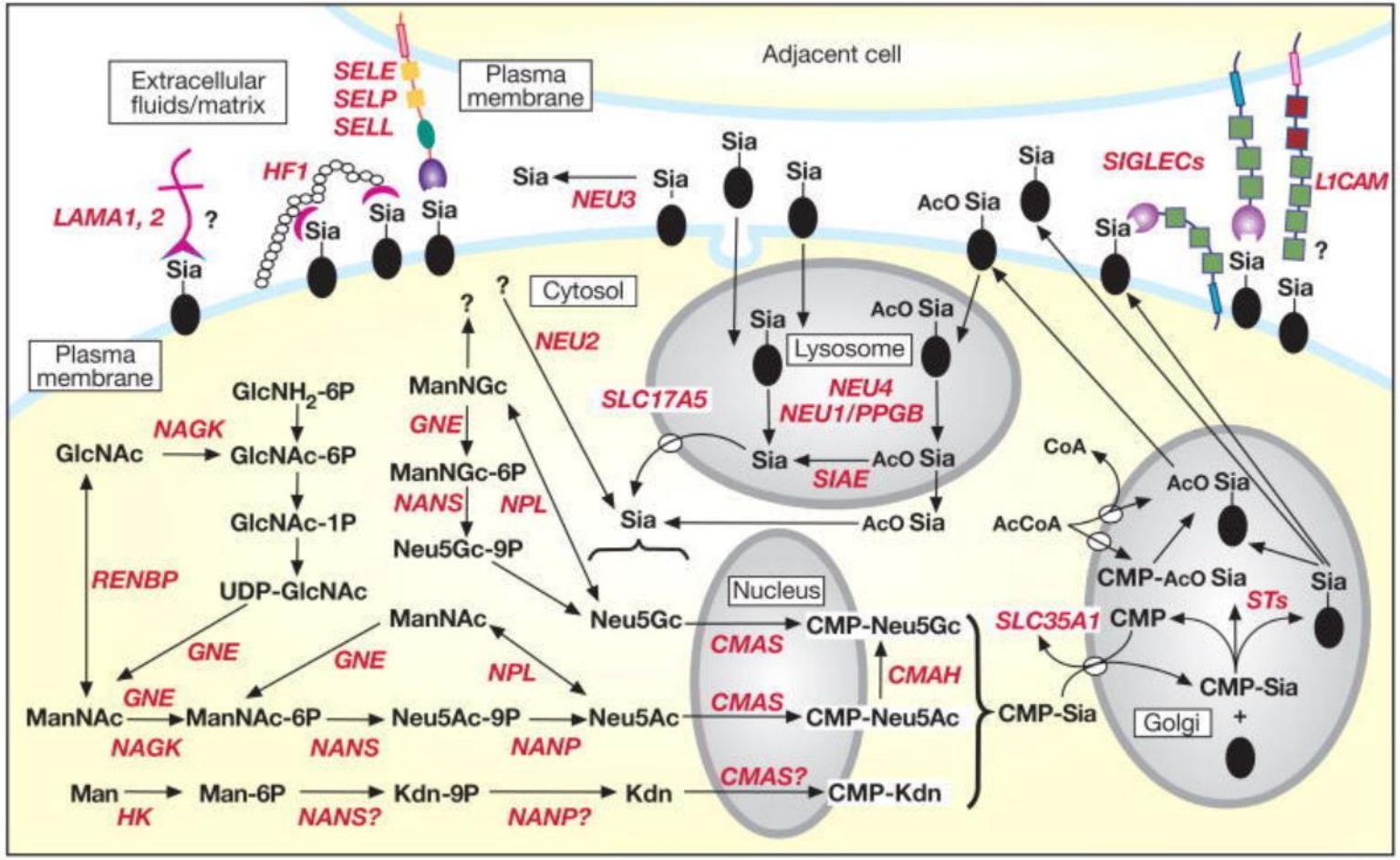
R5 = Amino; N-acetyl; N-glycolyl; hydroxyl; N-acetimidoyl; N-glycolyl-O-acetyl; N-glycolyl-O-methyl; N-glycolyl-O-2-Neu5Gc.

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.

The Sialome: Varki



Less than 60 genes for the sialome show more than 10 uniquely human genetic changes in comparison with our closest evolutionary relatives.

SIGLECS


Siglecs common to mammals
CD33-related Siglecs
Human
Structure
Common name
Sialoadhesin
CD22
MAG
Siglec-15
CD33
Siglec-5
Siglec-6
Siglec-7
Siglec-8
Siglec-9
Siglec-10
Siglec-11
Siglec-14
Alternative name
Siglec-1, CD169
Siglec-2
Siglec-4
–
Siglec-3
CD170
CD327
CD328
–
CD329
–
–
–
Expression
Mac
B
OligoD, Schw
ND
MyP, Mo
N, Mo, (B)
Troph (B)
NK (Mo)
Eo (Ba)
Mo, N, cDC (NK)
B (Mo, Eo)
Mac
ND
Mouse
Structure
Common name
Sialoadhesin
CD22
MAG
Siglec-15
CD33
Siglec-E
Siglec-F
Siglec-G
Siglec-H
Alternative name
Siglec-1, CD169
Siglec-2
Siglec-4
–
Siglec-3
–
–
–
–
Expression
Mac
B
OligoD, Schw
ND
N
N, Mo, cDC
Eo
B
pDC (Mac)
V-set immunoglobulin domain, sialic-acid binding
C2-set immunoglobulin domain
ITIM
ITIM-like
GRB2-binding motif
FYN kinase phosphorylation site

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.

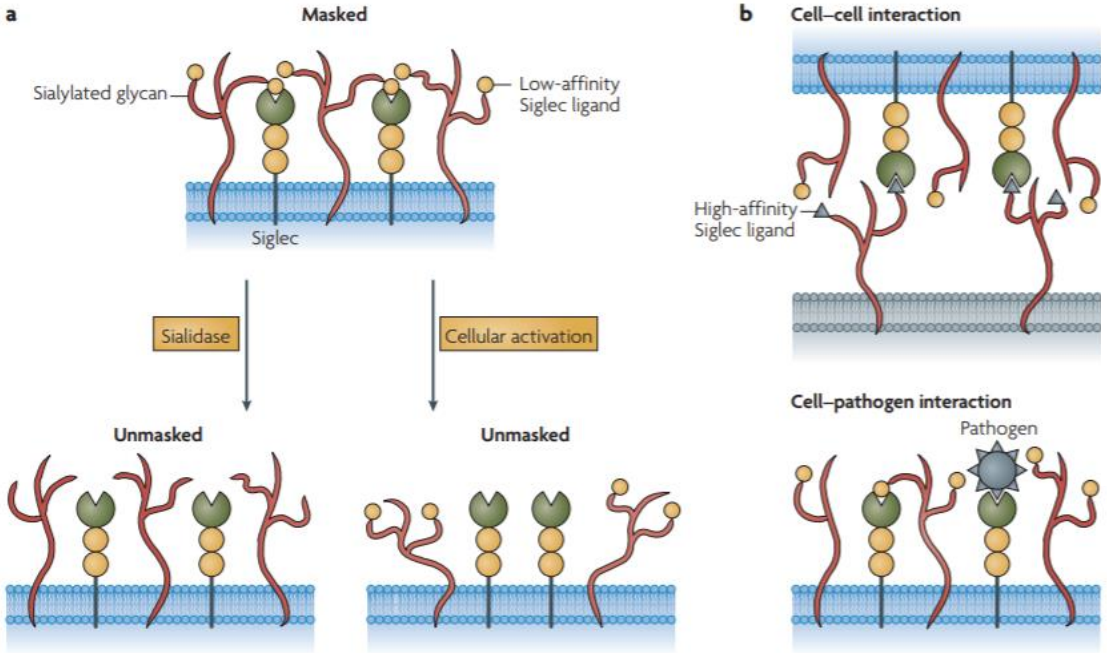


Figure 4 | ***Cis* and *trans* interactions of Siglecs. a** | Most sialic-acid-binding immunoglobulin-like lectins (Siglecs) are masked at the cell surface owing to *cis* interactions with abundantly expressed sialic acids. Following exposure of cells to sialidase, which cleaves the *cis*-interacting 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.

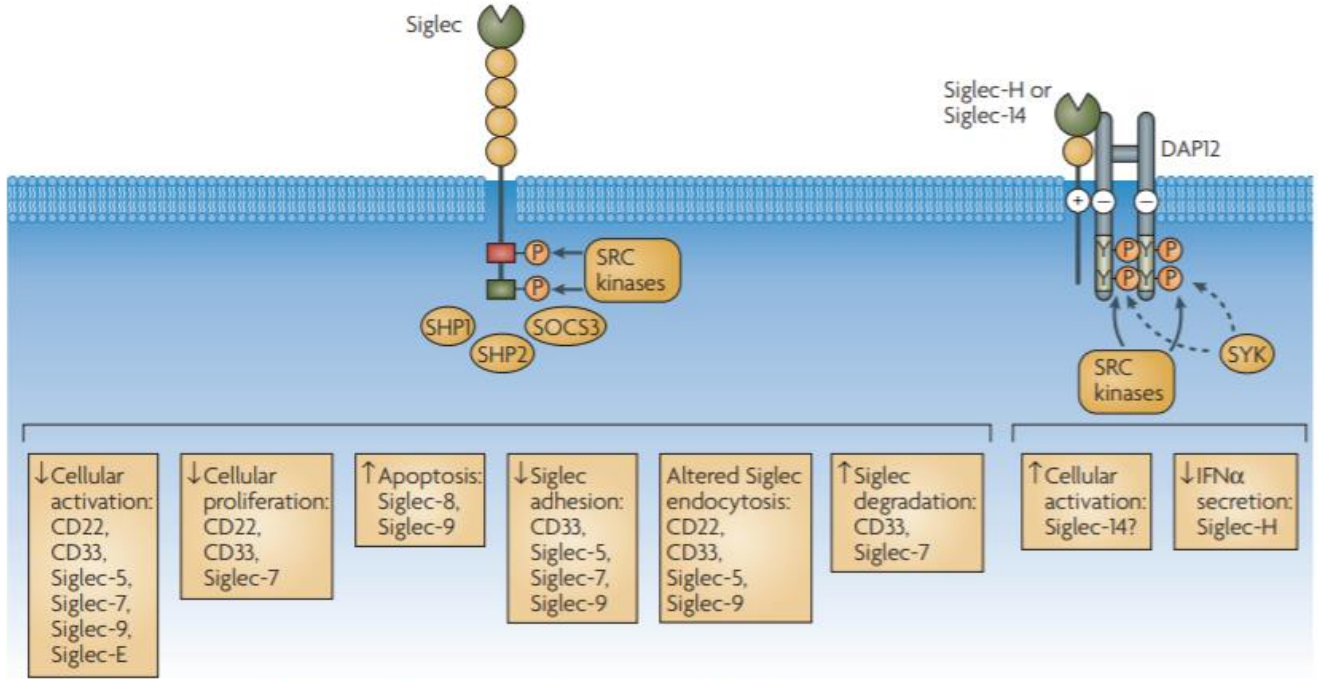


Figure 6 | **Signalling mediated by CD22 and the CD33-related Siglecs.** On phosphorylation of cytoplasmic tyrosine-based signalling motifs by SRC-family tyrosine kinases, sialic-acid-binding immunoglobulin-like lectins (Siglecs) recruit and activate SRC homology 2 (SH2)-domain-containing proteins, notably 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; IFNα, interferon-α.

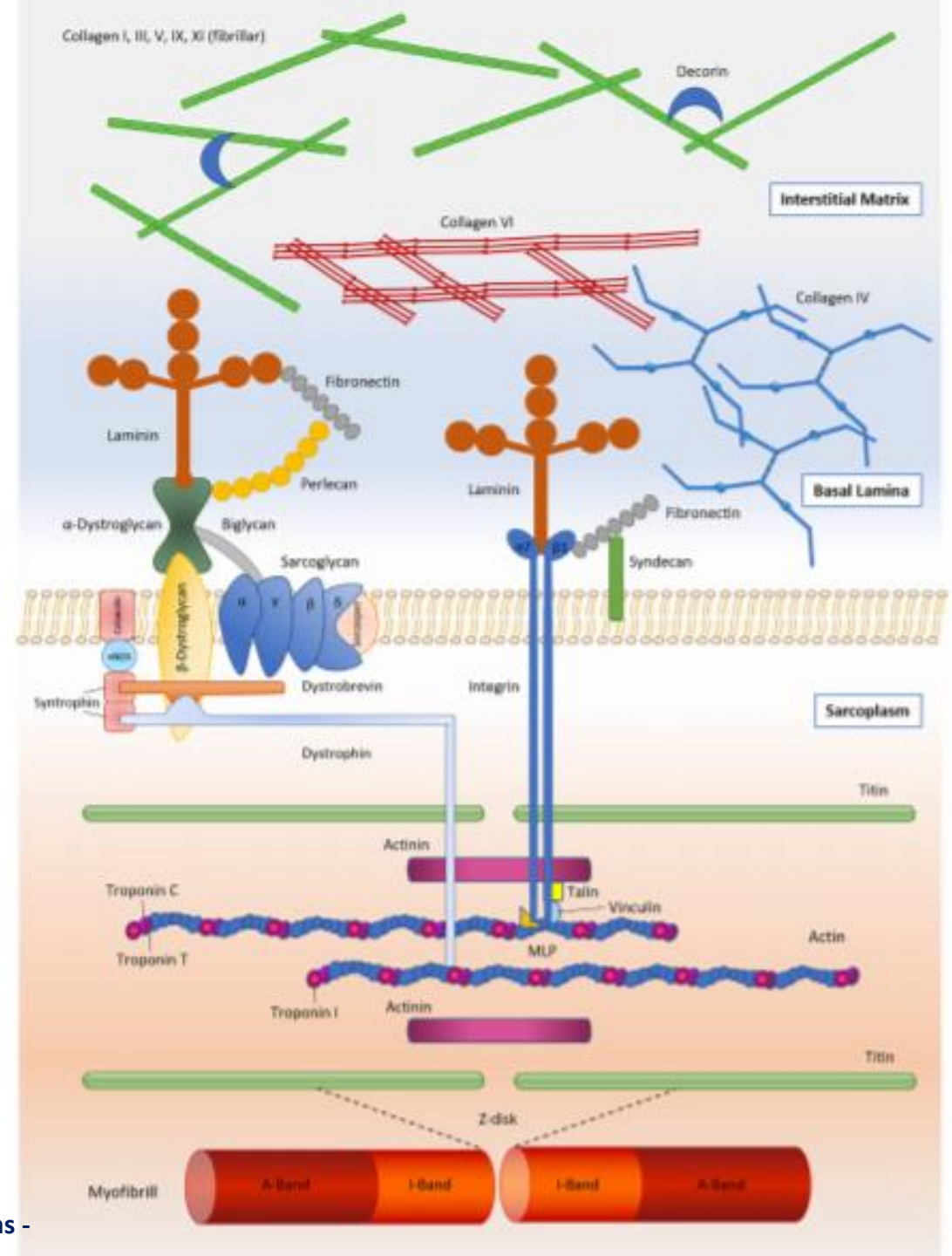
Skeletal Muscle Extracellular Matrix – What Do We Know About Its Composition, Regulation, and Physiological Roles? A Narrative Review

Robert Csapo[1], Matthias Gumpenberger[1] and Barbara Wessner[2]*

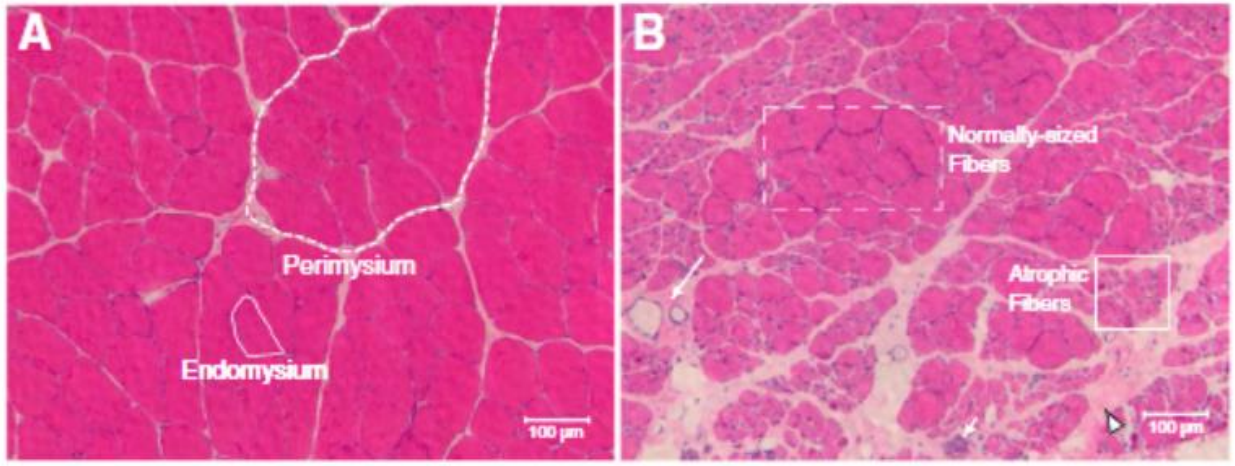
Skeletal muscle represents ~40% of human body mass in men,
~30% in women.
Critical in human evolution.
Muscle is also a secretory organ.
The extracellular matrix is critical to force transduction and encasing muscle cells, with complex responses to training and ageing. Crucial to passive load bearing.
The ECM is a support during both sarcopenia and ALS.

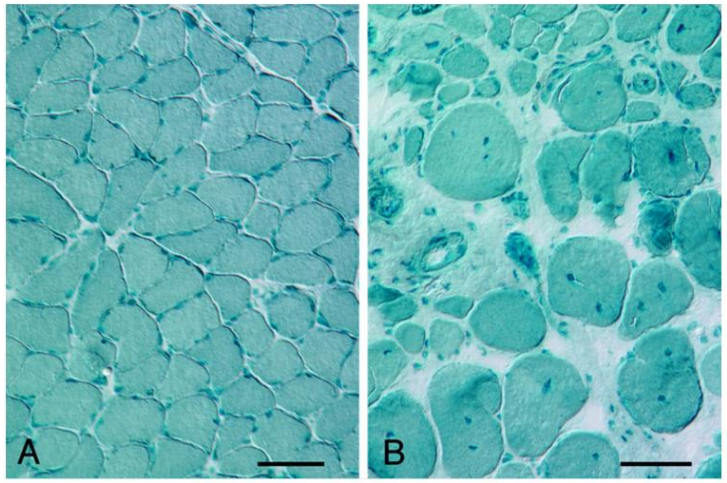
Fibrosis is related to
ageing,
- repair (e.g. damage*), and
- neuromuscular diseases.

* 117,000 kms run?



Skeletal Muscle Fibrosis





Duchenne Muscular Dystrophy model
B mdx mouse.

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

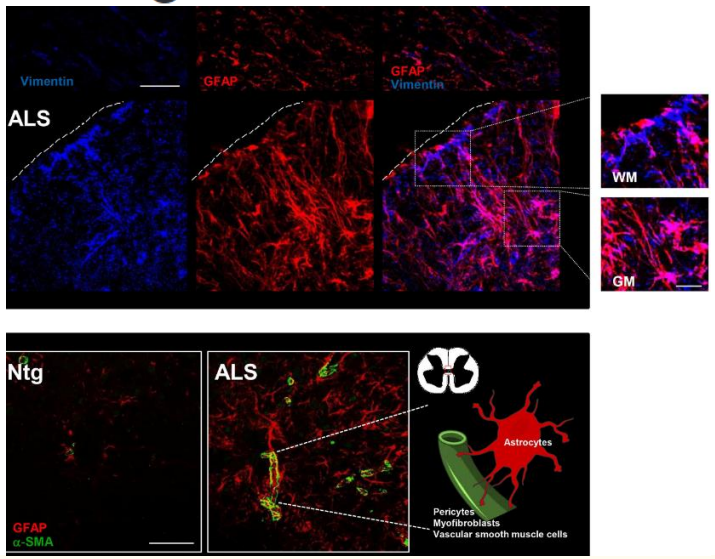
Also measure by collagen mass, hydroxyproline etc.

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**



CNS disease	Responder cells	Mediators	ECM component
Acute damage	Astrocytes, microglia, leukocytes, meningeal cells, fibroblasts, pericytes	Thrombin, MMP-9, ATP, PDGFRβ, TGFβ	Fibronectin, laminin, collagen, CSPGs, tenascin, HSPGs
ALS	Astrocytes, microglia, leukocytes, oligodendrocytes, meningeal cells, fibroblasts, pericytes	IL-6, CXCL1, CXCL10, CXCL12, TNFα, TGFβ, NGF, INFγ, PGD2, ADAMTS-4, CTGF, S100A4, MMP-9	Fibronectin, collagen IV, CSPGs, Sema3A, fibrin, vimentin, thrombin
MS	Astrocytes, microglia, leukocytes, endothelial cells, meningeal cells, fibroblasts, pericytes, oligodendrocytes	PDGFRβ, TGFβ, myelin	Collagen, fibronectin, biglycan, decorin, CSPGs
AD	Astrocytes, microglia, leukocytes, smooth muscle cells, fibroblasts, pericytes	PDGFRβ, TGFβ	GAGs, HSPGs

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,*]

TGFβ
Connective tissue growth factor (CTGF/CCN2) inc. (Ab FG-3019)

Pamrevlumab, FG-3019
phase III: IPF; COVID-19, DMD,

Evolution, Man

Noakes and Spedding, 2012, Nature.

1. Metabolic evolution to triple VO2 max in ~1 Myears ~3M years ago
   AND prolong lifespan.
1. Evolution of brain size and circuits
2. Very recent evolution (100K years) to occupy all planetary niches (SNPs, epigenetics, bacteriome and virome) which « hides » #1.
4. Modern lifestyle and modern diseases.

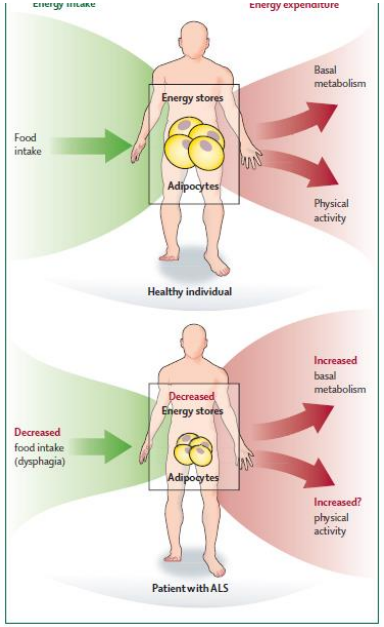
ALS Program

Mitochondria & Lipid Metabolism (Khaitovic)

Servier lipidomics
3000 lipids

Energy metabolism in amyotrophic lateral sclerosis

Luc Dupuis, Pierre-François Pradat, Albert C Ludolph, Jean-Philippe Loeffler

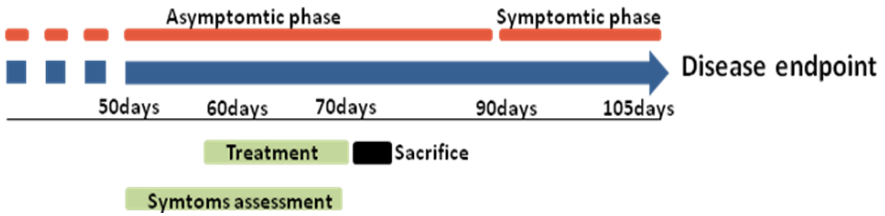


Superoxide dismutase (SOD1G86R) Tg model
Metabolomic & transcriptomic analysis
Ceramide/glucosylceramide ratio critical
Human patient tissue.

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

Other Screens
CHMP2B
C9orf72
TDP43

New enzymatic target (GCase)



Powerful phenotypical screens

The ALS preclinical consortium, thanks!

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

Florey Institute, Melbourne
Brad TURNER

University of Queensland
Shyuan NGO

Spedding Research Solutions
Michael SPEDDING
Alexandre HENRIQUES

IGBMC
Céline Keime

Servier
Vincent Croixmarie

University of Oxford
Mylene HÜBECKER
David PRIESTMAN
Frances PLATT

University of Tours
Hélène BLASCO
Philippe CORCIA
Christian ANDRES

Ambroxol ALS

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

- Spedding Research solutions -

TRIGLYCERIDES

Massive reduction of triglycerides in late stage SOD1 mice

PLASMA

RT	Pre	Par	HMDB
3,43	1,88	###	TG(20:4/18:1/20:5
3,56	1,73	###	TG(22:6/20:4/22:6
3,88	1,73	3,45	TG(22:6/18:2/22:6
4,26	1,84	2,49	TG(20:5/18:2/22:5
4,48	1,48	0,00	TG(20:4/14:0/20:4
4,52	1,29	2,64	TG(18:2/18:2/22:4
4,60	2,06	0,88	TG(20:4/18:1/20:5
4,93	1,20	2,00	TG(18:2/18:2/20:2
5,08	2,00	0,10	TG(18:3/16:0/18:3
5,15	1,26	0,35	TG(20:4/18:1/18:3
5,22	2,14	0,32	TG(18:1/14:0/18:1
5,28	1,63	0,53	TG(20:4/18:1/20:4
5,36	1,70	0,50	TG(20:5/18:1/20:5
5,39	2,56	0,04	TG(16:0/14:0/18:0
5,70	1,23	0,41	TG(18:1/16:0/18:1
5,79	1,96	0,38	TG(18:3/18:0/18:3
6,07	1,47	0,11	TG(18:2/18:1/18:2
6,14	2,05	0,10	TG(18:1/16:0/18:3
6,17	1,03	0,26	TG(18:2/18:1/22:4
6,18	1,99	0,04	TG(18:1/14:0/18:2
6,25	2,12	0,15	TG(16:1/14:0/18:1
6,48	1,56	0,02	TG(15:0/18:1/18:1
6,53	1,41	0,05	TG(15:0/20:1/14:0
6,85	1,08	0,00	TG(18:0/18:0/18:1
6,85	1,39	0,07	TG(18:1/16:1/18:1
6,93	1,93	0,07	TG(18:1/14:0/18:1
7,01	1,90	0,22	TG(18:0/14:0/16:1
7,46	0,95	0,12	TG(18:1/18:1/18:1
7,56	1,00	0,00	TG(18:1/18:1/20:0
7,80	1,44	0,30	TG(16:0/14:0/18:0
8,17	1,14	0,25	TG(18:1/18:0/18:1
8,30	1,23	0,36	TG(18:0/16:0/18:1
8,89	1,37	0,50	TG(18:0/18:0/18:1
9,46	1,41	0,19	TG(16:1/20:0/20:0

SOLEUS

RT	Pre	Par	HMDB
3,16	1,78	0,16	TG(12:0/12:0/12:0)
4,60	3,04	0,00	TG(20:4/18:1/20:5)
4,61	2,97	0,00	TG(20:4/18:2/18:3)
4,63	3,39	0,00	TG(20:4/14:0/20:4)
4,78	1,65	0,11	TG(20:4/16:0/18:3)
4,79	1,68	0,05	TG(18:3/16:0/18:3)
4,79	1,59	0,00	TG(18:3/16:0/18:3)
5,28	2,55	0,37	TG(20:4/18:1/20:4)
5,33	2,66	0,28	TG(20:4/18:1/18:3)
5,39	1,63	0,24	TG(18:3/18:0/18:3)
5,41	1,74	0,16	TG(18:2/16:0/18:3)
5,47	1,86	0,09	TG(15:0/18:2/16:0)
5,56	1,30	0,17	TG(16:1/14:0/16:1)
5,71	1,72	0,19	TG(20:4/18:0/18:3)
5,75	1,56	0,11	TG(15:0/18:1/18:2)
6,06	1,67	0,92	TG(18:2/18:1/22:4)
6,07	1,62	0,35	TG(18:2/18:1/18:2)
6,13	1,57	0,29	TG(18:1/16:0/18:3)
6,19	1,54	0,25	TG(18:1/14:0/18:2)
6,35	1,22	0,24	TG(16:0/14:0/16:1)
6,45	1,28	0,14	TG(15:0/18:1/18:1)
6,53	1,34	0,13	TG(15:0/20:1/14:0)
6,68	1,84	0,00	TG(18:2/18:1/20:2)
6,75	1,59	0,69	TG(18:1/18:1/22:4)
6,86	1,46	0,39	TG(18:1/16:1/18:1)
6,95	1,45	0,32	TG(18:1/14:0/18:1)
7,04	1,30	0,29	TG(18:0/14:0/16:1)
7,27	1,27	0,21	TG(15:0/18:1/18:1)
7,37	1,41	0,27	TG(15:0/18:2/18:2)
7,38	1,81	0,52	TG(18:1/18:1/20:2)
7,38	1,79	0,55	TG(18:2/18:1/22:4)
7,59	1,50	0,53	TG(18:1/18:1/20:0)
7,67	1,23	1,40	TG(18:1/14:0/18:2)
7,70	1,28	0,34	TG(18:0/14:0/18:1)
7,70	1,47	0,47	TG(18:0/14:0/18:1)

TIBIALIS ANTERIOR

RT	Pre	Par	HMDB
5,47	0,85	0,07	TG(18:3/14:0/18:3)
5,56	0,93	###	TG(16:0/14:0/18:0)
5,57	0,79	0,00	TG(16:1/14:0/16:1)

Pre – presymptomatic
Par - end stage
just before paralysed

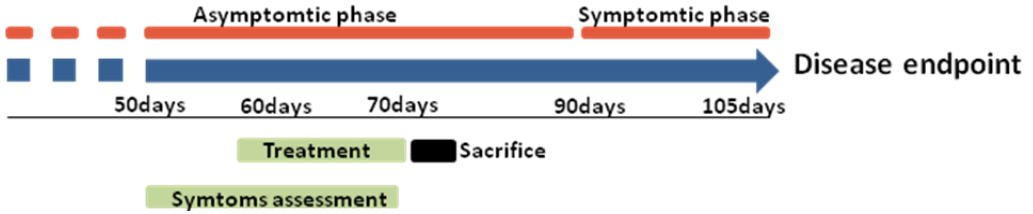
LUMBAR SPINAL CORD

RT	Pre	Par	HMDB
6,09	1,81	0,25	TG(18:3/16:0/18:3)
6,40	0,97	0,68	TG(18:2/18:1/18:2)
6,77	1,62	0,41	TG(18:3/18:0/18:3)
6,94	1,46	0,09	TG(18:0/14:0/16:1)
7,03	1,12	0,63	TG(18:2/18:1/20:2)
7,40	1,29	0,23	TG(18:1/18:1/18:1)
7,59	1,29	0,45	TG(18:0/14:0/18:1)
7,60	1,30	1,28	TG(18:0/14:0/18:1)
7,75	1,75	1,46	TG(18:2/16:0/18:3)
7,76	1,08	0,43	TG(18:1/14:0/18:2)
8,03	1,00	0,58	TG(18:1/18:0/18:1)
8,21	1,09	0,67	TG(18:0/16:0/18:1)
8,77	1,12	0,73	TG(18:0/18:0/18:1)

BRAIN

RT	Par

Late generalized decrease of triglycerides corroborates the hypolipidemic trait of mutant SOD1 mice



CERAMIDES AND GLUCOSYLCERAMIDES

PLASMA

RT	Pre	Par	HMDB

SOLEUS

RT	Pre	Par	HMDB
0,63	1,41	1,34	CerP(d18:0/16:0)
0,64	1,53	1,38	CerP(d18:1/16:0)
0,77	0,33	1,08	CerP(d18:1/24:0)
1,90	1,80	1,41	Glucosylcer(d18:1/9Z-18:1)
2,19	1,34	1,00	Cer(d18:1/9Z-18:1)
2,77	1,67	0,81	Cer(d18:1/18:0)
2,77	1,57	0,84	Cer(d18:1/18:0)
2,78	1,51	0,95	Cer(d18:1/18:0)
3,25	1,29	1,27	Cer(d18:0/22:0)
3,43	1,42	0,98	Cer(d18:1/20:0)
4,02	1,15	1,13	Cer(d18:1/24:1)
4,04	1,34	1,15	Cer(d18:1/24:1)
4,08	1,37	1,33	CerP(d18:1/24:0)
4,17	1,53	0,99	Cer(d18:1/22:0)
4,56	1,18	0,79	Cer(d18:1/23:0)
4,93	1,42	0,23	Cer(d18:1/24:0)
4,94	0,90	0,37	Glucosylcer(d18:1/9Z-18:1)
4,96	1,38	0,75	Cer(d18:1/24:0)
5,05	0,89	0,16	Glucosylcer(d18:1/9Z-18:1)
5,42	1,92	0,13	Cer(d18:1/14:0)
5,56	1,46	1,06	Cer(d18:1/24:1)
5,73	0,38	0,18	Glucosylcer(d18:1/18:0)
6,16	1,62	0,22	Cer(d18:0/14:0)
6,80	1,43	0,33	Cer(d18:0/16:0)
6,82	1,51	0,28	Cer(d18:0/14:0)
7,47	1,27	0,34	Cer(d18:0/16:0)

TIBIALIS ANTERIOR

RT	Pre	Par	HMDB
4,02	0,93	1,41	Cer(d18:1/24:1
4,04	0,84	1,83	Cer(d18:1/24:1
4,17	1,98	1,88	Cer(d18:1/22:0

LUMBAR SPINAL CORD

RT	Pre	Par	HMDB
1,94	0,56	0,30	Glucosylcer(d18:1/9Z-18:
2,01	0,23	0,74	CerP(d18:1/18:0)
2,13	0,43	0,13	Glucosylcer(d18:1/9Z-18:
2,15	0,64	1,02	Glucosylcer(d18:1/12:0)
2,16	0,45	0,88	Glucosylcer(d18:1/18:0)
2,33	0,45	0,43	Glucosylcer(d18:1/9Z-18:1)
2,48	0,44	0,43	Glucosylcer(d18:1/9Z-18:1)
2,65	0,48	1,08	Glucosylcer(d18:1/20:0)
2,67	0,50	0,53	Glucosylcer(d18:1/16:0)
2,82	0,36	0,68	Glucosylcer(d18:1/22:0)
3,15	0,61	0,65	Cer(d18:1/24:1)
3,18	1,64	1,21	Cer(d18:0/22:0)
3,23	0,65	0,80	Cer(d18:1/24:1)
3,25	0,47	0,27	Glucosylcer(d18:1/18:0)
3,31	0,37	2,86	Glucosylcer(d18:1/22:0)
3,35	1,29	0,49	Cer(d18:1/20:0)
3,36	1,12	0,53	Cer(d18:1/20:0)
3,49	0,52	2,16	Glucosylcer(d18:1/22:0)
3,55	0,51	0,07	Cer(d18:1/22:0)
3,88	0,50	0,10	Glucosylcer(d18:1/22:0)
4,04	0,45	1,23	CerP(d18:1/24:0)
4,07	0,87	0,48	Cer(d18:1/22:0)
4,26	0,84	1,71	Glucosylcer(d18:1/18:0)
4,29	0,75	0,55	Cer(d18:1/24:0)
5,21	0,00	1,49	Glucosylcer(d18:1/22:0)

BRAIN

RT	Par
3,42	1,50
4,19	2,28
4,21	1,69

Glucosyl ceramide is reduced in spinal cord presymptomatic,
- GSLs increased in muscle

SPHINGOMYELINS

PLASMA

RT	Pre	Par	HMDB
5,48	1,88	0,05	SM(d18:1/26:0

SOLEUS

RT	Pre	Par	HMDB
0,70	1,56	1,38	SM(d18:1/18:0)
1,90	1,71	1,69	SM(d18:0/16:0)
3,11	0,99	0,13	SM(d18:0/16:0)
3,21	1,64	1,27	SM(d18:0/20:2)
4,79	1,13	0,08	SM(d18:1/24:0)
5,05	1,47	1,61	SM(d18:0/26:1)
5,06	1,63	1,22	SM(d18:0/24:0)
6,45	1,75	0,82	SM(d18:1/18:1)

TIBIALIS ANTERIOR

RT	Pre	Par	HMDB
5,05	1,63	3,07	SM(d18:0/26:1
5,06	1,53	3,15	SM(d18:0/24:0

LUMBAR SPINAL CORD

RT	Pre	Par	HMDB
1,87	0,72	0,99	SM(d18:0/14:0)
2,06	0,49	0,56	SM(d18:0/22:3)
2,22	1,54	3,19	SM(d18:0/16:1)
2,55	0,46	0,35	SM(d18:1/16:0)
2,62	1,87	1,77	SM(d18:0/22:3)
2,63	1,99	2,19	SM(d18:0/18:1)
2,65	0,56	0,39	SM(d18:0/16:1)
3,02	1,75	0,76	SM(d18:0/20:0)
3,06	1,50	1,12	SM(d17:1/24:0)
3,15	0,59	0,67	SM(d18:1/18:0)
3,16	3,57	1,53	SM(d18:1/22:1)
3,26	2,56	1,75	SM(d18:1/22:1)
4,58	0,46	0,00	SM(d18:0/23:0)
7,75	0,92	1,64	SM(d18:1/24:1)
7,94	0,84	1,22	SM(d18:0/16:0)

BRAIN

RT	I

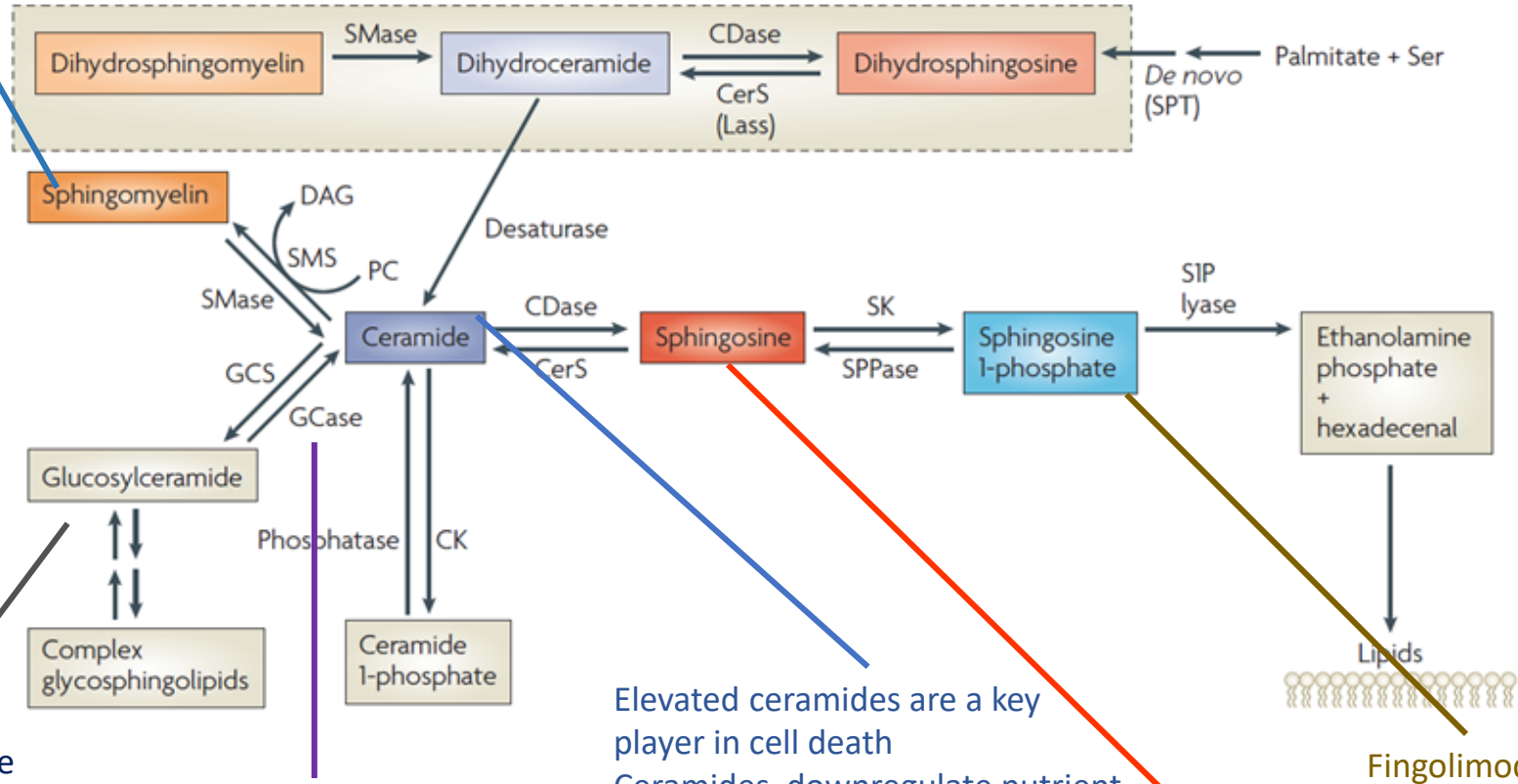
Sphingolipid metabolism is early altered in a tissue-specific manner in mutant SOD1 mice

Overview of ceramide and sphingolipid changes in all studies

One form of sphingomyelin increased in spinal cord 70, 77d agonist. Massive changes at 105d

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

Henriques et al. 2015, 2017, 2018



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

Increased 77d, trend 70d

Fingolimod, weakly protective, acts as S1P agonist

Amyotrophic lateral sclerosis and denervation alter sphingolipids and up-regulate glucosylceramide synthase

Alexandre Henriques[1,2], Vincent Croixmarie[3], David A. Priestman[4], Angela Rosenbohm[5], Sylvie Dirrig-Grosch[1,2], Eleonora D'Ambra[1,2], Mylene Huebecker[4], Ghulam Hussain[1,2,6], Claire Boursier-Neyret[3], Andoni Echaniz-Laguna[1,2,7], Albert C. Ludolph[5], Frances M. Platt[4], Bernard Walther[3], Michael Spedding[8,9], Jean-Philippe Loeffler[1,2] and Jose-Luis Gonzalez De Aguilar[1,2,*]

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

Alexandre Henriques[1,2,3], Mylene Huebecker[4], Hélène Blasco[5,6], Céline Keime[7], Christian R. Andres[5,6], Philippe Corcia[5,8], David A. Priestman[4], Frances M. Platt[4], Michael Spedding[3] & Jean-Philippe Loeffler[1,2]

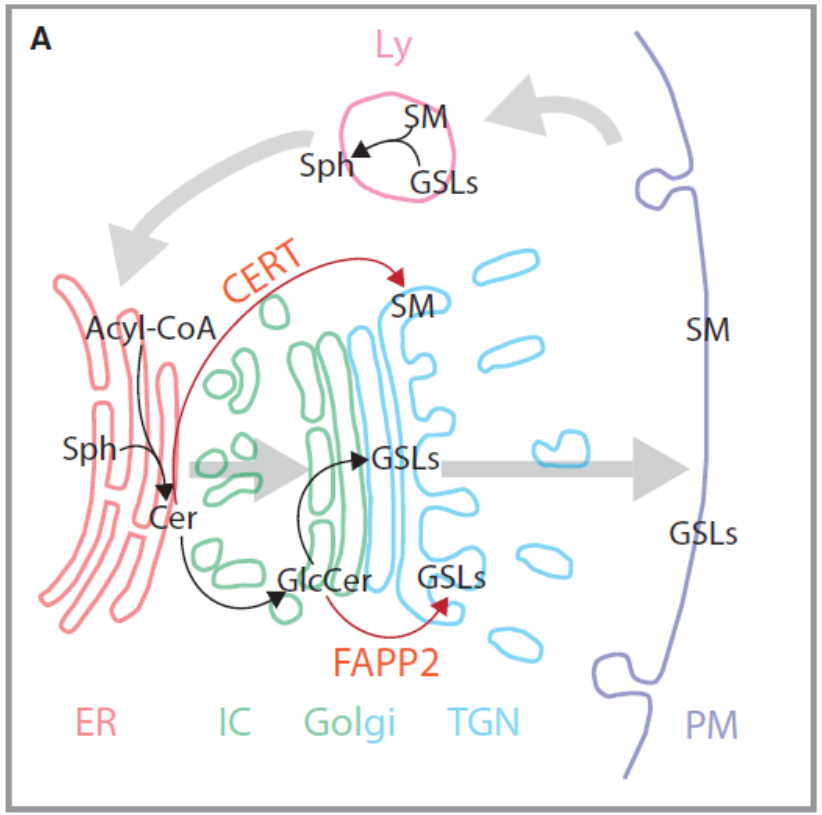
Sphingolipid Metabolism Is Dysregulated at Transcriptomic and Metabolic Levels in the Spinal Cord of an Animal Model of Amyotrophic Lateral Sclerosis

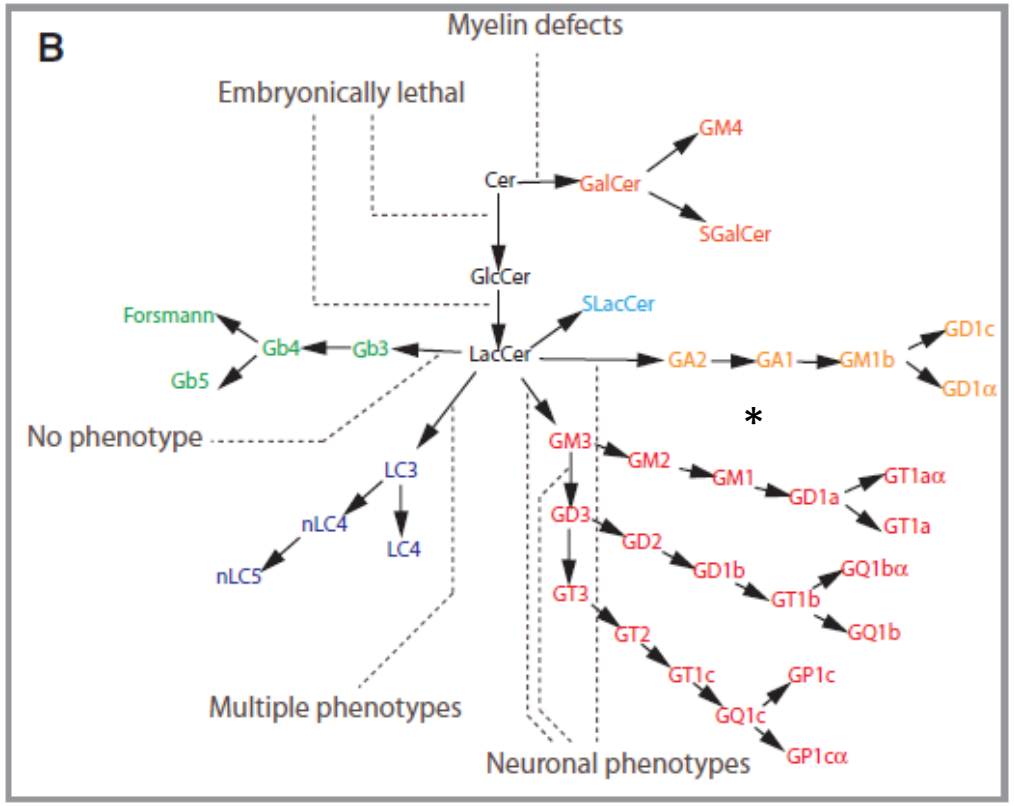
Alexandre Henriques[1,2,3], Vincent Croixmarie[4], Alexandra Bouscary[1,2], Althéa Mosbach[1,2], Céline Keime[5], Claire Boursier-Neyret[6], Bernard Walter[6], Michael Spedding[3] and Jean-Philippe Loeffler[1,2*]*

Ambroxol Hydrochloride Improves Motor Functions and Extends Survival in a Mouse Model of Familial Amyotrophic Lateral Sclerosis

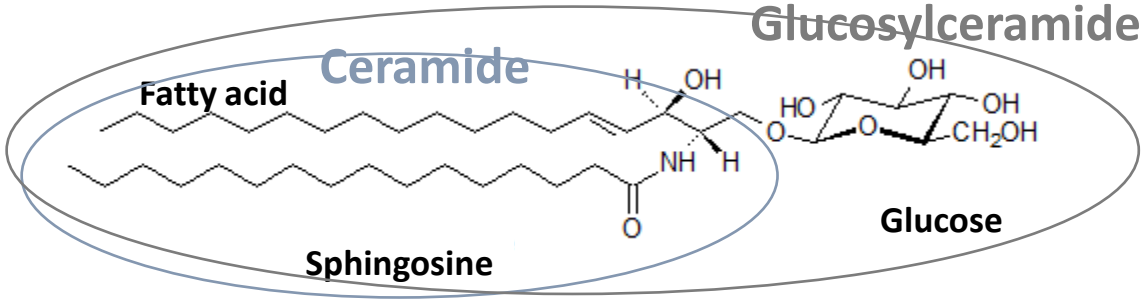
Alexandra Bouscary[1,2], Cyril Quessada[1,2], Althéa Mosbach[1,2], Noëlle Callizot[3], Michael Spedding[4], Jean-Philippe Loeffler[1,2*] and Alexandre Henriques[1,2,4*†]*

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

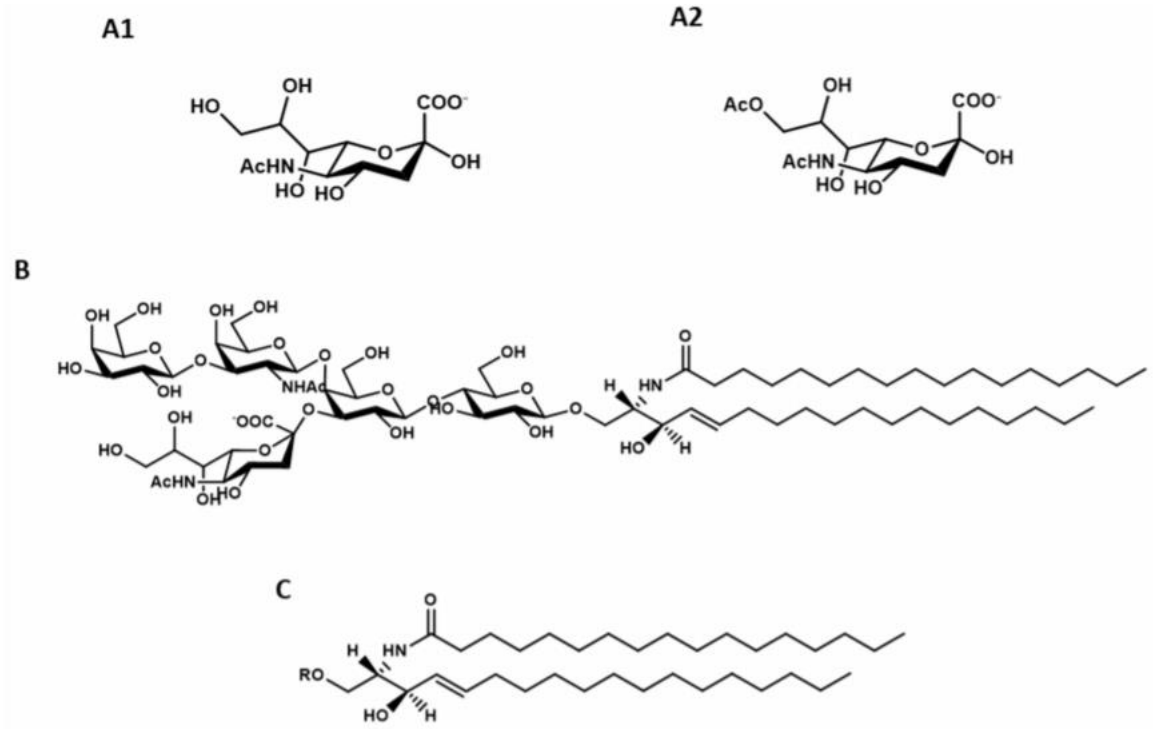
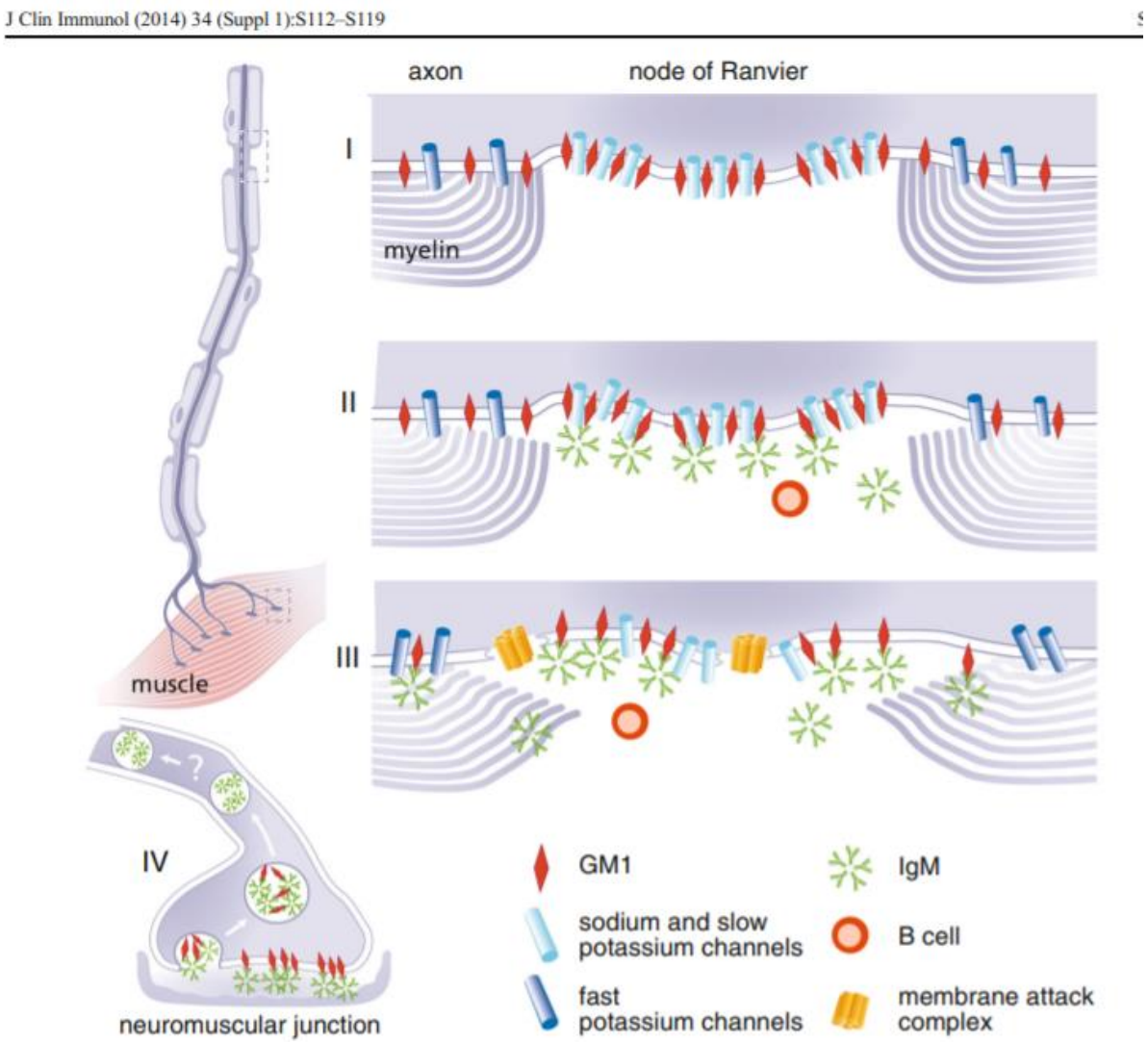


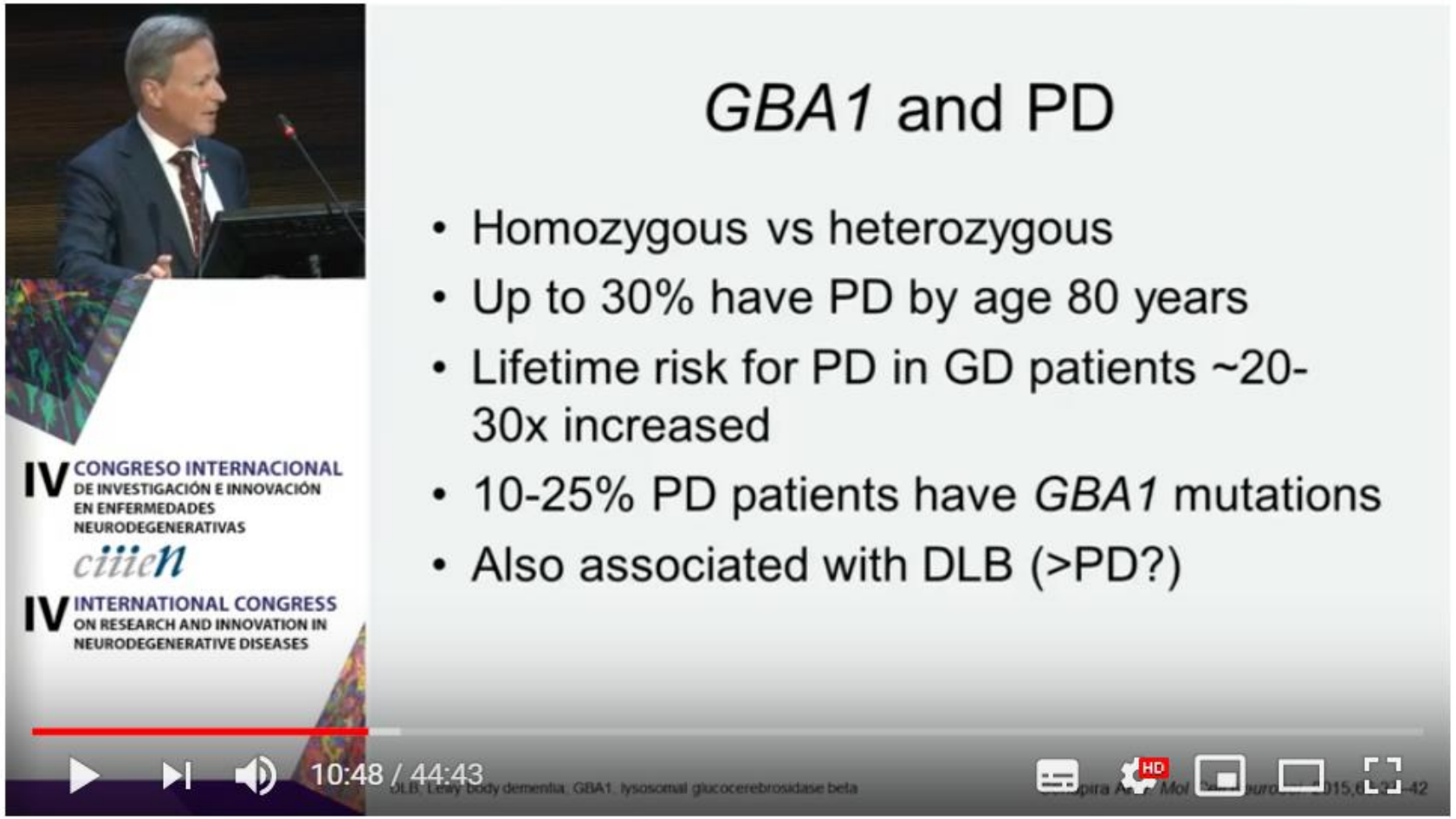
Figure 1. Structure of: *N*-acetylneuraminic acid, Neu5Ac (**A1**), *N*-acetyl-9-acetylneuraminic acid, $Neu5,9Ac_2$ (**A2**), ganglioside GM1, $II^3Neu5AcGg_4Cer$ (**B**), ceramide, Cer R = oligosaccharide chain (**C**).

GM1 can be labelled with cholera toxin

One of the binding sites for influenza virus.

GM1 antibodies
cause multifocal motor neuropathy (MMN)

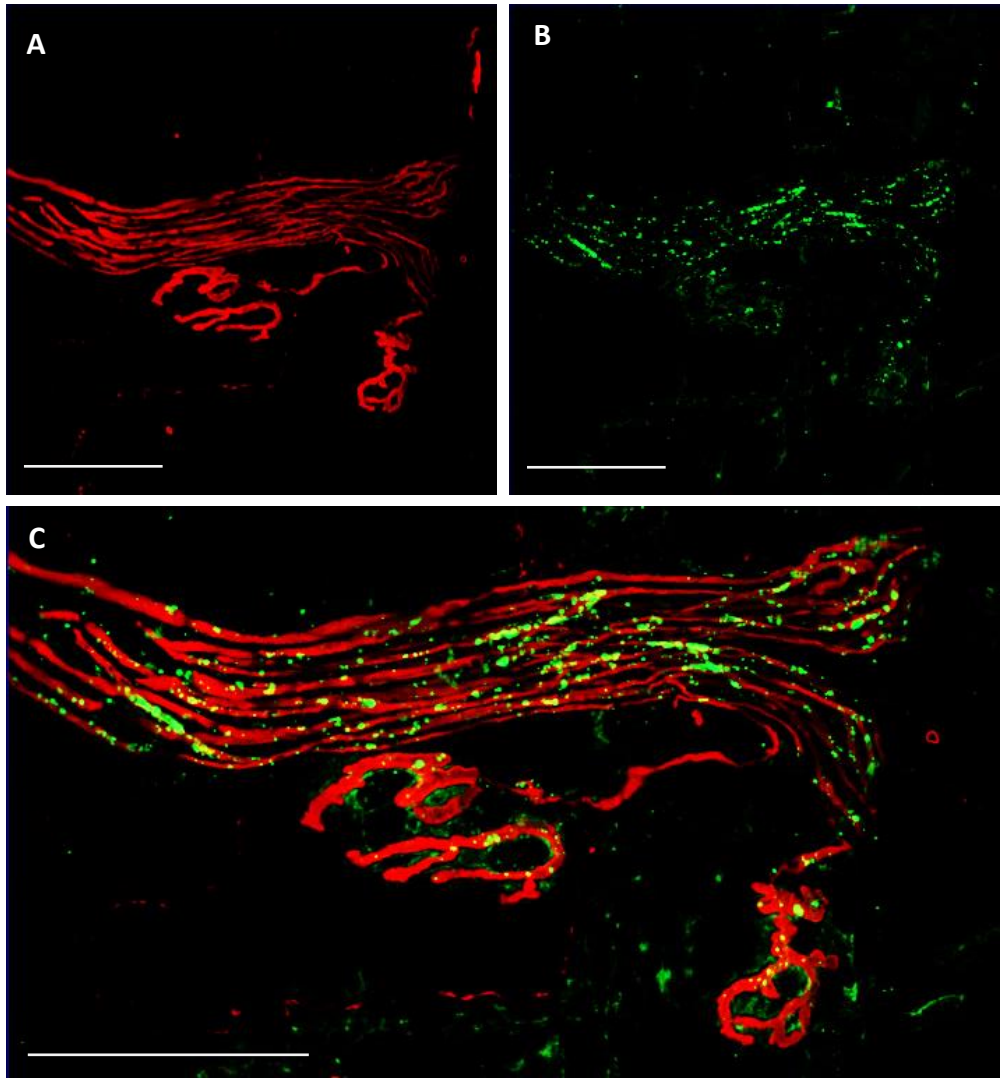




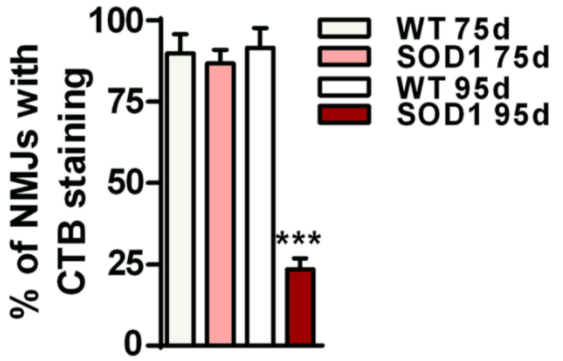
Anthony Schapira: The Glucocerebrosidase pathway: a paradigm for pathogenesis and treatment...

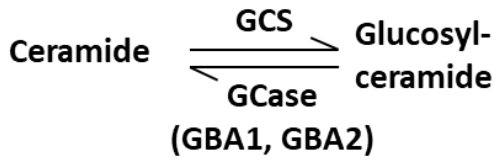
There are two enzymes Glucocerebrosidase : GBA1 (lysosomal) and GBA2, non-lysosomal

Loss of gangliosides on neuromuscular junctions of SOD1 mice

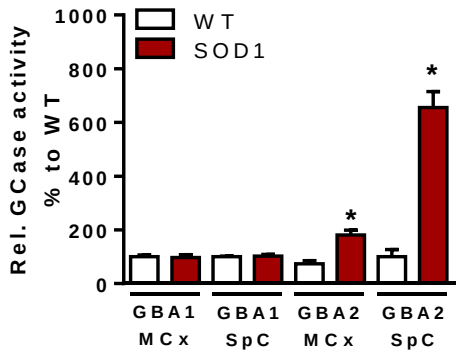


Proportion of NMJs with presynaptic gangliosides



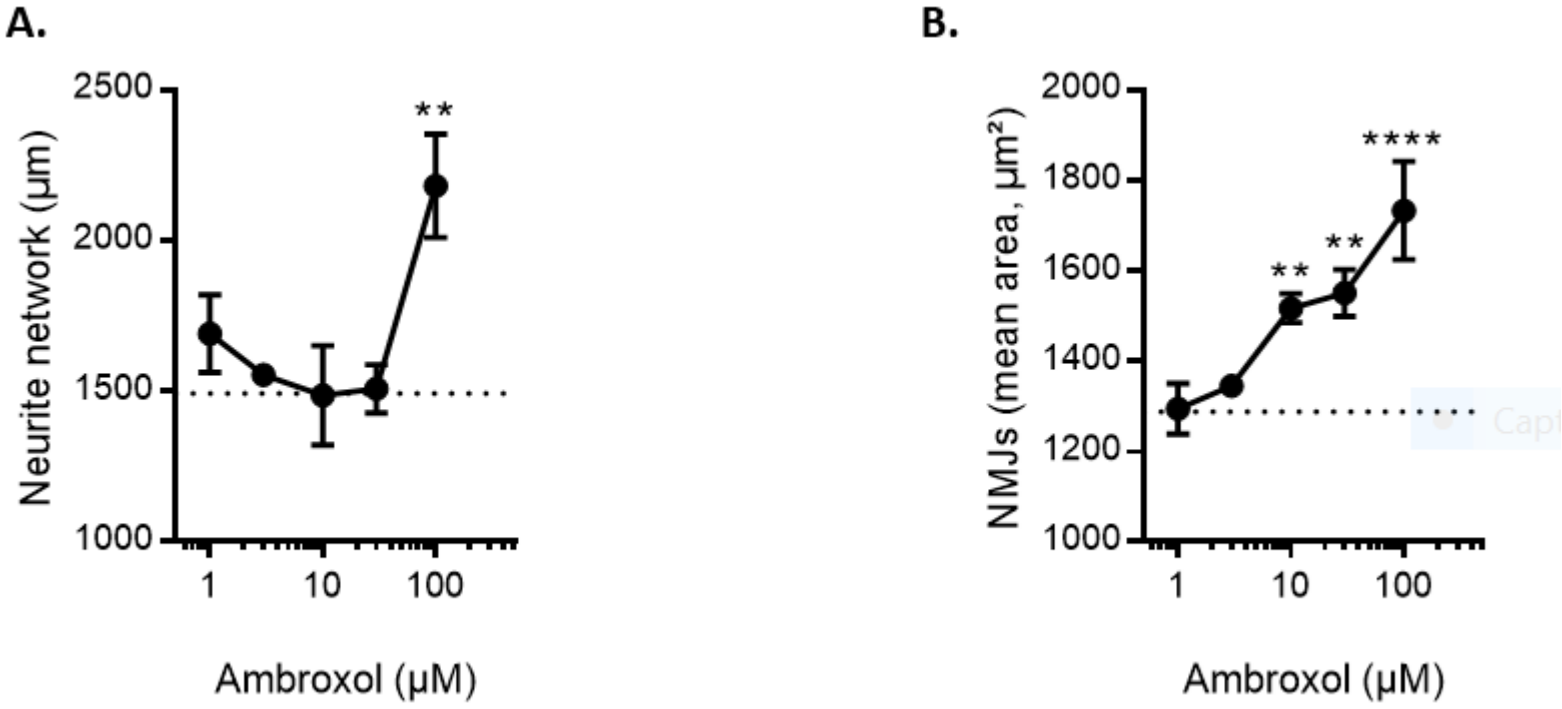


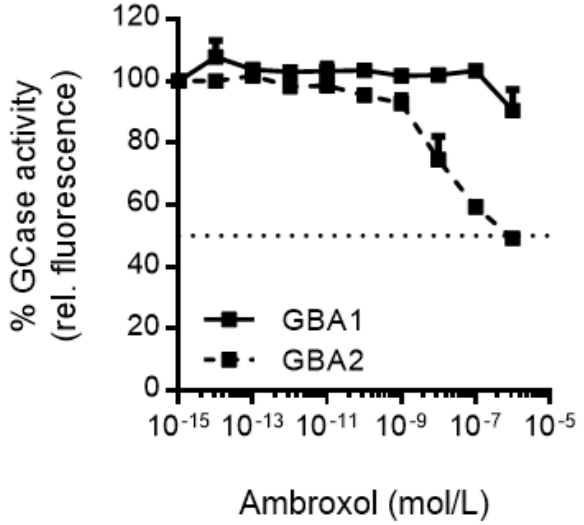
ONLY GBA2 Increased in spinal cord



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





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
a-synuclein.

(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).

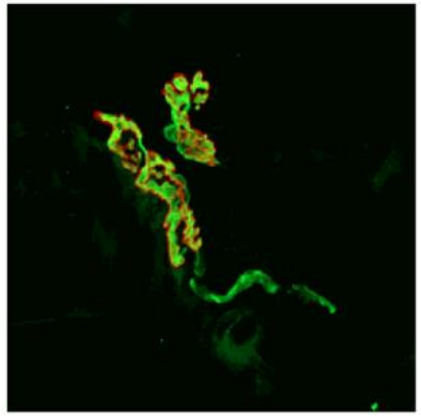
Ambroxol hydrochloride delays disease onset in SOD1 mice

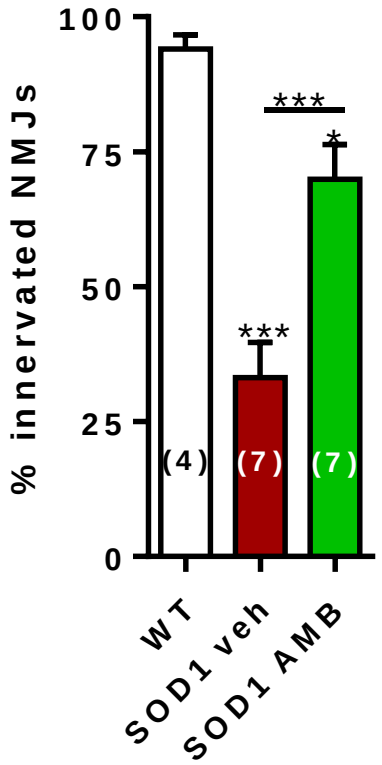
Ambroxol - Presymptomatic study (120mg/kg/d)

Preserved neuromuscular junction integrity

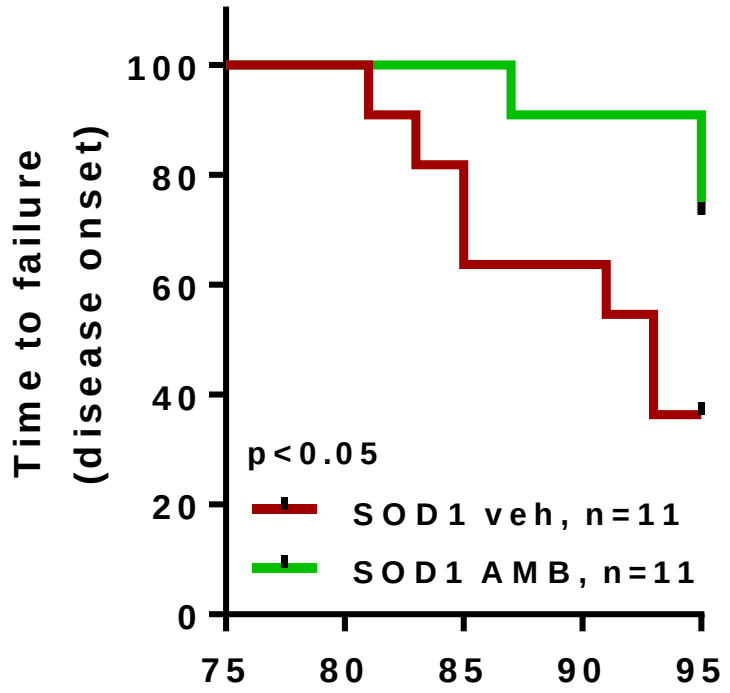
Delays disease onset (and delays decline after disease onset, grip strength)

In vivo – neuromuscular junctions





In vivo – Disease onset



Bouscary et al. 2000

Fibrosis

Myriocin treatment of CF lung infection and inflammation: complex analyses for enigmatic lipids

Anna Caretti[1] · Michele Vasso[2] · Fabiola Tecla Bonezzi[1] · Andrea Gallina[1] · Marco Trinchera[3] · Alice Rossi[4] · Raffaella Adami[1] · Josefina Casas[5] · Monica Falleni[1] · Delfina Tosi[1] · Alessandra Bragonzi[4] · Riccardo Ghidoni[1] · Cecilia Gelfi[2] · Paola Signorelli[1]

The distribution of ceramide versus sphingomyelin and sphingosine contributes to the delicate balance of antiinflammatory 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-inflammatory 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

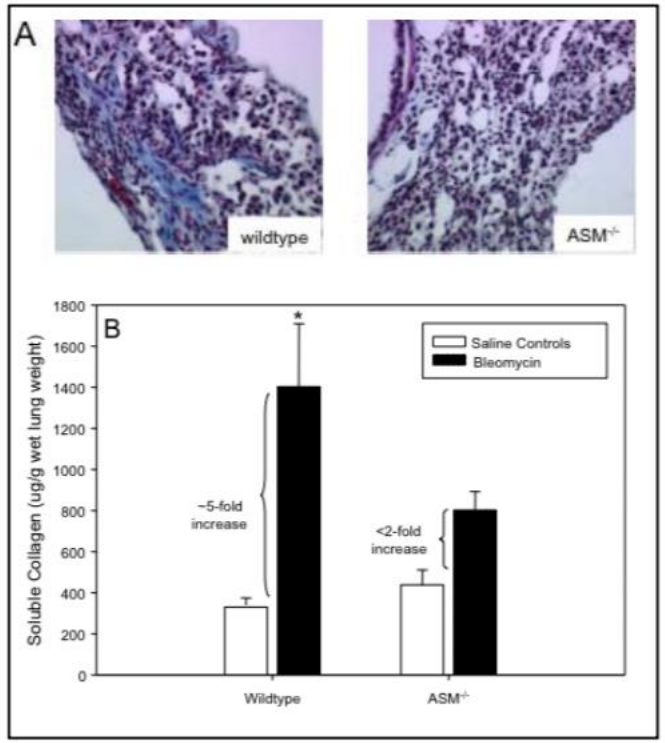
Katrin Anne Becker[1], Rabea Verhaegh[1], Hedda-Luise Verhasselt[2], Simone Keitsch[1], Matthias Soddemann[1], Barbara Wilker[1], Gregory C. Wilson[3], Jan Buer[2], Syed A. Ahmad[3], Michael J. Edwards[3], Erich Gulbins[1,3]

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 Li[1], Shanshan Guo[1], Wenyuan Pang[1,2] and Zhigang Zhao[1]**

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

Alessandra Mingione[a] Michele Dei Cas[b] Fabiola Bonezzi[c] Anna Caretti[a] Marco Piccoli[c] Luigi Anastasia[d] Riccardo Ghidoni[a] Rita Paroni[b] Paola Signorelli[a]

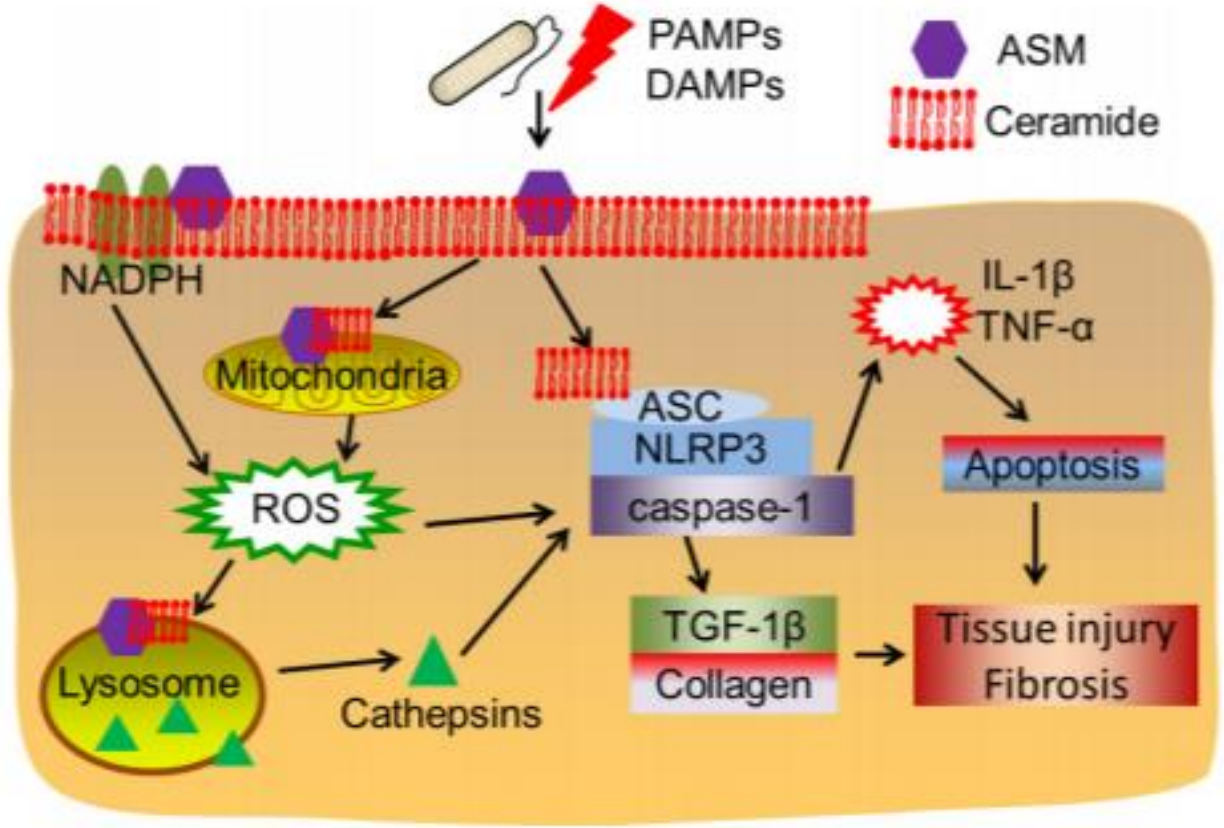
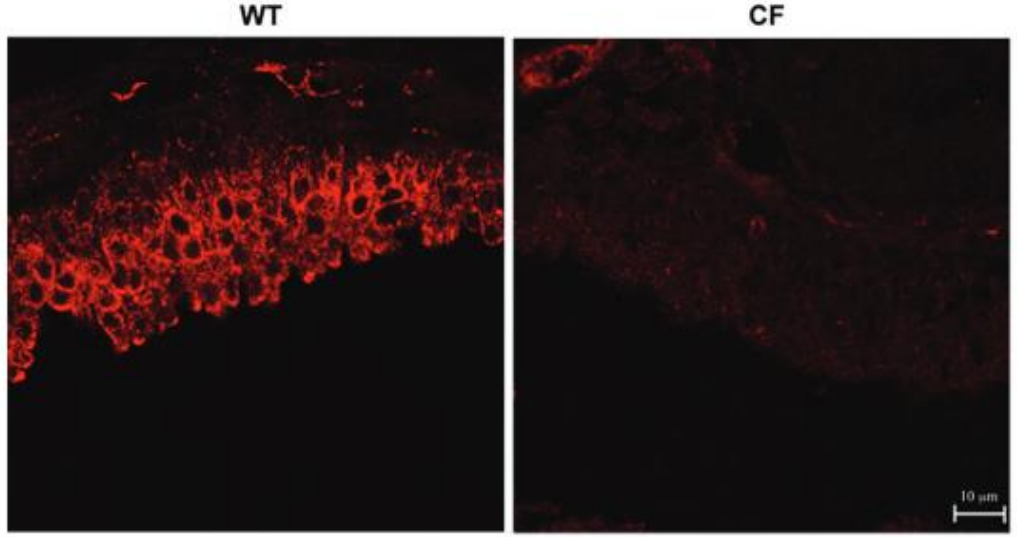


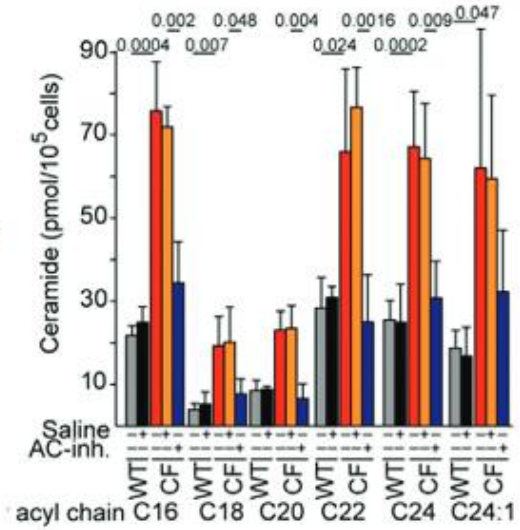
FIGURE 1 | Acid sphingomyelinase/ceramide system in inflammasome signaling and tissue fibrosis. Upon PAMPs or DAMPs exposure, ASM is activated and ceramide enriched domains are formed. Ceramide enriched domains may either directly interact with the ASC-NLRP3-caspase-1 inflammasome complex, or, ASM-ceramide 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

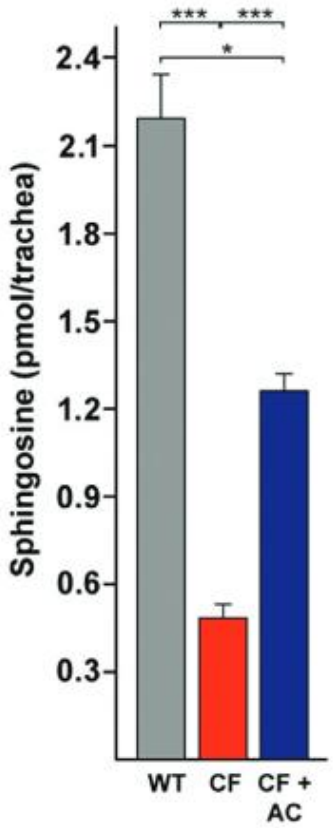
Confocal microscopy

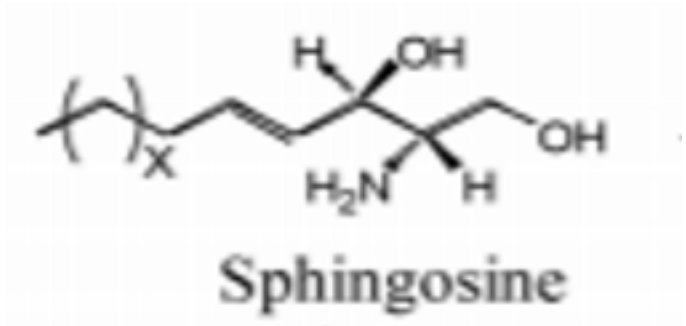


Mass spectrometry



Kinase-assay



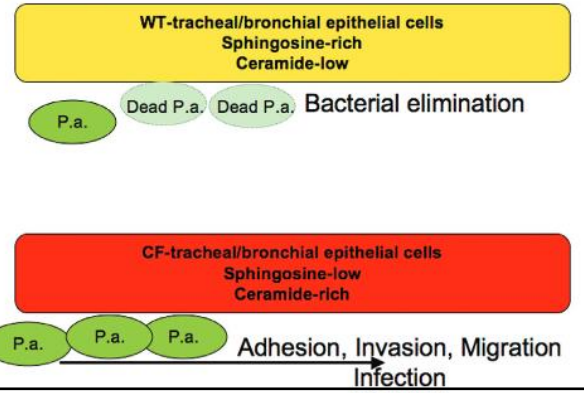


DE GRUYTER Biol. Chem. 2015; 396(6-7): 611–620

Review

Aaron P. Seitz, Heike Grassmé, Michael J. Edwards, Yael Pewzner-Jung and Erich Gulbins*

Ceramide and sphingosine in pulmonary infections



Glucosylceramide Critically Contributes to the Host Defense of Cystic Fibrosis Lungs

Barbara Kovacic[a] Carolin Sehl[a] Barbara Wilker[a] Markus Kamler[b] Erich Gulbins[a,c] Katrin Anne Becker[a]

Abstract

***Background*:** Cystic fibrosis (CF) is the most common autosomal-recessive disorder in western countries. Previous studies have demonstrated an important role of sphingolipids in the pathophysiology of cystic fibrosis. It has been shown that ceramide has a central role in various pulmonary infections, including those with *Pseudomonas aeruginosa* (*P. aeruginosa*). Ceramide is accumulated in the airways of CF mice and patients. However, little is known about a potential role of glucosylceramide in cystic fibrosis. ***Methods*:** We investigated the expression of glucosylceramide and lactosylceramide in the respiratory tract of murine and human CF samples by immunohistochemistry and analyzed effects of glucosylceramide on *P. aeruginosa in vitro*. We performed pulmonary infections with *P. aeruginosa* and tested inhalation with glucosylceramide. ***Results*:** We demonstrate that glucosylceramide is down-regulated on the apical surface of bronchial and tracheal epithelial cells in cystic fibrosis mice. Although glucosylceramide did not have a direct bactericidal effect on *Pseudomonas aeruginosa in vitro*, inhalation of CF mice with glucosylceramide protected these mice from infection with *P. aeruginosa*, while non-inhaled CF mice developed severe pneumonia. ***Conclusion*:** Our data suggest that glucosylceramide acts *in vivo* in concert with ceramide and sphingosine to determine the pulmonary defense against *P. aeruginosa*.

Fig. 2. Immunohistochemical analysis of glucosylceramide in human lungs. Human lung biopsies were stained with anti-GlcCer-antibody (A, B). Panel B shows a magnification of an area of interest from panel A. Samples were analyzed by confocal microscopy with a 63x objective. Shown are representative images from 3 human lungs.

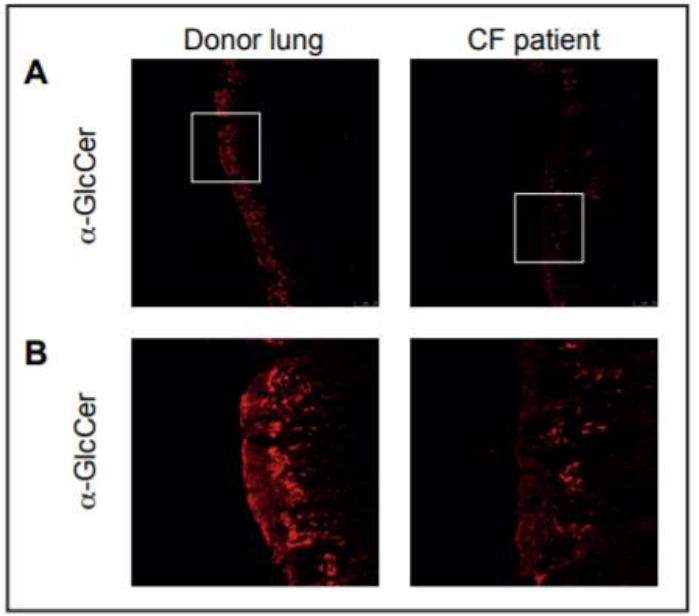
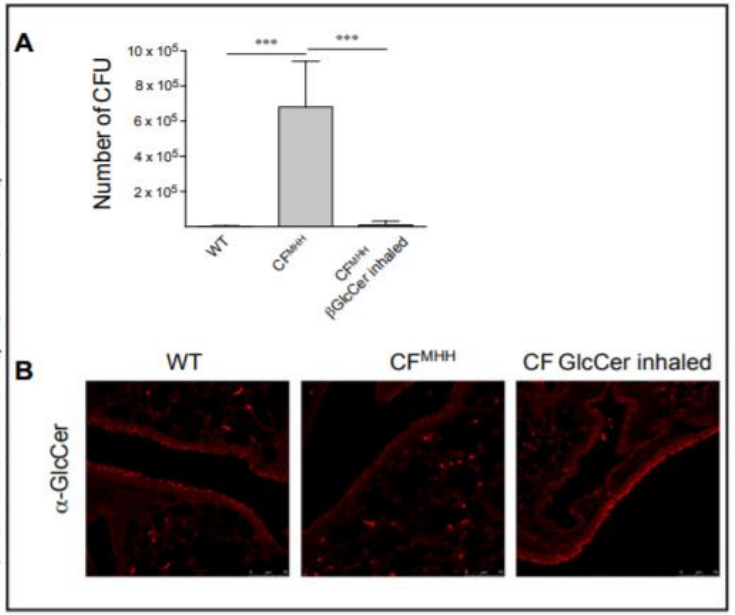


Fig. 4. Inhalation of β-glucosylceramide prevents *in vivo* infection with *P. aeruginosa*. Inhalation of β-GlcCer significantly reduced the number of bacteria in cystic fibrosis (CF) lungs (A) and elevated levels of GlcCer in apical membrane of CF bronchial epithelial cells (B). CF mice were inhaled with β-GlcCer 30 min prior to infection with *P. aeruginosa*. Infection was compared to that in non-inhaled CF and of WT mice. The bacterial load of lungs was determined 2 hrs after infection of the mice. Displayed are means ± SD, n≥3, ***p<0.001, ANOVA and Bonferroni post-hoc test (A). Sections of lungs of a different set of mice that were sacrificed 30 min after inhalation of β-GlcCer were stained with anti-GlcCer (B).

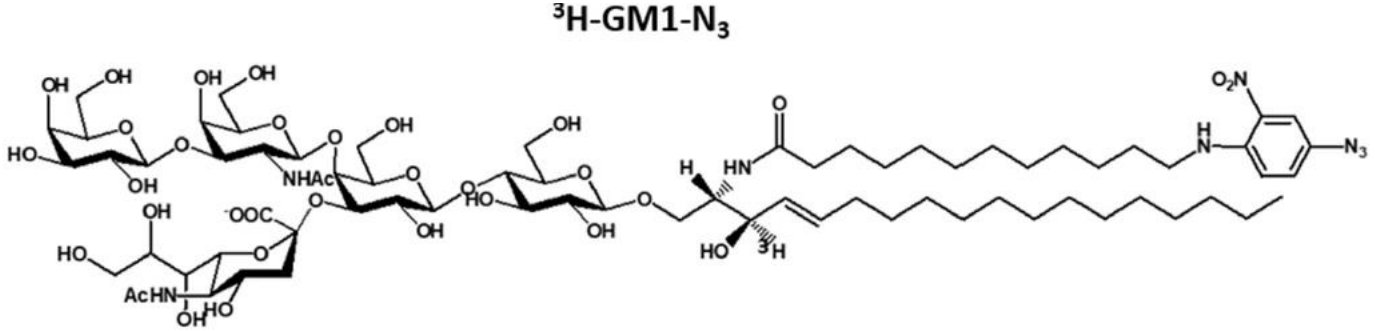


Cell stress induces the membrane-based sphingomyelin pathway, whereas p53-dependent apoptosis occurs secondary to DNA damage.

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,*]

a



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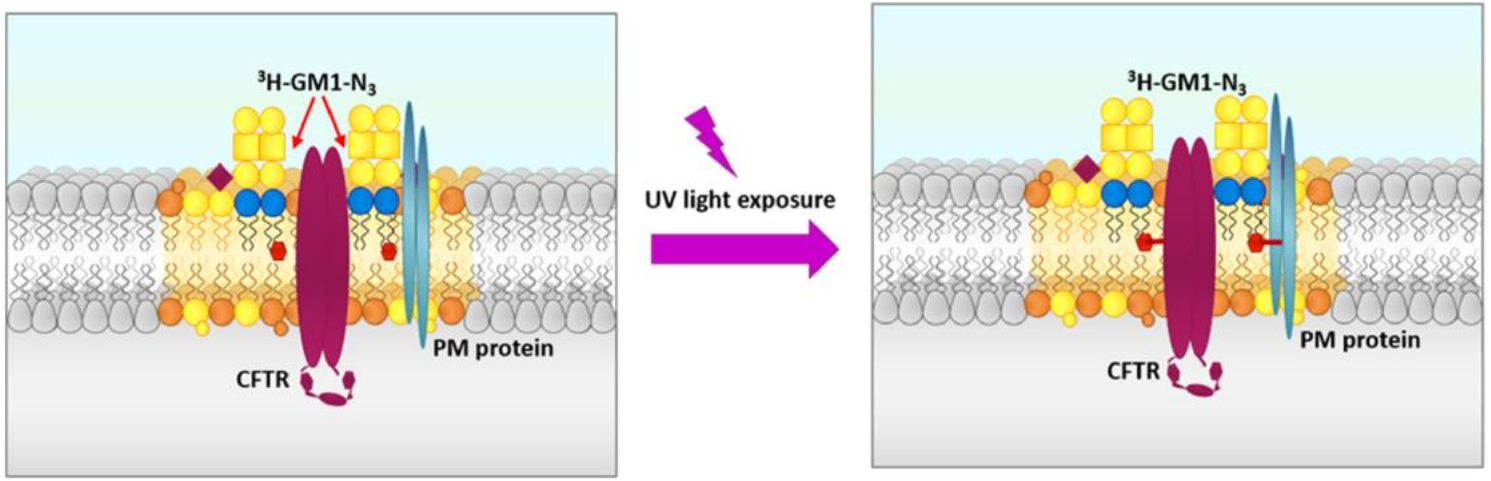

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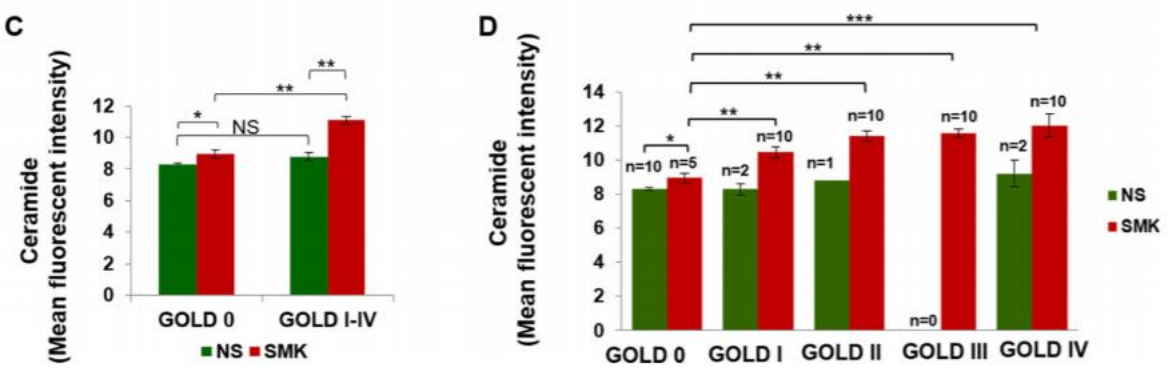
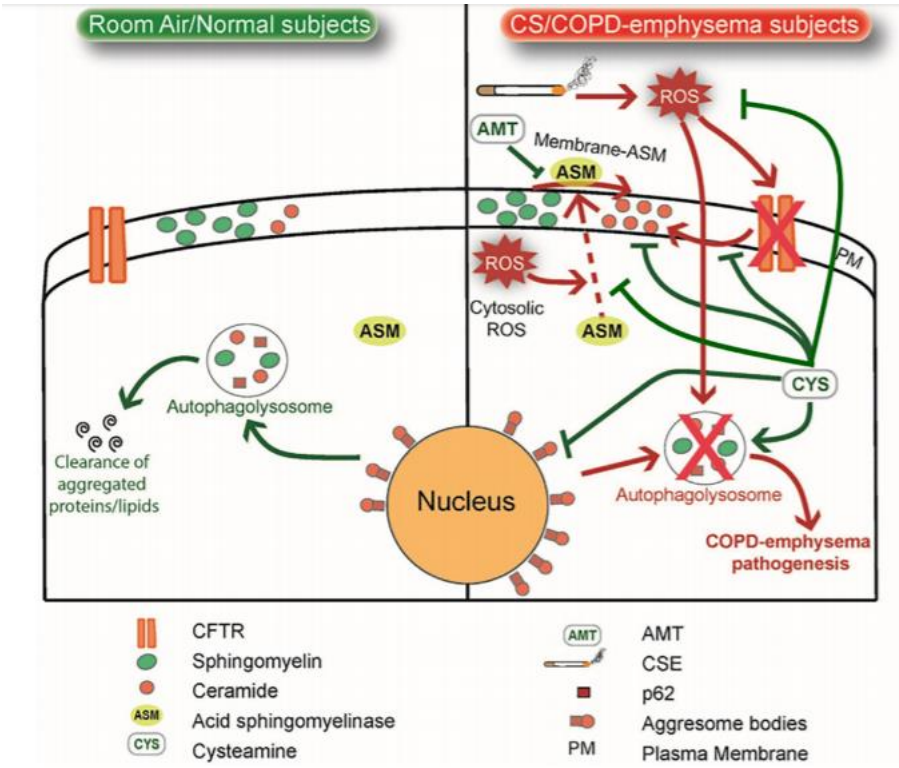
Original article

Autophagy augmentation alleviates cigarette smoke-induced CFTR-dysfunction, ceramide-accumulation and COPD-emphysema pathogenesis

Manish Bodas[a], Garrett Pehote[a], David Silverberg[a], Erich Gulbins[b], Neeraj Vij[a,c,d,*]

In this study, we aimed to investigate precise mechanism(s) of sphingolipid-imbalance and resulting ceramide-accumulation in COPD-emphysema. 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-emphysema/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/lipophagy-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 redox impaired-autophagy dependent membrane-ceramide accumulation. In summary, our data shows that CS-mediated autophagy/lipophagy-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-emphysema pathogenesis that is alleviated by treatment with cysteamine, a potent anti-oxidant with CFTR/autophagy-augmenting properties.





Ceramide and Sphingosine 1-Phosphate in Liver Diseases

Woo-Jae Park[1,*], Jae-Hwi Song[2], Goon-Tae Kim[2], and Tae-Sik Park[2,*]

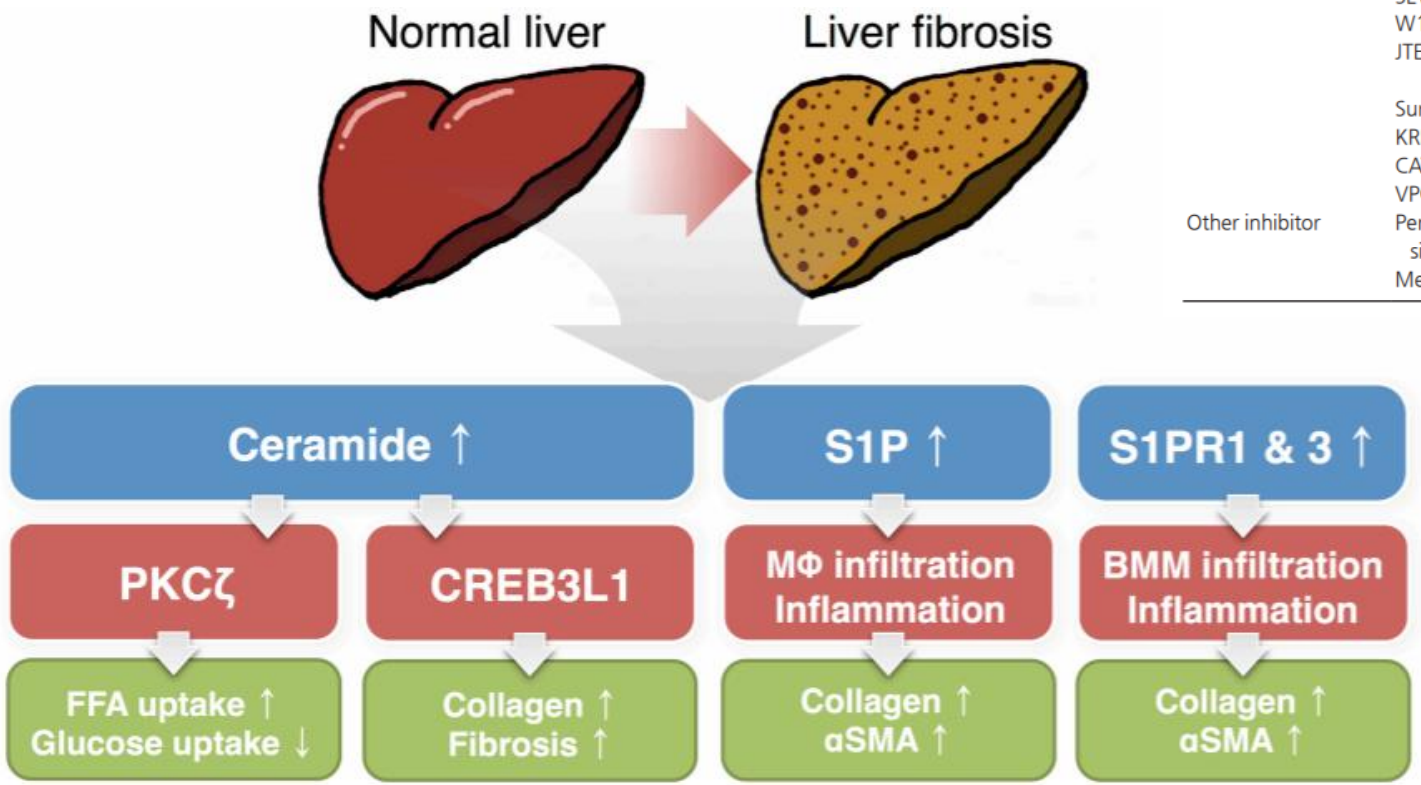
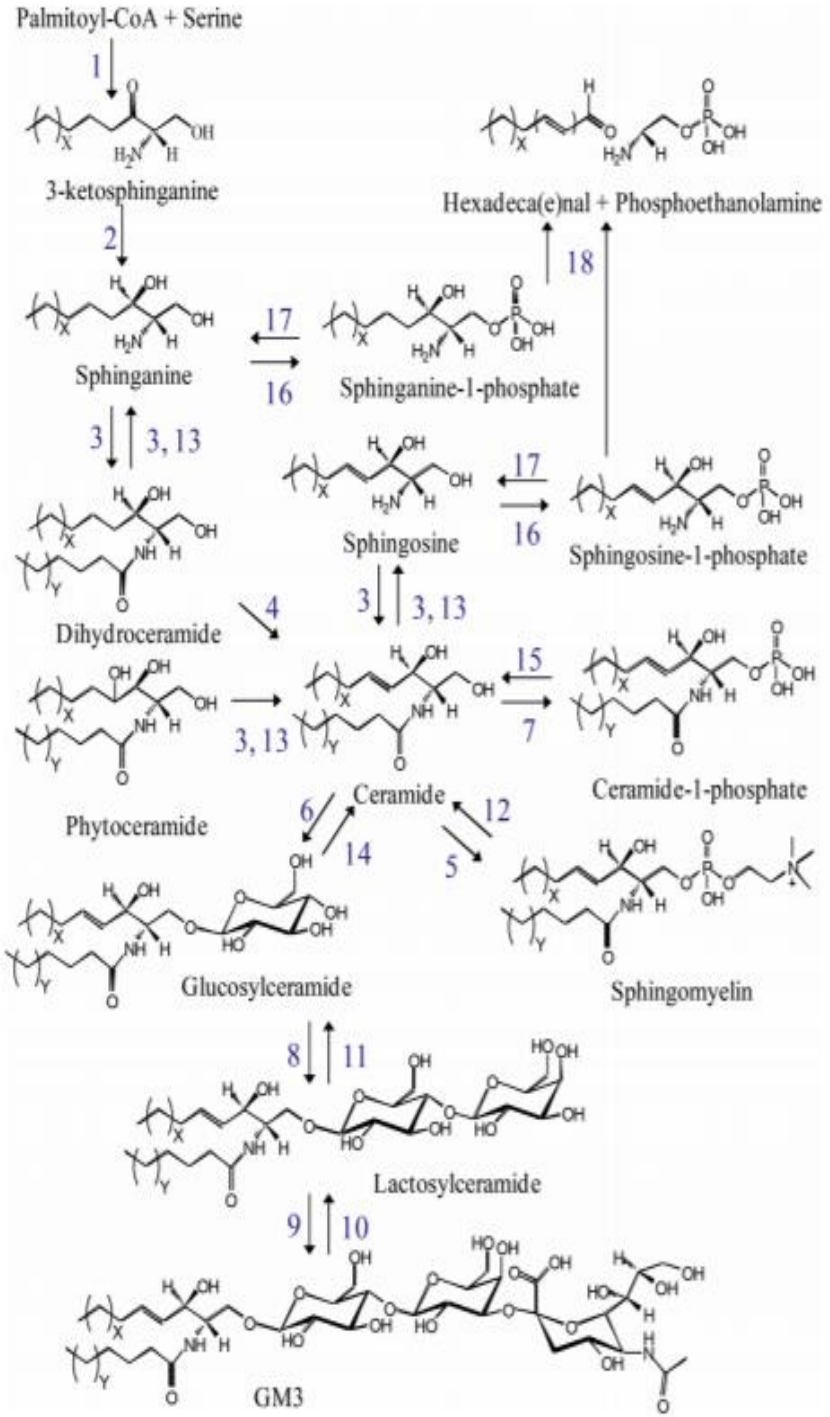


Table 2. The candidate drugs/agents of antifibrotic activity

Action	Drug/agent	Reference
Sphingosine kinase inhibitor	PF543 (SphK inhibitor)	(Gonzalez-Fernandez et al., 2017)
	SKI-II (SphK inhibitor, non-selective)	(Yang et al., 2013)
	N,N-dimethylsphingosine (DMS, SphK inhibitor)	(Brunati et al., 2008; Wang et al., 2017b; Xiu et al., 2015)
S1P receptor agonist/ antagonist	FTY720 (S1PR1 and S1PR3 agonist)	(Brunati et al., 2008; King et al., 2017; Kong et al., 2014)
	VPC23019 (S1PR1 and S1PR3 antagonist)	(Brunati et al., 2008; Yang et al., 2012; 2013)
	SEW2871 (S1PR1 agonist)	(Ding et al., 2016)
	W146 (S1PR1 antagonist)	(King et al., 2017; Liu et al., 2011; Yang et al., 2012; 2013)
	JTE-013 (S1PR2 antagonist)	(Kageyama et al., 2012; Wang et al., 2017a; Xu et al., 2016; Yang et al., 2015)
	Suramin (S1PR3 antagonist)	(Li et al., 2009a; 2009b)
	KRP203 (FTY720 analog)	(Kaneko et al., 2006; Khattar et al., 2013)
	CAY-10444 (S1PR3 antagonist)	(Yang et al., 2015)
	VPC24191 (S1PR1 and S1PR3 antagonist)	(Al Fadel et al., 2016)
Other inhibitor	Pertussis toxin (PTX; G-protein-coupled receptor signaling inhibitor)	(Brunati et al., 2008; Gonzalez-Fernandez et al., 2017; Yang et al., 2015)
	Melatonin (melatonin receptors agonist)	

"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 Diferent roles and sites of action of ceramide.

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

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, b, *]

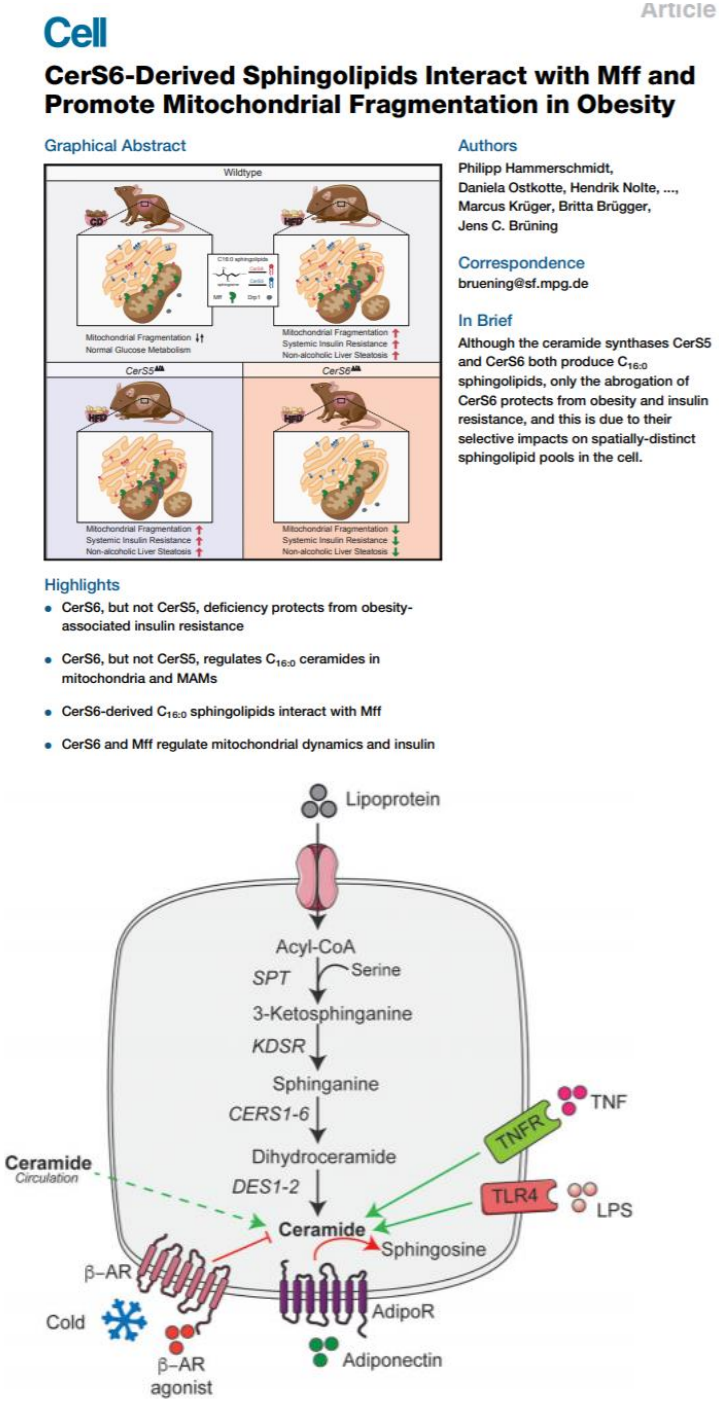
Ceramides in Adipose Tissue

Ying Li[1], Chad Lamar Talbot[1] and Bhagirath Chaurasia[1,2]*

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)

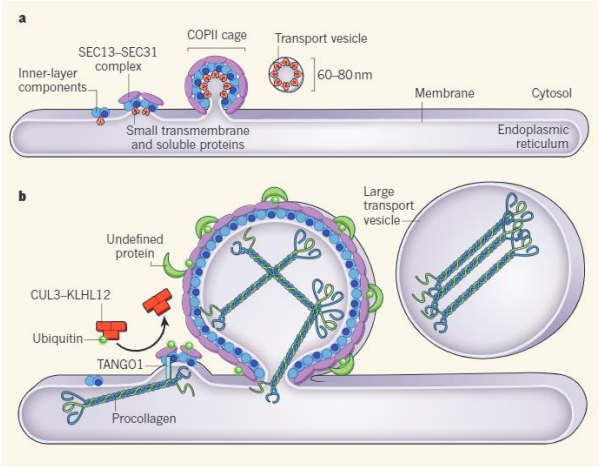


Inhibiting Ceramide Synthesis Attenuates Hepatic Steatosis and Fibrosis in Rats With Non-alcoholic Fatty Liver Disease

*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 ameliorated 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[a,b,*], Irene Pierantonelli[a,c,1], Pierangelo Torquato[d,1], Rita Marinelli[d], Carla Ferreri[e], Chryssostomos Chatgilialoglu[e], Desirée Bartolini[d], Francesco Galli[d]

FIBROSIS

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

Sarah Alsamman[1], Stephanie A. Christenson[2], Amy Yu[1], Nadia M. E. Ayad[3,4], Meghan S. Mooring[5], Joe M. Segal[1], Jimmy Kuang-Hsien Hu[6], Johanna R. Schaub[7], Steve S. Ho[7], Vikram Rao[7], Megan M. Marlow[7], Scott M. Turner[7], Mai Sedki[8], Lorena Pantano[9], Sarani Ghoshal[10], Diego Dos Santos Ferreira[11], Hsiao-Yen Ma[12], Caroline C. Duwaerts[1,13], Regina Espanol-Suner[14], Lan Wei[10], Benjamin Newcomb[15], Izolda Mileva[15], Daniel Canals[15], Yusuf A. Hannun[15], Raymond T. Chung[16], Aras N. Mattis[13,17], Bryan C. Fuchs[10], Andrew M. Tager[18]*, Dean Yimlamai[5], Valerie M. Weaver[3,4,14,19,20,21], Alan C. Mullen[16], Dean Sheppard[2,12†], Jennifer Y. Chen[1,13†]

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

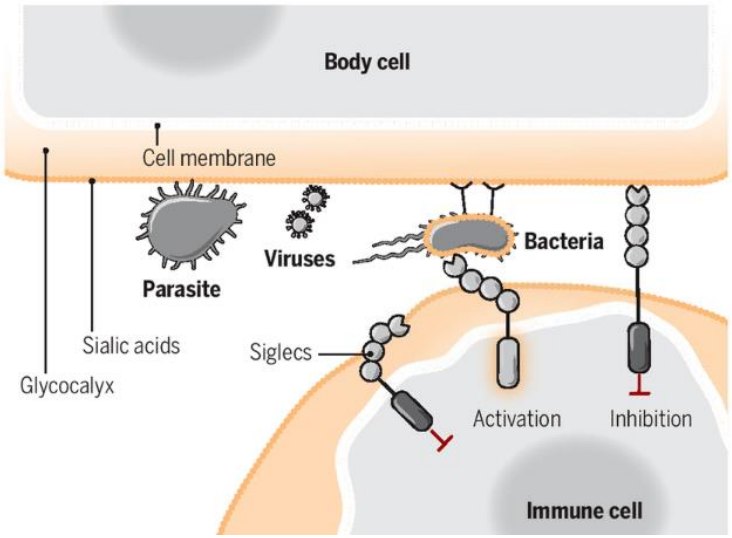
HUMAN EVOLUTION

How an ancient microbial arms race remodeled human cells

By Ann Gibbons

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 (Siglecs) into inhibiting immune responses. But other Siglecs can instead turn on an immune response if they sense sialic acids on pathogens.



VIEWPOINT: COVID-19

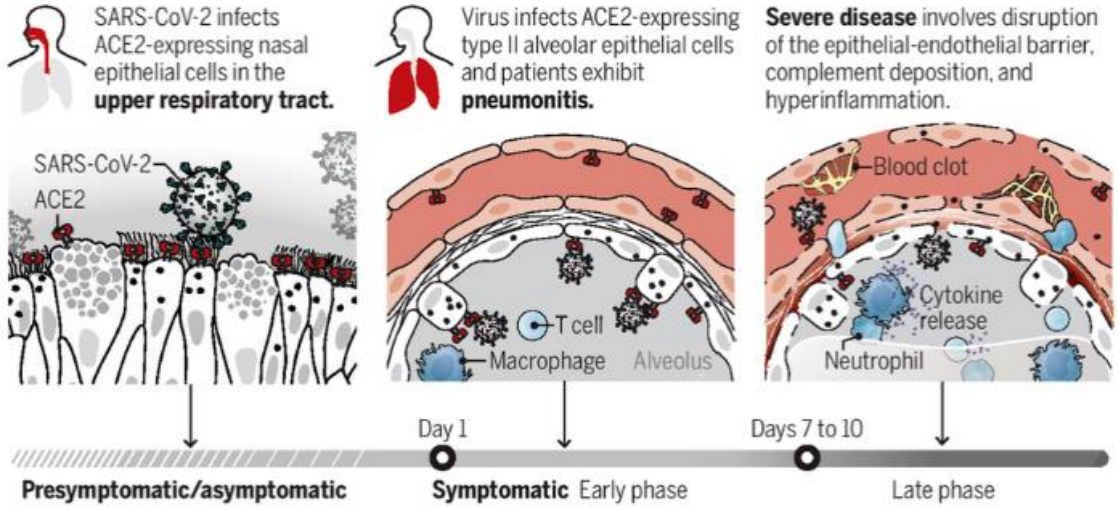
How does SARS-CoV-2 cause COVID-19?

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

By Nicholas J. Matheson[1,2] and Paul J. Lehner[1]

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 pneumonitis, 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.



Altered Lipid Metabolism in Recovered SARS Patients Twelve Years after Infection

SCIENTIFIC REPORTS | 7: 9110 | DOI:10.1038/s41598-017-09536-z

Qi Wu[1], Lina Zhou[2], Xin Sun[3], Zhongfang Yan[4], Chunxiu Hu[2], Junping Wu[3], Long Xu[3], Xue Li[5], Huiling Liu[6], Peiyuan Yin[2], Kuan Li[5], Jieyu Zhao[2], Yanli Li[2], Xiaolin Wang[2], Yu Li[5], Qiuyang Zhang[5], Guowang Xu[2] & Huaiyong Chen[1,5]

Severe acute respiratory syndrome-coronavirus (SARS-CoV) and SARS-like coronavirus are a potential threat to global health. However, reviews of the long-term effects of clinical treatments in SARS patients are lacking. Here a total of 25 recovered SARS patients were recruited 12 years after infection. Clinical questionnaire responses and examination findings indicated that the patients had experienced various diseases, including lung susceptibility to infections, tumors, cardiovascular disorders, and abnormal glucose metabolism. As compared to healthy controls, metabolomic analyses identified significant differences in the serum metabolomes of SARS survivors. The most significant metabolic disruptions were the comprehensive increase of phosphatidylinositol and lysophospha tidylinositol levels in recovered SARS patients, which coincided with the effect of methylprednisolone administration investigated further in the steroid treated non-SARS patients with severe pneumonia. These results suggested that high-dose pulses of methylprednisolone might cause long-term systemic damage associated with serum metabolic alterations. The present study provided information for an improved understanding of coronavirus-associated pathologies, which might permit further optimization of clinical treatments.

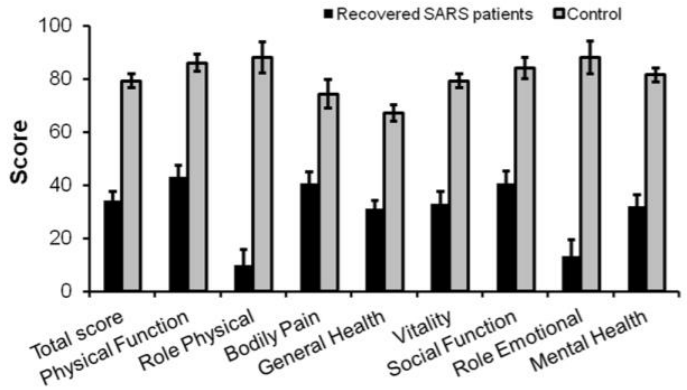
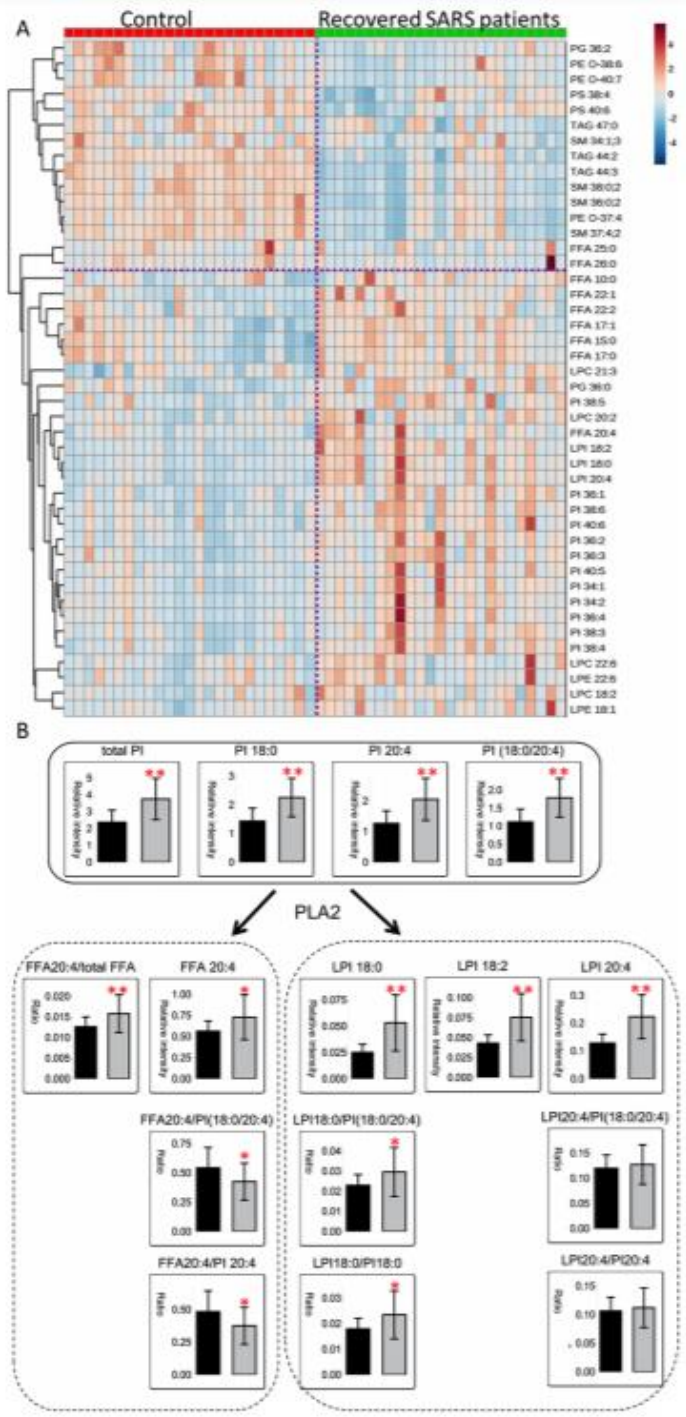


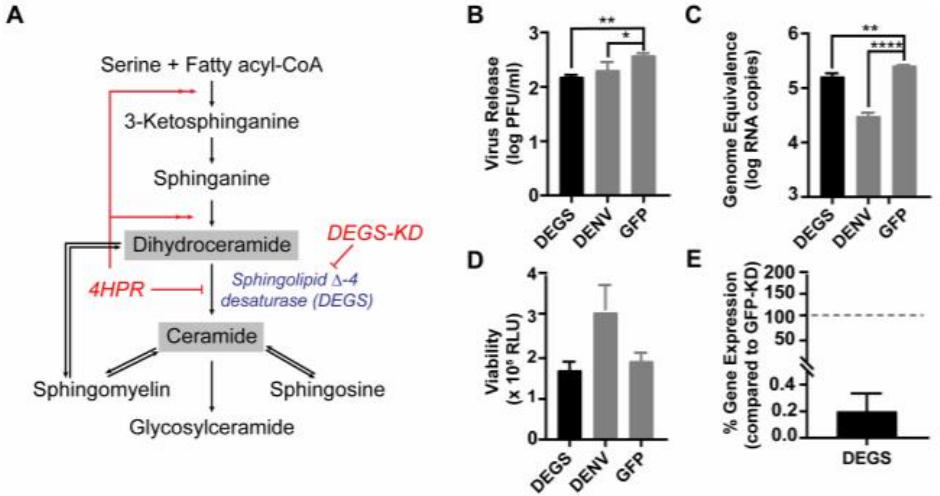
Figure 1. MOS 36-item Short Form Health Survey (SF-36) results for severe recovered respiratory syndrome patients 12 years after recovery.

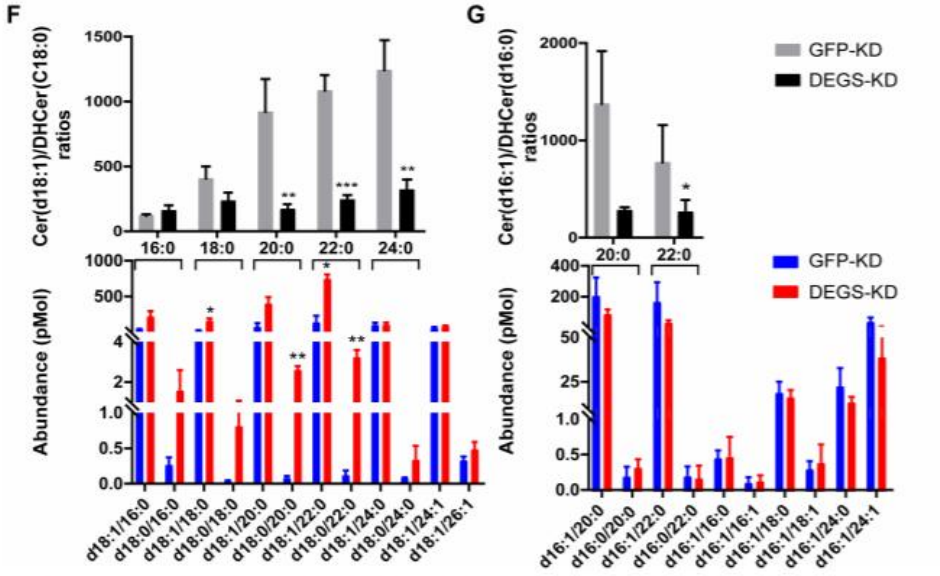
25 patients recovered from SARS-CoV-1 had very poor health, only partially recovered lung function, and major changes in serum metabolomics due to changes in lipid metabolism:
Lipid metabolomics are critical markers

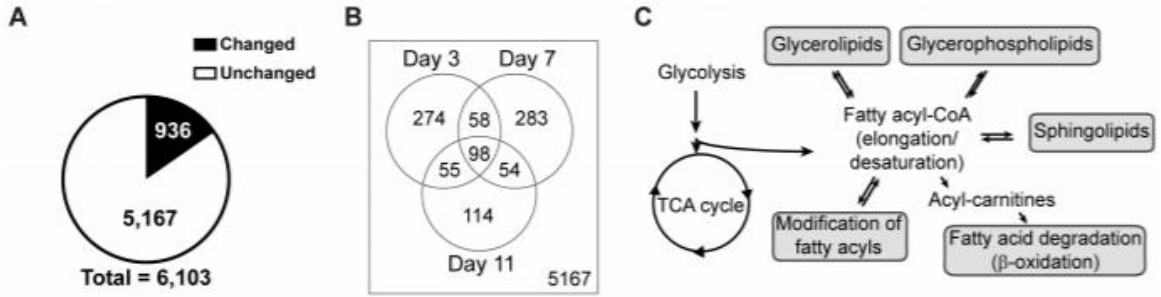


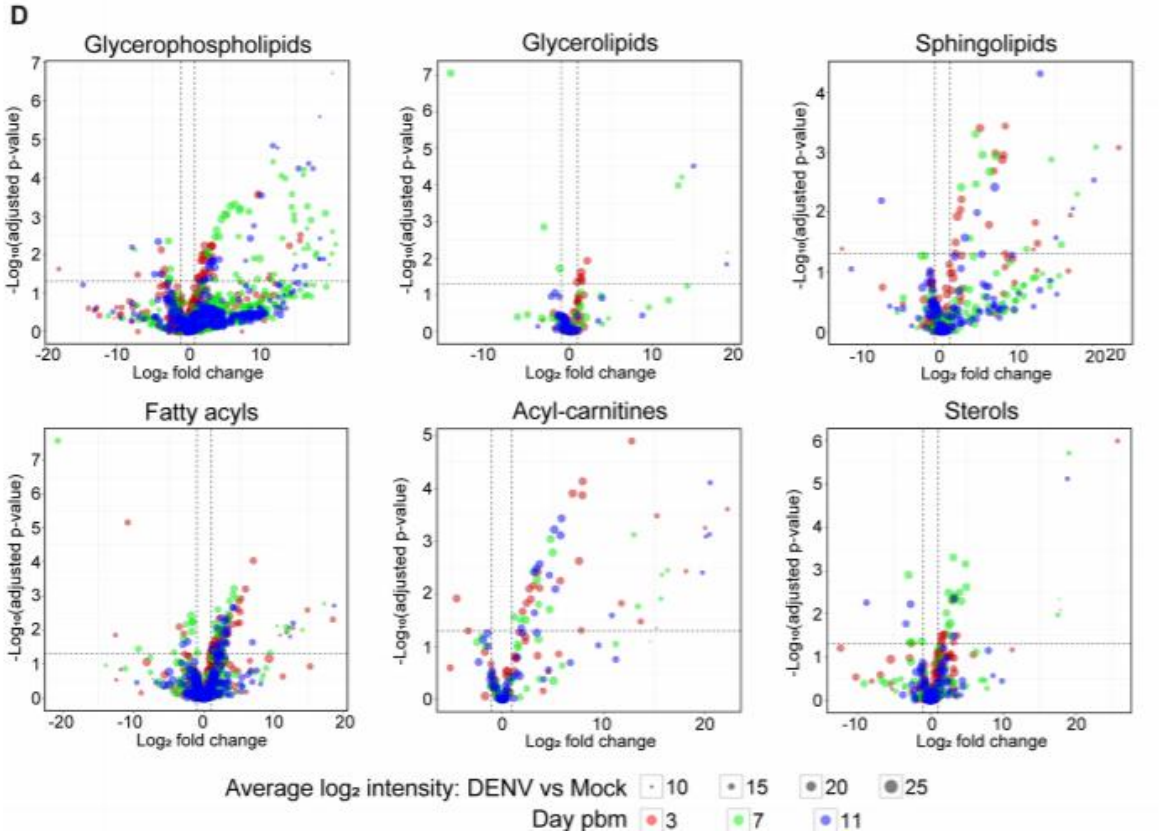
Dynamic remodeling of lipids coincides with dengue virus replication in the midgut of *Aedes aegypti* mosquitoes

Nunya Chotiwan[1], Barbara G. Andre[1], Irma Sanchez-Vargas[1], M. Nurul Islam[1], Jeffrey M. Grabowski[2,3¤a], Amber Hopf-Jannasch[4], Erik Gough[5], Ernesto Nakayasu[4¤b], Carol D. Blair[1], John T. Belisle[1], Catherine A. Hill[3,6], Richard J. Kuhn[2,6], Rushika Perera[1]*







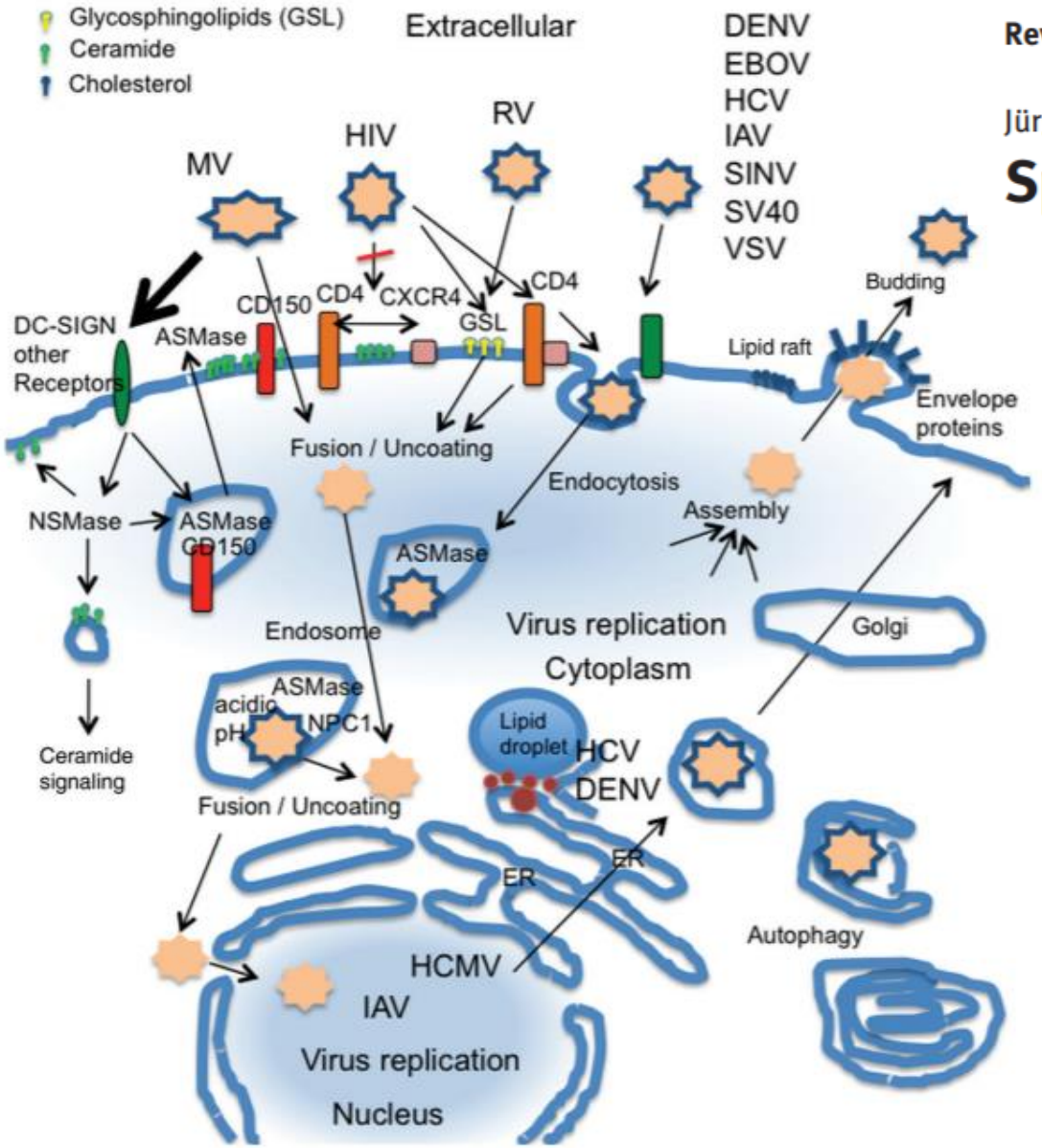


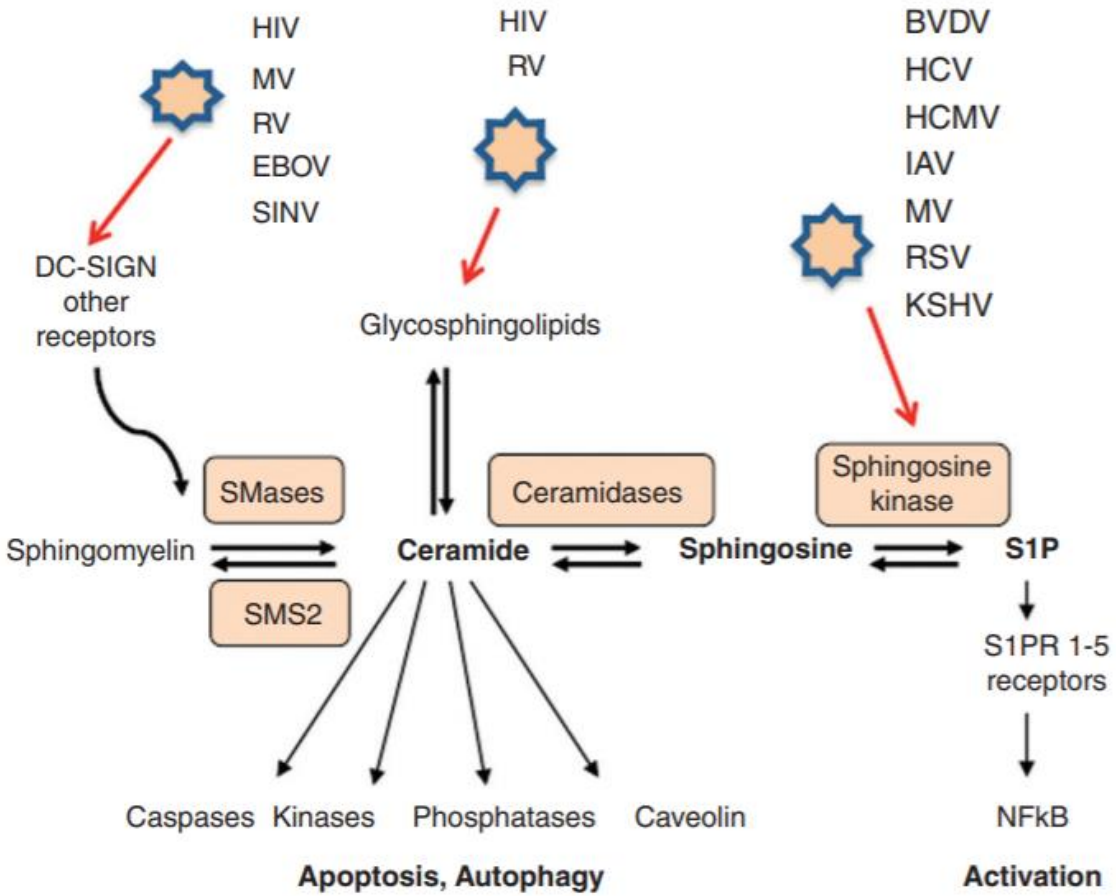
DE GRUYTER Biol. Chem. 2015; 396(6-7): 585–595

Review

Jürgen Schneider-Schaulies* and Sibylle Schneider-Schaulies

Sphingolipids in viral infection

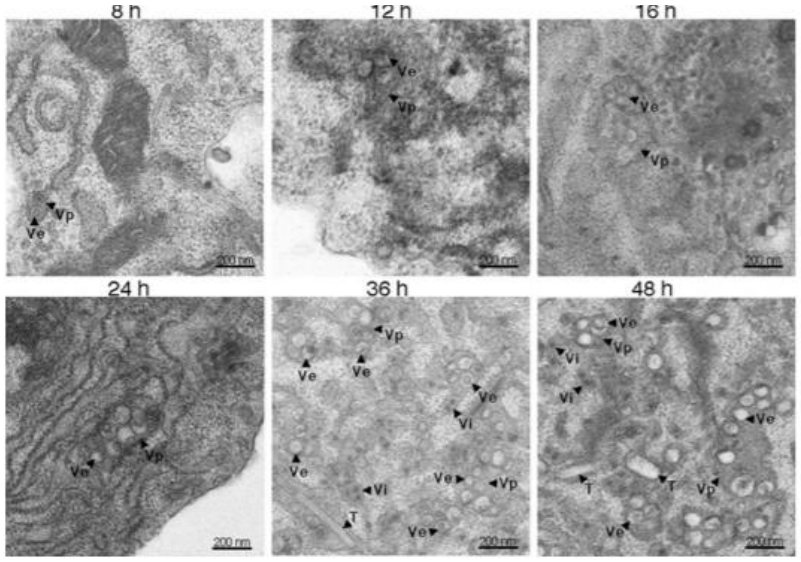




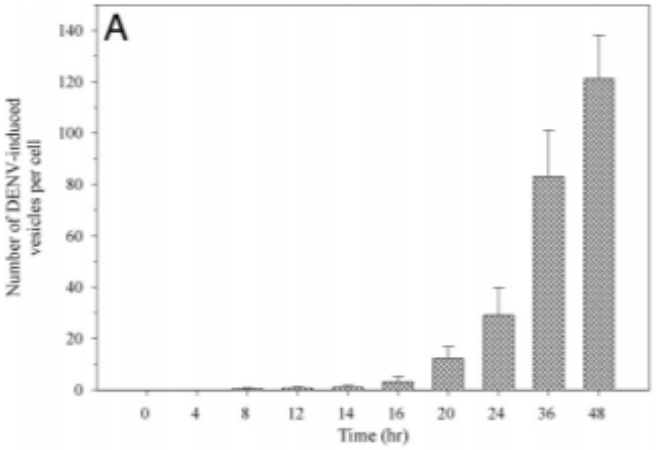
Ultrastructural Characterization and Three-Dimensional Architecture of Replication Sites in Dengue Virus-Infected Mosquito Cells

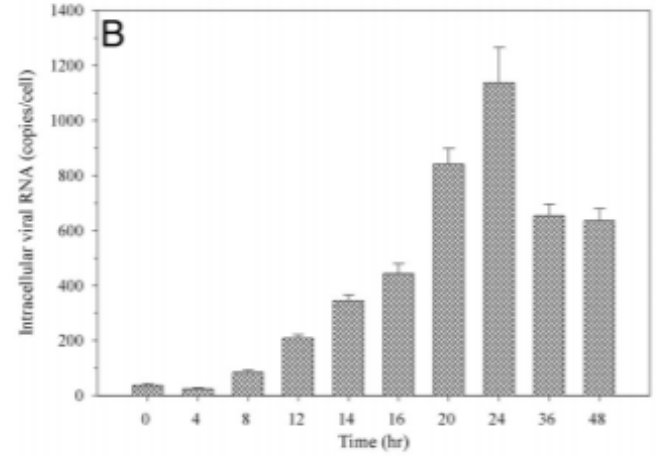
Jiraphan Junjhon,[a*] Janice G. Pennington,[a*] Thomas J. Edwards,[a] Rushika Perera,[a*] Jason Lanman,[a] Richard J. Kuhn[a,b]

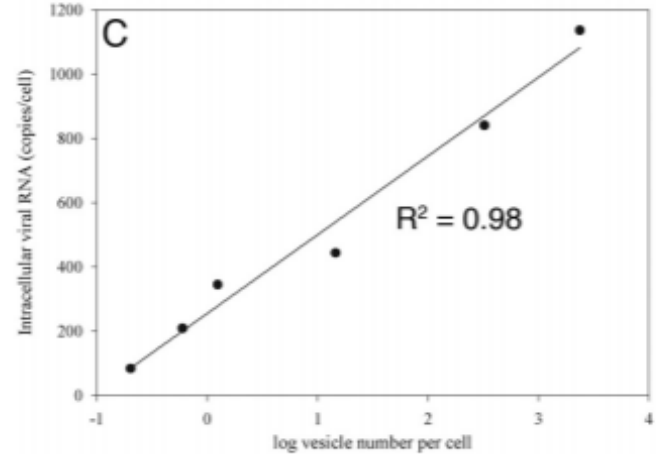
Markey Center for Structural Biology, Department of Biological Sciences, Purdue University, West Lafayette, Indiana, USA[a]; Bindley Bioscience Center, Purdue University, West Lafayette, Indiana, USA[b]



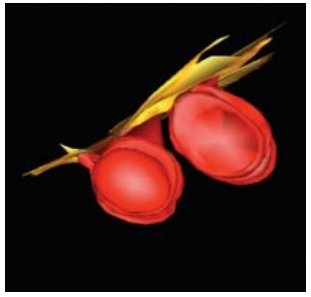
Time-dependent 'chaos' in the endoplasmic-reticulum/Golgi after infection







Reconstruction of Viral particles budding from the ER



SARS-CoV-1 – ER budding

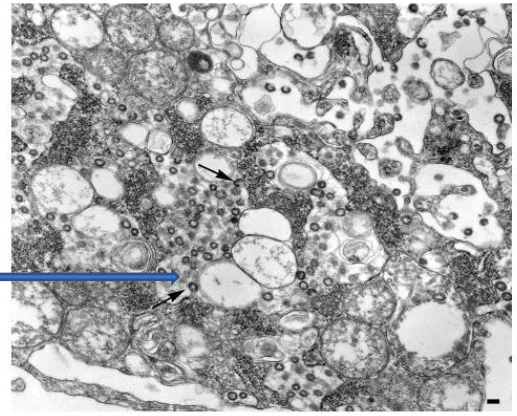


FIG. 12. Thin section of an RNA virus produced in the cytoplasm. SARS coronavirus particles (arrows) obtain their envelope by budding through the membranes of the endoplasmic reticulum. Bar, 100 nm. Magnification, ×20,000.

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Modern Uses of Electron Microscopy for Detection of Viruses

Cynthia S. Goldsmith[1]* and Sara E. Miller[2]

Infectious Disease Pathology Branch, Centers for Disease Control and Prevention, Atlanta, Georgia,[1] and Department of Pathology, Duke University Medical Center, Durham, North Carolina[2]

Quantitative analysis of the lipidomes of the influenza virus envelope and MDCK cell apical membrane

Mathias J. Gerl,[1] Julio L. Sampaio,[1] Severino Urban,[1] Lucie Kalvodova,[1] Jean-Marc Verbavatz,[1] Beth Binnington,[2] Dirk Lindemann,[3] Clifford A. Lingwood,[2,4,5] Andrej Shevchenko,[1] Cornelia Schroeder,[1] and Kai Simons[1]

[1]Max Planck Institute of Molecular Cell Biology and Genetics, 01307 Dresden, Germany
[2]Research Institute, Division of Molecular Structure and Function, The Hospital for Sick Children, Toronto, Ontario M5G 1X8, Canada
[3]Institut für Virologie, Medizinische Fakultät Carl Gustav Carus, Technische Universität Dresden, 01062 Dresden, Germany
[4]Department of Biochemistry and [5]Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Ontario M5S 1A1, Canada

The influenza virus (IFV) acquires its envelope by budding from host cell plasma membranes. Using quantitative shotgun mass spectrometry, we determined the lipidomes of the host Madin–Darby canine kidney cell, its apical membrane, and the IFV budding from it. We found the apical membrane to be enriched in sphingolipids (SPs) and cholesterol, whereas glycerophospholipids were reduced, and storage lipids were depleted compared with the whole-cell membranes. The virus membrane exhibited a further enrichment of SPs and cholesterol compared with the donor membrane at the expense of phosphatidylcholines. Our data are consistent with and extend existing models of membrane raft-based biogenesis of the apical membrane and IFV envelope.

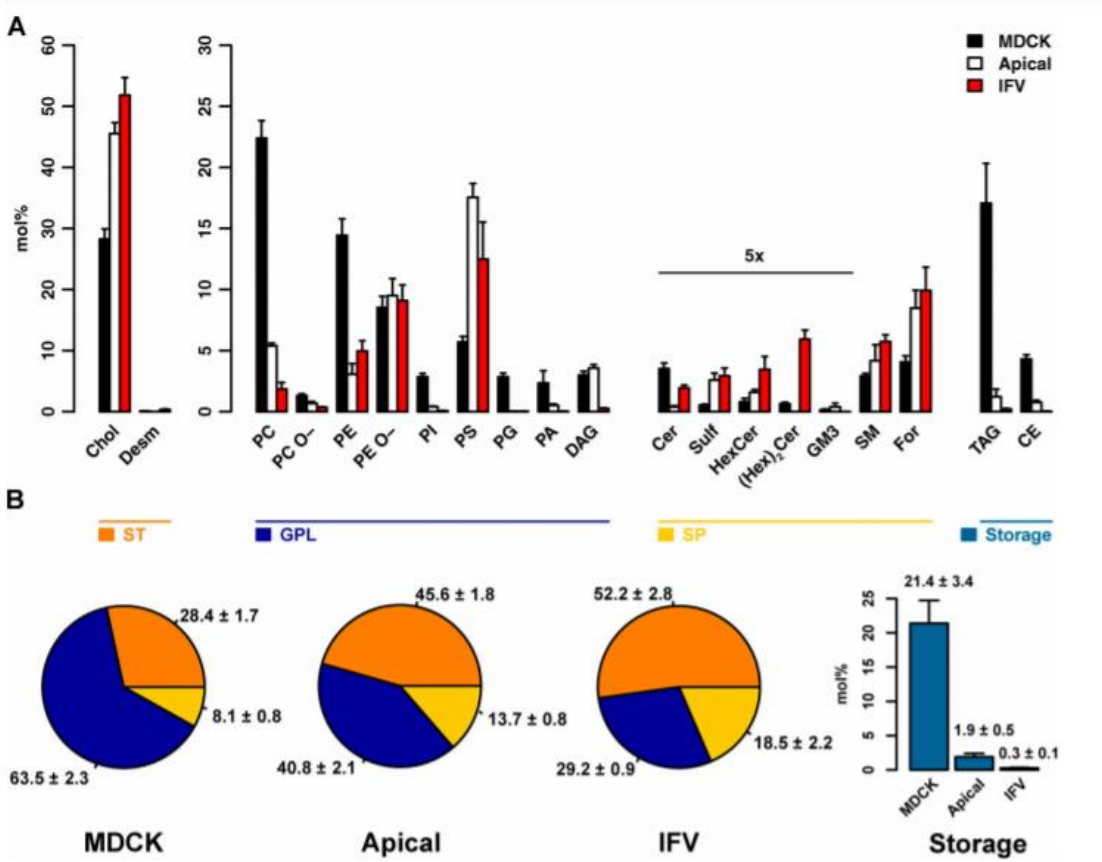


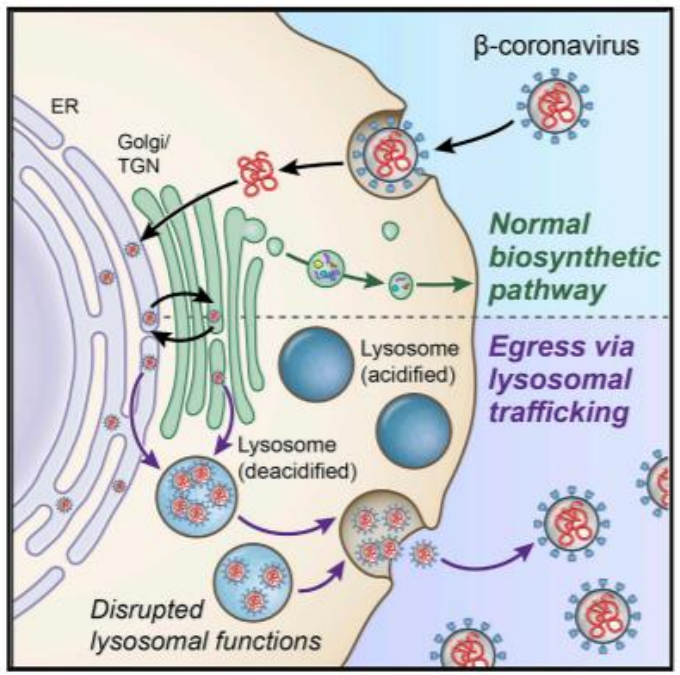
Figure 2. **Lipid class composition and functional categories.** (A and B) The content of individual lipid classes (A) and functional lipid categories (B) of total cells (MDCK), the AP (Apical), and influenza virus (IFV) was determined by summing up absolute abundances of all identified species. Values are standardized to mole percentage of all membrane lipids within the sample without storage lipids. Indicated values have been multiplied by five for visibility. Sterols (ST): cholesterol (Chol) and desmosterol (Desm). GPLs: DAG, phosphatidic acid (PA), phosphatidylcholine/-ethanolamine/-glycerol/-inositol/-serine (PC/PE/PG/PI/PS), and ether linked PC/PE (PC O-/PE O-). Sphingolipids (SP): ceramide (Cer), Forssman glycolipid (For), hexosylceramide (HexCer), dihexosylceramide ($(Hex)_2Cer$), sphingomyelin (SM), and sulfatide (Sulf). Storage: cholesterol esters (CE) and triacylglycerol (TAG). Error bars correspond to SDs (n = 3).

- ***Budding from lipid rafts.***
- ***'the outer influenza virus leaflet should consist almost entirely of sphingolipids and cholesterol'***
- ***Enrichment of sphinglipids (Lac-Cer) – from GM3.***
- ***The presence of neuraminidase on envelope (to 'deforest' host cell glycocalyx) will also affect the envelope GSLs (eg GM3).***

Cell

β-Coronaviruses Use Lysosomes for Egress Instead of the Biosynthetic Secretory Pathway

Graphical Abstract



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. Dellibovi-Ragheb,
Adeline Kerviel, ..., 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.)

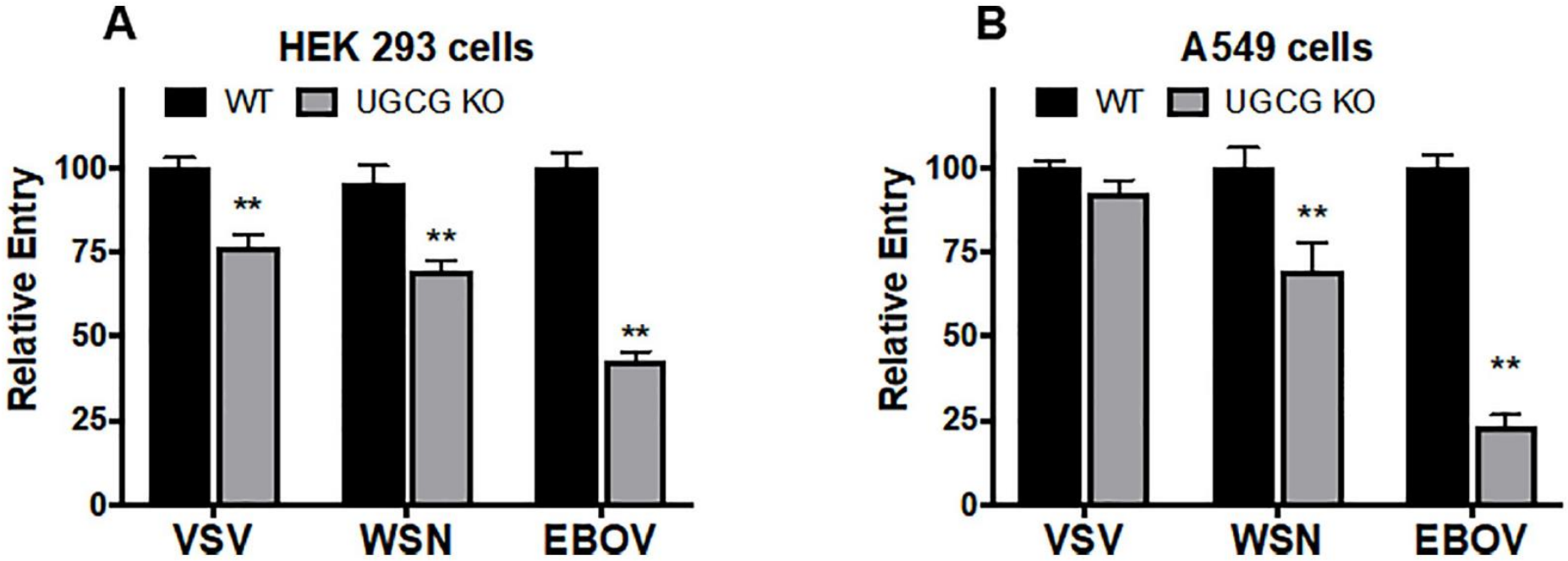
In Brief

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 Drews[1], Michael P. Calgi[2], William Casey Harrison[2¤a], Camille M. Drews[1], Pedro Costa-Pinheiro[1], Jeremy Joseph Porter Shaw[1], Kendra A. Jobe[3¤b], John D. Han[4], Todd E. Fox[5], Judith M. White[6,7], Mark Kester[1,2,5]*

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.



Glucosylceramidase Maintains Influenza Virus Infection by Regulating Endocytosis

Kelly Drews,[a] Michael P. Calgi,[b] William Casey Harrison,[b]* Camille M. Drews,[a] Pedro Costa-Pinheiro,[a] Jeremy Joseph Porter Shaw,[a] Kendra A. Jobe,[c]* Elizabeth A. Nelson,[d] John D. Han,[e] Todd Fox,[f] Judith M. White,[d,g] Mark Kester[a,b,f]

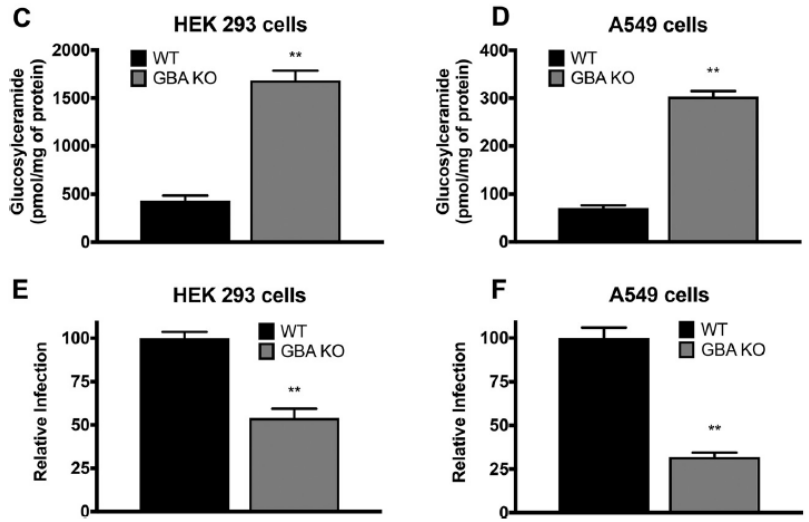
ABSTRACT Influenza virus is an RNA virus encapsulated in a lipid bilayer derived from the host cell plasma membrane. Previous studies showed that influenza virus infection depends on cellular lipids, including the sphingolipids sphingomyelin and sphingosine. Here we examined the role of a third sphingolipid, 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.

A

	HEK 293 cells (pmol/mg of protein)		
	WT	GBA KO	Fold Change
Glucosylceramide	430.00	1664.37	3.87
Glucosylsphingosine	0.70	55.50	79.29
Dihydrosphingosine	5.15	13.43	2.61
Dihydrosphingosine-1-Phosphate	0.90	1.15	1.28
Ceramide	790.87	1008.08	1.27
Sphingosine	113.22	134.92	1.19
Sphingomyelin	13011.30	14909.43	1.15
Sphingosine-1-Phosphate	1.84	1.60	0.87

B

	A549 cells (pmol/mg of protein)		
	WT	GBA KO	Fold Change
Glucosylceramide	71.33	333.34	4.67
Glucosylsphingosine	0.16	1.67	10.44
Dihydrosphingosine	12.19	25.79	2.12
Dihydrosphingosine-1-Phosphate	0.71	0.99	1.39
Ceramide	87.46	186.55	2.13
Sphingosine	86.26	115.10	1.33
Sphingomyelin	6770.50	5284.65	0.78
Sphingosine-1-Phosphate	0.51	1.45	2.84



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

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https://doi.org/10.1080/07391102.2020.1798813

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

Rupesh V. Chikhale[a], Vivek K. Gupta[b], Gaber E. Eldesoky[c], Saikh M. Wabaidur[c], Shripad A. Patil[b] and Md Ataul Islam[d,e,f]

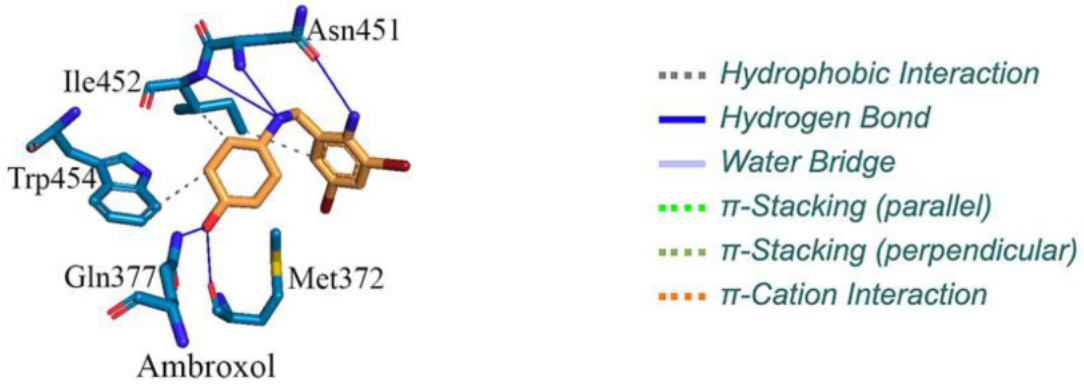


Figure 7. Binding interactions of final phytochemicals with TMPRSS2.

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).

	Virtual screening workflow (VSW)			Molecular dynamics simulations (MDS)	
Molecule name	GScore	DockScore	Prime MM-GBSA (ΔG_{bind})	MM-GBSA (ΔG_{bind})	MM-PBSA (ΔG_{bind})
Natural products					
Neohesperidin	−12.77	−12.77	−66.53	−72.84 (7.73)	−15.83 (8.82)
Myricitrin	−11.52	−11.55	−51.70	−49.52 (3.56)	−7.28 (4.83)
Quercitrin	−10.78	−10.81	−55.01	−50.57 (4.73)	−14.61 (6.90)
Naringin	−10.73	−11.25	−37.11	−50.29 (5.28)	7.45 (7.57)
Icariin	−10.10	−10.11	−56.83	−60.34 (4.26)	−9.52 (8.83)
Standard drugs					
Camostat	−6.23	−4.62	−25.00	−25.00 (2.67)	11.99 (4.44)
Ambroxol	−7.21	−3.61	−44.48	−44.48 (4.94)	−11.36 (7.59)

ΔG_{bind}=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.

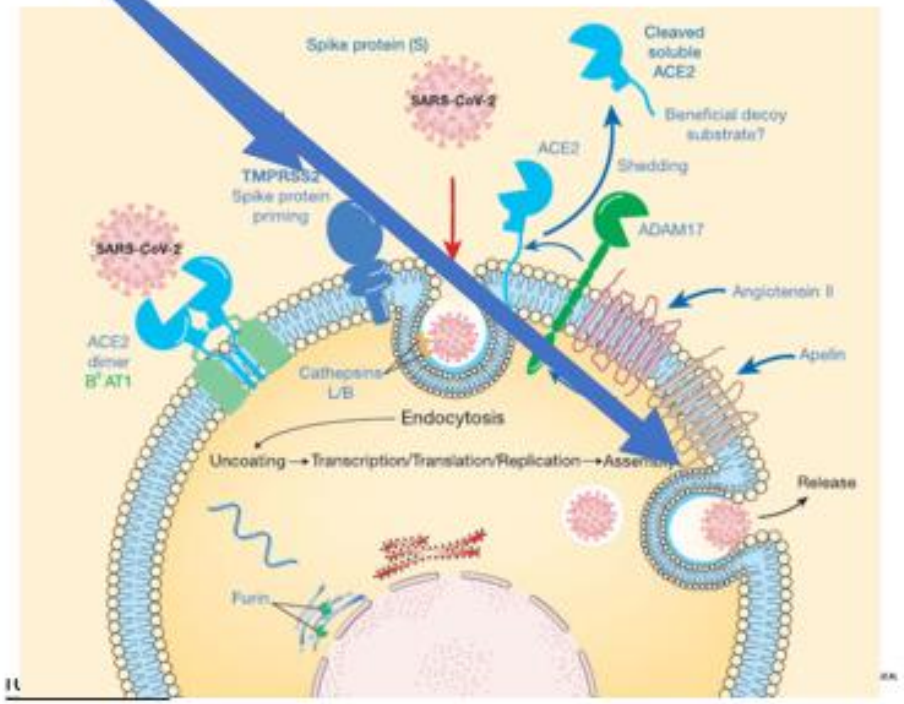
Orphan Drug Designation from EMA for ALS.

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.*
6. *Modulation of mitochondrial function*.

Ambroxol



A rational roadmap for SARS-CoV-2/COVID-19 pharmacotherapeutic research and development: IUPHAR Review 29

Steve P.H. Alexander[1] | Jane F. Armstrong[2] | Anthony P. Davenport[3] | Jamie A. Davies[4] | Elena Faccenda[2] | Simon D. Harding[5] | Francesca Levi-Schaffer[6] | Janet J. Maguire[7] | Adam J. Pawson[8] | Christopher Southan[9,10] | Michael Spedding[11]

Ambroxol for the treatment of fibromyalgia: science or fiction? Kern et al. 2017

Ambroxol inhalation ameliorates LPS-induced airway inflammation and mucus secretion through the extracellular signal-regulated kinase 1/2 signaling pathway. Zhang et al. 2016

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)*

Reduction of cytokine release of blood and bronchoalveolar mononuclear cells by ambroxol. *Pfeifer et al. 1997*

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_1 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*

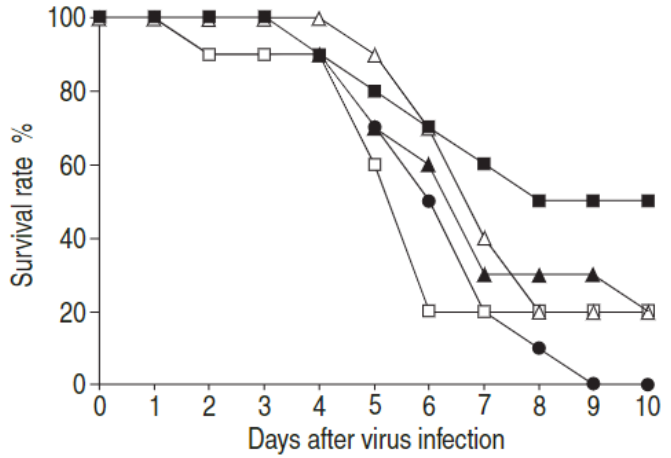
ABSTRACT: The protective effect of ambroxol, a mucolytic agent which has anti-oxidant properties and stimulates the release of pulmonary surfactant, against influenza-virus proliferation in the airway was investigated in mice.

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 $mg \cdot kg^{-1} \cdot day^{-1}$, 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.

Eur Respir J 2002; 19: 952–958.

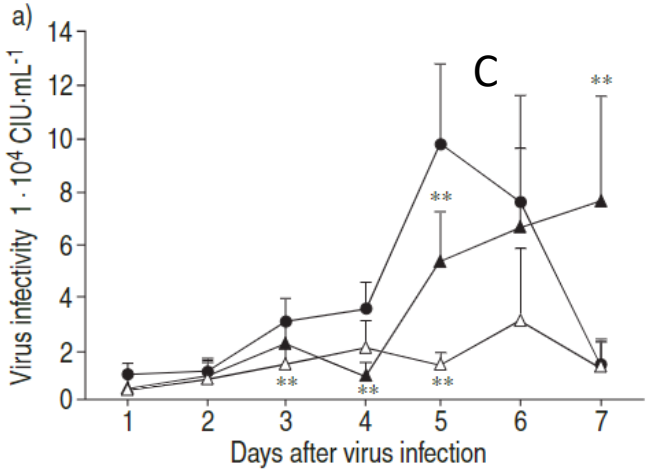


10 mg/kg ip bd ambroxol

20,30

C

Fig. 1.—The effect of ambroxol on the survival rate of mice infected with influenza-A virus. Mice were infected with 6.6×10^4 plaque forming units of influenza A/Aichi/68 virus and then injected with saline (●) or ambroxol *i.p.* twice daily, at a total daily dose of 4 (▲), 10 (■), 20 (△), and 30 (□) $mg \cdot kg^{-1} \cdot day^{-1}$, respectively.



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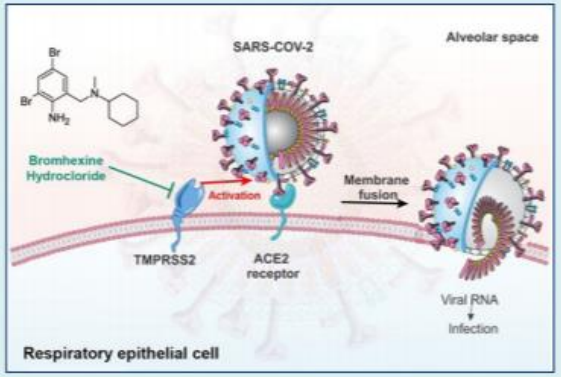
10

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

Khalil Ansarin[1,2], Ramin Tolouian[3], Mohammadreza Ardalan[4*], Ali Taghizadieh[2,5], Mojtaba Varshochi[6], Soheil Teimouri[5], Tahere Vaezi[5], Hamed Valizadeh[2,5], Parviz Saleh[4], Saeid Safiri[7,8], Kenneth R. Chapman[9]

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.

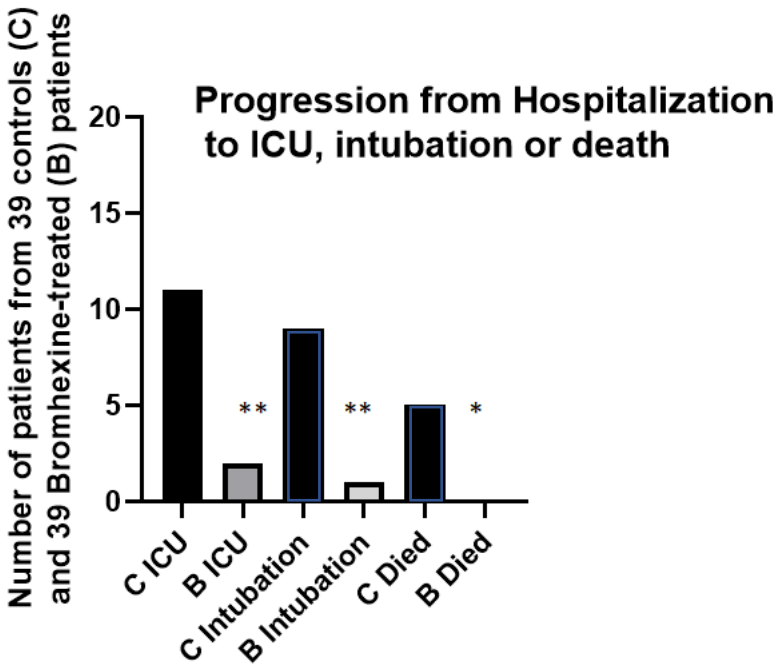


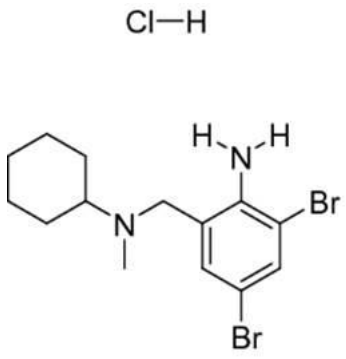
Methods: An open-label randomized clinical trial study was performed in Tabriz, North-West of Iran. They were randomized to either the treatment with the bromhexine group or the control group, in a 1:1 ratio with 39 patients in each arm. Standard therapy was used in both groups and those patients in 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.

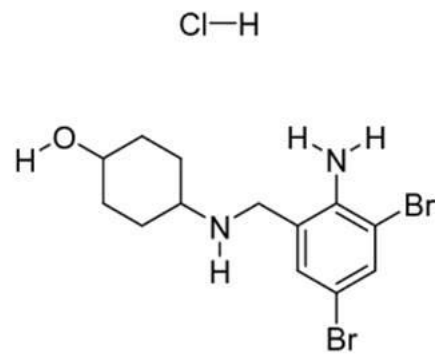
Conclusion: The early administration of oral bromhexine reduces the ICU transfer, intubation, and the mortality rate in patients with COVID-19. This affordable medication can easily be administered everywhere with a huge positive impact(s) on public health and the world economy. Altogether, the verification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

Trial Registration: IRCT20200311704679 7N4; https://irct.ir/trial/46969





Bromhexine HCl



Ambroxol HCl

Investigator-driven trials in ALS and COVID-19