Chemistry and Physics of Lipids 200 (2016) 94–103

Contents lists available at ScienceDirect

Chemistry and Physics of Lipids

journal homepage: www.elsevier.com/locate/chemphyslip

Review

Unravelling the role of sphingolipids in cystic fibrosis lung disease

Massimo Aureli[a,1,*], Domitilla Schiumarini[a,1], Nicoletta Loberto[a], Rosaria Bassi[a], Anna Tamanini[b], Giulia Mancini[a], Matteo Tironi[a], Silvia Munari[b], Giulio Cabrini[b], Maria Cristina Dechecchi[b,*], Sandro Sonnino[a]

[a] Department of Medical Biotechnology and Translational Medicine, University of Milano, Italy
[b] Department of Pathology and Diagnostics, Laboratory of Molecular Pathology, University Hospital of Verona, Italy

ARTICLE INFO

Article history:
Received 6 July 2016
Received in revised form 22 August 2016
Accepted 25 August 2016
Available online 31 August 2016

Keywords:
Cystic fibrosis
Sphingolipids
Lipid rafts
Inflammation

ABSTRACT

Cystic fibrosis (CF), one of the most common lethal hereditary diseases of white European populations, is caused by loss-of-function mutations in the CF Transmembrane conductance Regulator (CFTR) gene. One of the main causes of mortality is the onset of CF lung disease, which is characterized by chronic infection and inflammation resulting in the progressive remodelling, irreversible damage and fibrosis of the airways. An increasing number of studies indicate that sphingolipids are crucial players in pulmonary manifestations of CF, even if their direct involvement in CF lung disease is still unclear. In this review, we give an overview of the role of sphingolipids in CF pulmonary disease, focusing on the relationship between glycosphingolipids and lung inflammation, which represents the main hallmark of this disease.

Contents

1. Cystic fibrosis lung disease

Cystic fibrosis (CF) is one of the most common lethal hereditary and monogenic disorders among people of white European descent, although it has been reported in all races. It is an inherited autosomal recessive disease caused by mutations in a single gene, consisting of 27 exons, which codes for the Cystic Fibrosis Transmembrane conductance Regulator (CFTR) protein (Riordan et al., 1989). It is a large multidomain protein comprising 1480 amino acid residues arranged in two membrane-spanning domains (MSD 1, 2), two nucleotide (ATP) binding domains (NBD1, 2), and a unique regulatory domain (RD) with multiple phosphorylation sites (Riordan, 2008; Billet et al., 2015). The CFTR protein is a cAMP-activated chloride channel expressed at the apical membrane of most of the surface epithelial cells lining the airways and the gastrointestinal tract, exocrine pancreas, airway submucosal and sweat glands. It plays a key role in hydrating airway secretions and regulating other cellular functions, including Na^+ transport in airway epithelia (Welsh and Smith, 2001; Cutting, 2010). There are more than 2000 sequence variations in the CFTR gene, many of which have been associated with causing diseases (see the Cystic Fibrosis Mutation Database of the Cystic Fibrosis Gene Analysis Consortium, www.genet.sickkids.on.ca/cftr/). The

* Corresponding authors.
E-mail addresses: massimo.aureli@unimi.it (M. Aureli), cristina.dechecchi@ospedaleuniverona.it (M.C. Dechecchi).
[1] These authors equally contributed to this work.

http://dx.doi.org/10.1016/j.chemphyslip.2016.08.002

most common CF mutation, a three base pair deletion resulting in the loss of phenylalanine at position 508 of the protein from the first NBD1, causes a folding defect in the protein and impaired localization of the CFTR channel on the apical surface of epithelial cells, leading to defective epithelial ion transport (Cheng et al., 1990; Du et al., 2005). This mutation occurs in approximately 70% of CF chromosomes worldwide, in at least one allele of 90% of CF patients, and it is associated with a severe clinical phenotype. The consequences of mutated CFTR are considerable in the respiratory tract (Wright et al., 2006). Here, mutations in CFTR impair the mucociliary clearance due to reduced periciliary fluid volume and increased viscosity of submucosal gland secretions, leading to chronic bacterial infections mostly caused by *Pseudomonas aeruginosa* (*P.aeruginosa*) (Mogayzel and Flume, 2010). The destruction of the airways in chronically colonized CF individuals is due to a massive influx of polymorphonuclear neutrophils (PMN) in the bronchial lumen. This is orchestrated by the bronchial epithelial cells secreting the neutrophilic chemokine interleukin 8 (IL-8) in response to continuous stimulation by bacteria or their products (Bezzerri et al., 2011; Cohen and Prince, 2012) which activate a series of kinases and transcription factors depending on Toll-like Receptor signalling (Bezzerri et al., 2011). Obstructive lung disease is currently the primary cause of morbidity and is responsible for about 80% of mortality (Cutting, 2015).

2. Overview of sphingolipid homeostasis

Sphingolipids (SLs) are minor cell components of all mammal cell membranes with the hydrophilic head group protruding toward the extracellular environment and the lipid moiety, the Ceramide (Cer), deeply inserted into the membrane bilayer (Feizi, 1985). In the plasma membrane (PM), they reside asymmetrically in the extracellular leaflet, where they are concentrated in restricted membrane areas known as “lipid rafts” or “sphingolipid and cholesterol enriched membrane domains” (Sonnino et al., 2006). It is believed that in lipid rafts, SLs modulate the functional features of several membrane proteins (Prinetti et al., 2009) by both direct lateral interactions between SLs and plasma membrane proteins (Kabayama et al., 2007), and by short-range alterations of the physico-chemical properties of the protein membrane microenvironment (Sonnino et al., 2009).

SLs are important in the regulation of cell functions, and cells employ different strategies to establish the proper pattern of SLs at the plasma membrane. SLs are synthesized through complex metabolic networks involving neo-biosynthesis, catabolism and complex intracellular trafficking, as well as exchanges with the extracellular environment. A general schematic of SL metabolism, as a well as of their intracellular transport, is depicted in Fig. 1.

Cer plays a key role in SL metabolism, since it is the common intermediary in both SL biosynthetic (sphingomyelin and glycosphingolipids biosynthesis) and catabolic pathways.

The *de novo* biosynthesis of Cer occurs at the cytosolic face of the endoplasmic reticulum and starts with the condensation of L-serine to a fatty acyl-coenzyme A, usually palmitoyl-CoA. This reaction is catalysed by vitamin B6-dependent serine palmitoyltransferase and produces 3-ketosphinganine (Mandon et al., 1991; Nagiec et al., 1994; Weiss and Stoffel, 1997), which is then reduced to D-*erythro*-sphinganine by 3-ketosphinganine reductase, a NADPH-dependent reaction (Stoffel et al., 1970). Sphinganine is subsequently acylated to dihydroCer by a family of six Cer synthases, integral membrane proteins, each of which displays a different specificity to Acyl-CoAs (Merrill and Wang, 1986; Rother et al., 1992; Shimeno et al., 1998). Most of the dihydroCer pool is then desaturated to Cer *via* the dihydroCer desaturase reaction (Geeraert and van Veldhoven, 1997; Michel et al., 1997; Mikami et al., 1998). Once synthesized, Cer is directly routed to the membrane or used as a precursor for glycosphingolipids (GSL) or sphingomyelin (SM) biosynthesis. In particular, SM can be formed at the Golgi apparatus or at the plasma membrane by SM synthase 1 and SM synthase 2, respectively. Both isoforms transfer

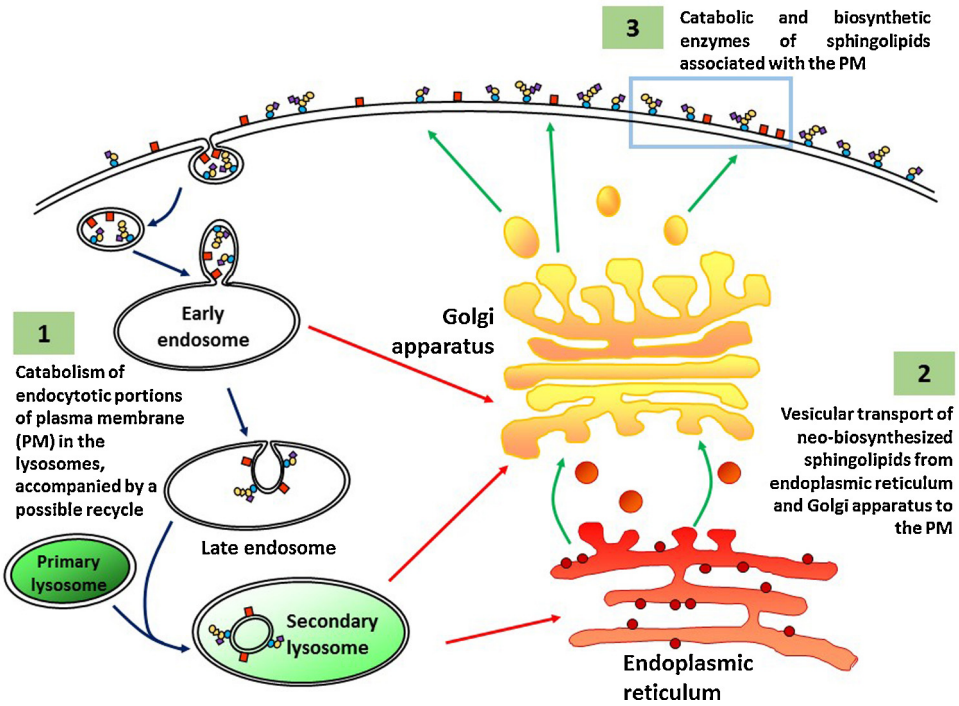

Fig. 1. Schematic representation of the major routes and the subcellular sites of sphingolipid metabolism.
The pathways of sphingolipid metabolism include their catabolism in the endolysosomal compartments (1) and their biosynthesis in the endoplasmic reticulum and Golgi apparatus (2). A fine-tuning of SL composition can also occur at the cell surface through the action of plasma membrane glycosyltransferases and glycosidases (3). -Glucose (light blue circle); galactose (yellow circle); *N*-acetyl-galactosamine (yellow square); sialic acid (purple diamond); methyl-choline (red rectangle).

phosphorylcholine from phosphatidylcholine to Cer, yielding diacylglycerol as a by-product of the reaction.

Conversely, GSL biosynthesis begins with glycosylation of Cer to glucosylCer (GlcCer) *via* the action of GlcCer synthase at the cytosolic side of early Golgi membranes (Jeckel et al., 1992). Once formed, GlcCer can be translocated to the luminal side of the Golgi where it is further metabolized by different membrane-bound glycosyltransferases, which catalyse the sequential addition of sugar residues, leading to the generation of GSL oligosaccharide chains (Fig. 2). GlcCer can also be directly transferred to the plasma membrane (Warnock et al., 1994).

SLs degradation take place in the acidic compartments of cells, the lysosomes, where PM SLs are transported by endocytic vesicular flow through early and late endosomal compartments. In lysosomes, GSLs are catabolized by different exoglycosidases that sequentially cleave off the sugar residues from the non-reducing end of their SL substrates. In the same compartment, SM is directly catabolized to Cer by the acid sphingomyelinase. Cer

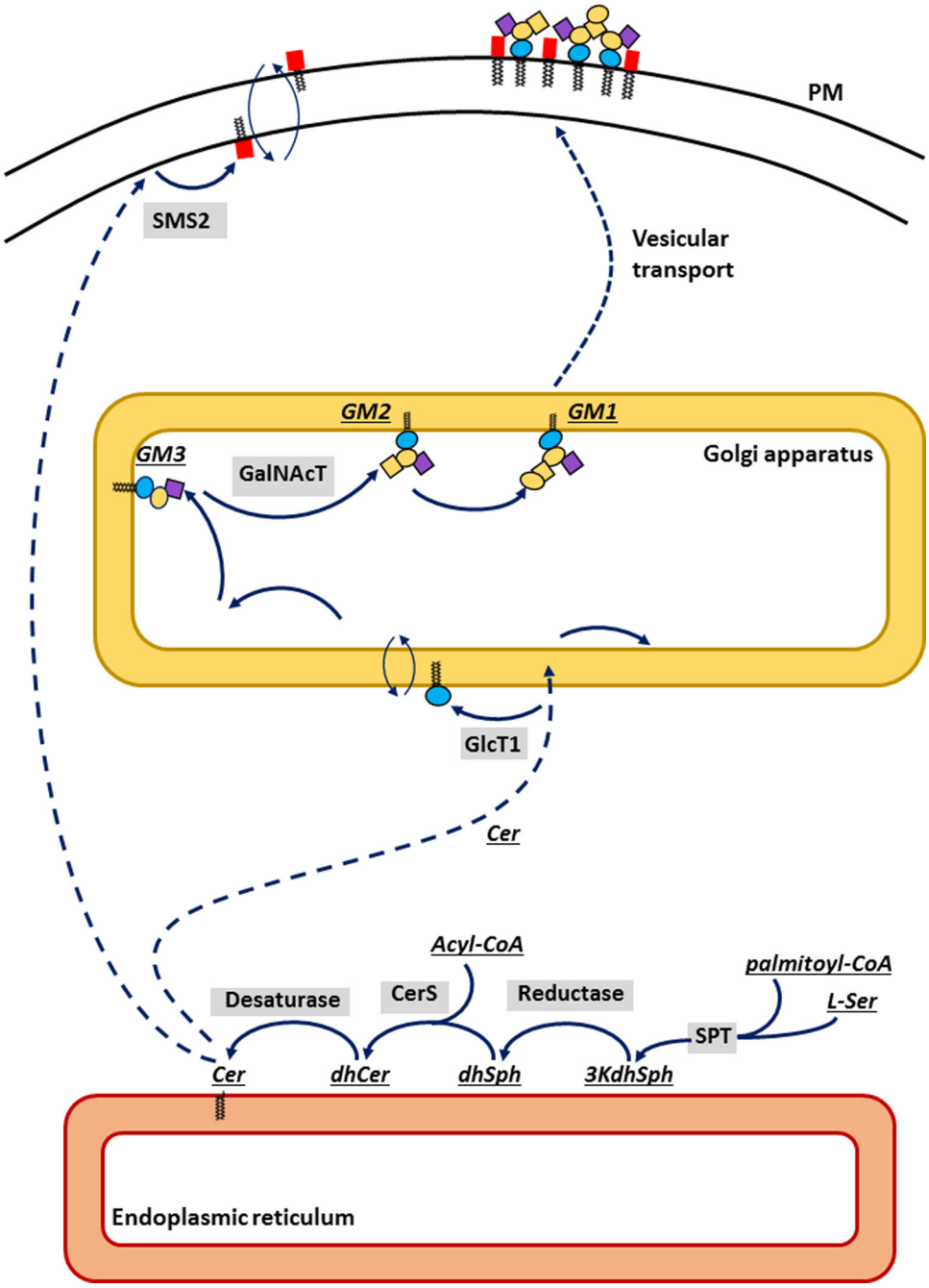

Fig. 2. Sphingolipid neo-biosynthetic pathway.
The *de novo* byosinthesis of ceramide (Cer) occurs at the endoplasmic reticulum by the sequential action of different enzymes (SPT: serine palmitoyl-transferase; 3KdhSph reductase: 3-ketosphinganine reductase; CerS: ceramide synthase; dhCer desaturase: dihydro ceramide desaturase).
Ceramide can then reach directly the inner leaflet of the PM, where it is converted by the sphingomyelin synthase 2 (SMS2) to SM. Conversely, Cer reaches the Golgi apparatus where it is converted to glycosphingolipids (GSL) or to SM by SM synthase-1 (SMS1). The GSL synthesis starts at the cytosolic side of the Golgi apparatus by the action of a transferase (GlcT1) with the formation of glucosylceramide (GlcCer). GlcCer is transferred inside the Golgi apparatus where different transferases catalyse the maturation of the hydrophilic head (GalT1: galactosyl transferase 1; SAT1: GM3 synthase; GalNacT: GM2 synthase; GalTII: GM1 synthase). GSL can then reach the PM by a vesicular transport.

deriving from both GSL and SM degradation is further catabolized to sphingosine and fatty acid by acid ceramidase. The resulting catabolites, including the sphingoid bases, leave the lysosomes and are either recycled or further degraded. During the catabolism of sphingosine, sphingosine kinases first phosphorylate it to sphingosine 1 phosphate (S1P) and then sphingosine-1-phosphate lyase degrades it to phosphoethanolamine and hexadecanal. It is widely documented that S1P is not only a catabolic intermediate, but also a secondary messenger playing a key role in the regulation of several signalling pathways (Hannun and Obeid, 2008) (Fig. 3).

In addition to these main SL metabolism pathways, it is nowadays accepted that SL synthesis and catabolism are triggered directly at the cell surface by plasma membrane-associated hydrolases and transferases (Fig. 4). Several enzymes for SL metabolism have been found at the plasma membrane level, including sialidase and sialyltransferase, sphingomyelinase and

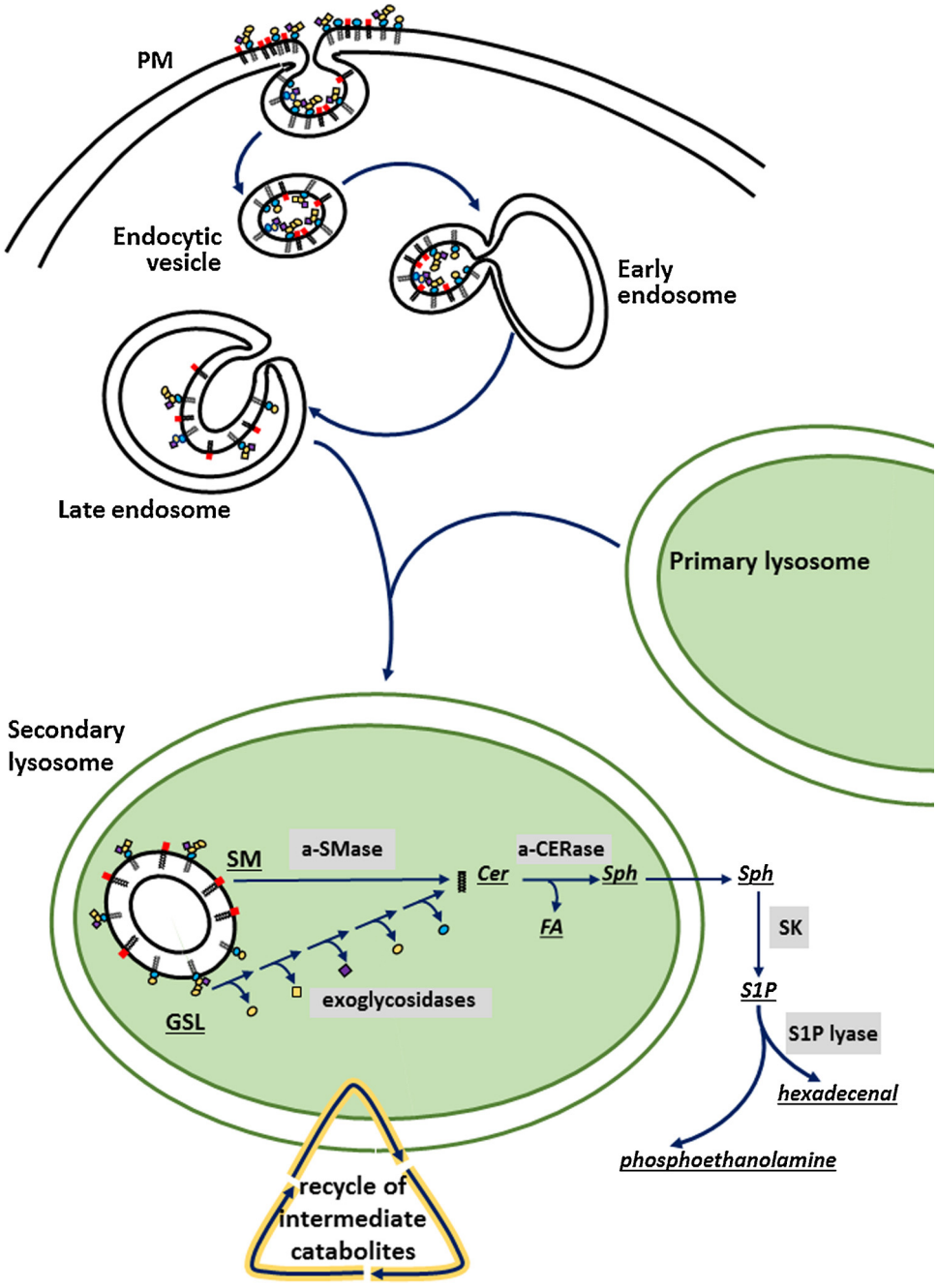

Fig. 3. Sphingolipid catabolism in the endo-lysosomal compartment.
Sphingolipids catabolism starts from invaginations of plasma membrane (PM) portion leading to the formation of endocytic vesicles, which are incorporated in the primary lysosome.
In secondary lysosome, different exoglycosidases sequentially cleave off the sugar residues from the non-reducing end of glycosphingolipids (GSL) producing ceramide (Cer). Cer can be also obtained from the sphingomyelin (SM) catabolism by the action of the acidic-sphingomyelinase (a-SMase).
Acid-ceramidase (a-CERase) catalyse Cer in sphingosine (Sph) and fatty acid (FA).
In cytosol, sphingosine kinase (SK) phosphorylates Sph in sphingosine-1-phosphate (S1P), which is degraded to phosphoethanolamine and hexedecenal by the action of a lyase (S1P lyase).

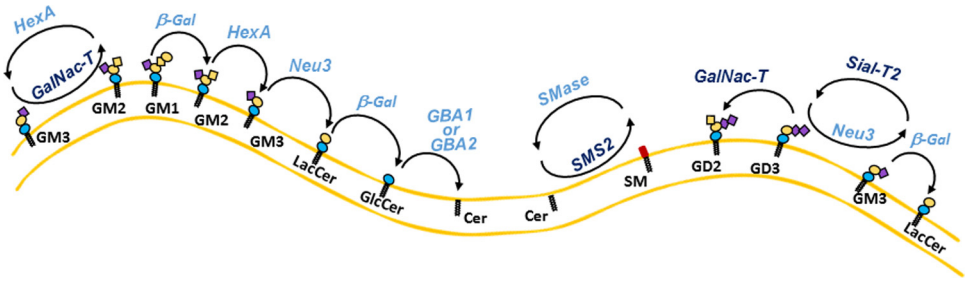

Fig. 4. Schematic representation of sphingolipid metabolism occurring at the cell surface.
The presence of both catabolic and anabolic enzymes of SL metabolism at the cell surface allows the *in situ* modulation of their composition at the plasma membrane level. Among glycosyltransferases, GD3 synthase (SAT2), GM2-GD2 synthase (GalNac-T) and sphingomyelin synthase 2 (SMS2) have been also described at the cell surface. Among the hydrolases found associated with plasma membrane, β-galactosidase (β-gal), β-galactocerebrosidase (β-gal), β-hexosaminidase A (HexA), β-glucocerebrosidase (GBA1) and acid-sphingomyelinase (SMase) are the same enzymes present in the lysosomes, whereas sialidase NEU3 and non-lysosomal β-glucosylceramidase GBA2 are mainly associated with the cell surface.

sphingomyelin synthase, β-hexosaminidase, β-galactosidase and β-glucosidases, ceramidase and two glycosyltransferases: GalNAc transferase and sialyltranferase 2.

Several lines of evidence indicate that most of the PM-associated hydrolases, such as β-galactosidase, β-galactocerebrosidase, β-hexosaminidase A, β-glucocerebrosidase GBA1, Neu1 and sphingomyelinase, are the same enzymes present in lysosomes. In contrast, sialidase NEU3 and non-lysosomal beta-glucosylceramidase (GBA2) are located almost exclusively at the plasma membrane. At this site, these enzymes may modulate the metabolism of SLs directly at the cell surface, thus possibly contributing in mediating the cellular effects exerted by SLs, as well as to the regulation of the level of bioactive sphingoid molecules, such as Cer and S1P.

3. Lung sphingolipids

The lung is characterized by the presence of a complex pattern of SLs including Cer, phospho-SLs, neutral GSLs and gangliosides (Hanqing et al., 1986). The adult human lung contains 85 nmol of gangliosides and 140 nmol of neutral GSLs per gram wet weight. Globosides, including globotetraosylCer and globotriaosylCer, are the major classes of neutral glycolipids, followed by lactosylCer (LacCer), glucosylCer (GlcCer) and tetahexosylCer (asialoGM1, A-GM1). Fourteen gangliosides with different carbohydrate moieties have been identified in sialylated GSLs in the adult human lung. Among these, the most abundant are monosialogangliosides GM3 and GM1, disialo GD3, and trisialosyllactosylCer GT3 (Mansson et al., 1986). The role of SLs and SL metabolism in lung development has been widely investigated. In particular, the preservation of SL rheostat synthesized by Cer, known to induce apoptosis, and S1P, which promotes cell survival, is fundamental for the formation of lung structure at all stages of lung development, as well as the preservation of pulmonary physiology (Lee et al., 2015). Furthermore, *de novo* synthesis of Cer has been linked to apoptotic endothelial cell death and a decreased pulmonary barrier function (Petrache et al., 2005; Medler et al., 2008). Moreover, an excess of Cer induces oxidative stress and apoptotic cell death leading to alveolar enlargement (Petrache et al., 2008). Overall, these data suggest that Cer regulation is necessary in maintaining lung homeostasis. Different findings have demonstrated the involvement of Cer in pulmonary infections caused by a variety of bacterial and viral pathogens, including *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Listeria monocytogenes*, *Salmonella typhimurium*, *Escherichia coli*, mycobacteria, measles virus, rhinovirus, and sindbis virus (Seitz et al., 2015). The important role of Cer in infections is due to its biophysical properties. Cer molecules, displaying high hydrophobicity and low amphiphilicity, bind strongly to each other by means of hydrophobic interactions, resulting in the formation of small Cer-enriched membrane domains, which form platforms that are larger than other parts of the cell membrane and characterized by different biophysical membrane properties (Grassmé et al., 2001). These Cer-enriched platforms trap and activate receptors and signalling molecules, thus amplifying initial signals (Grassme et al., 2003a,b). It has been shown that Cer-enriched platforms are formed in cells after the application of many different stimuli, including bacterial infections (Seitz et al., 2015). However, several questions remain to be addressed; in particular, it is not clear if SL misregulation is the cause or the consequence of these disorders.

4. Sphingolipid involvement in cystic fibrosis lung infections

The airways of CF patients are characterized by large amounts of pro-inflammatory mediators, such as interleukin-(IL)-8, IL-6 and tumor necrosis factor-(TNF)-α, which continuously recruit neutrophils unable to kill bacteria, resulting in a vicious circle that amplifies inflammation and perpetuates infection (Mogayzel and Flume, 2010). Binding of bacterial components to pattern-recognition receptors expressed on epithelial cell surface, such as Toll-like receptors (TLRs) 2, 4, and 5, activates a series of kinases and adaptors, ultimately triggering nuclear translocation of transcription factors and expression of pro-inflammatory genes (Kawai and Akira, 2010; Bezzerri et al., 2011).

An increasing number of studies indicate that SLs play an important regulatory role in CF with respect to pulmonary infections and inflammation (Lahiri and Futerman, 2007; Guilbault et al., 2008; Teichgraber et al., 2008; Uhlig and Gulbins, 2008; Guilbault et al., 2009; Yu et al., 2009; Brodlie et al., 2010; Bodas et al., 2011; Yang and Uhlig, 2011; Nahrlich et al., 2013).

In particular, some evidence suggests that GSLs associated with the cell surface of epithelial cells can serve as receptors for bacterial adhesion. In the case of *P. aeruginosa*, it has been shown, by TLC binding assay using standard lipids, that several clinically isolated strains of *P. aeruginosa* bind to A-GM1 and asialo-GM2 (A-GM2). It is quite clear that they could be considered as receptors for the *P. aeruginosa*; however, more confusing are the data reporting the presence of these GSLs in the epithelial cells of lung and bronchi. To our knowledge, no data are available regarding A-GM2, whereas the presence of A-GM1 has been detected by the use of antibodies on bronchial epithelial cells in culture (Krivan et al., 1988a,b; Baker et al., 1990; de Bentzmann et al., 1996). Using flow cytometry on primary respiratory epithelial cells, Saiman and colleagues showed that superficial amounts of A-GM1 were present in 12% of primary CF airway cells, compared to 2.9% of cells in normal control subjects (Saiman and Prince, 1993). More recently, Adamo and colleagues showed that *P. aeruginosa* flagella activate airway epithelial cells through binding with A-GM1 and

TLR2 and 5 (Adamo et al., 2004). The role of proteins such as antibodies in recognizing SLs remains one of the most debated issues in the scientific community working on SLs. One of the main criticisms, already confirmed for several SL-binding proteins such as cholera toxin, is related to their capability to recognize single molecules or a cluster of different species of lipids (Krivan et al., 1988a,b; de Bentzmann et al., 1996; Adamo et al., 2004; McNamara et al., 2006).

Also under debate is the role of Cer in CF inflammation and infection. Guilbault et al. (Guilbault et al., 2008, 2009) demonstrated, using mass spectroscopy that the plasma of CF patients displays significantly low levels of several Cer species, in particular, C14:0, C20:0, C22:0, C22:1 and C24:0 Cer and dihidroxy Cer (DHC 16:0). Using TLC-ELISA, they found an overall reduction (about 4 times less) of Cer content in lung, plasma, ileum and pancreas in C57BL/6-CFTR$^{-/-}$ mice compared to WT mice. Interestingly, heterozygous mice, which possess one normal and one ablated allele of CFTR gene, had a 1.9-fold reduction of Cer in the lungs, a 2-fold reduction in the plasma, a 3.4-fold reduction in the ileum, and a 1.9-fold reduction in the pancreas compared to WT mice.

Using immortalized human respiratory tracheal epithelial cells, (Vilela et al., 2006) tried to elucidate the possible molecular mechanism responsible for Cer reduction in CF. They found that CFTR-deficient tracheal epithelial cells are characterized by high glutathione levels that decrease the intracellular content of Cer through the inhibition of neutral sphingomyelinase. Cer deficiency occurring in CF seems to be responsible for the increased activation of the pro-inflammatory transcriptional nuclear factor (NF)-kB that in turn is responsible for the abnormally high inflammatory response in CF respiratory epithelial cells. In fact, increasing Cer levels by treating CF$^{-/-}$ mice or CFTR-deficient tracheal epithelial cells with fenretinide, results in an improved ability to control *P. aeruginosa* infections (Lavrentiadou et al., 2001; Vilela et al., 2006; Guilbault et al., 2008). However, the mechanism underlying this treatment is unknown. Further studies on the involvement of Cer in the control of inflammatory response is needed to detail, in particular, the mechanism involving the glutathione-dependent neutral sphingomyelinase inhibition as well as the subcellular localization of the signalling Cer.

Different results were reported by Teichgraber and colleagues showing an accumulation of Cer in CF lung disease (Teichgraber et al., 2008). In fact, the authors showed an age-dependent accumulation of Cer in the respiratory tract of uninfected mutant mice lacking functional CFTR. In addition, they found Cer accumulation in ciliated respiratory cells and in the submucosa. From a mechanistic point of view, they suggested that the loss of CFTR induces an alkalinization of intracellular vesicles that strongly reductes or reverses acid ceramidase activity without substantial modifications to the activity of the acid sphingomyelinase (ASMase) (Teichgraber et al., 2008). The imbalance of ASMase and acid ceramidase activity results in a net accumulation of Cer. The accumulation of Cer is also responsible for age-dependent pulmonary inflammation, the death of respiratory epithelial cells, and the high susceptibility to severe *P. aeruginosa* infections. Interestingly, the genetic or pharmacological inhibition of the acid sphingomyelinase causes a reduction of Cer levels, resulting in an amelioration of the inflammatory response to *P. aeruginosa* infections (Becker et al., 2010). These controversial findings seem to be due to the intrinsic differences among the different mouse models used, as well as the special diet required for CFTR knockout mice. Mice completely lacking CFTR need a Peptamen^{R}-based diet in order to prevent intestinal obstruction. Peptamen^{R} is normally used for the dietary management of patients with compromised or impaired gastro-intestinal function. This includes patients suffering from conditions such as malabsorption, diarrhea, intolerance to standard enteral formulas, delayed gastric emptying, short-bowel syndrome, inflammatory bowel disease and pancreatic insufficiency. Teichgraber and colleagues demonstrate that this particular diet induces an accumulation of cholesterol in the lung that is responsible for the 60% inhibition of ASMase, which in turn causes a reduction of Cer levels in the lung (Teichgraber et al., 2008). Interestingly, the effect of a Peptamen^{R} diet on Cer and ASMase in the lung was abrogated by treatment with simvastatin, a known blocker of cholesterol biosynthesis (Mol et al., 1986; Teichgraber et al., 2008).

Despite these contradictory results, probably due to different analytical approaches used, Brodlie and colleagues, described an increase in Cer content in the lower airway epithelium of CF patients with advanced lung disease for the first time. The results were obtained using different experimental approaches, ranging from immunochemistry to high-performance liquid chromatography-mass spectrometry. In particular, they found significantly elevated levels of Cer C16:0, C18:0 and C20:0. Additionally, Cer was significantly increased in lungs colonized by *P. aeruginosa* (Brodlie et al., 2010).

The role of Cer in determining the host defence against *P. aeruginosa* represents one of the hot topics in CF research. In Grassmé et al. (2001) is described how *P. aeruginosa* infection triggers the activation of ASMase through its translocation to the plasma membrane in normal epithelial cells. At this site it catalyzes SM hydrolysis, resulting in the production of Cer that, together with CD95 clusterization, promotes *P. aeruginosa* internalization, induces apoptosis, and regulates the cytokine response in infected cells.

Cer seems to be also involved in the susceptibility of CF patients to microbial colonization, in particular to *P. aeruginosa*, which might be explained, at least in part, by the alteration of its levels in lung macrophages. Zhang and colleagues demonstrated that in *CFTR*-deficient macrophages, alkalinization of intracellular acid vesicles is correlated with the lack of response of these cells to infections caused by various strains of *P. aeruginosa* (Zhang et al., 2010). In contrast to the wild type, *CFTR*-deficient macrophages are defective in the acute activation of ASMase, which produces Cer that serves to cluster gp91^{phox} (an important membrane-bound subunit of the NADPH oxidase) and to release reactive oxygen species. The absence of these responses to bacterial infection may result in a persistence of *P. aeruginosa* in the lung.

These findings open the possibility of targeting Cer metabolism as new therapeutic strategies for CF lung disease (Teichgraber et al., 2008; Guilbault et al., 2009; Yu et al., 2009; Bodas et al., 2010; Brodlie et al., 2010; Dechecchi et al., 2011; Nahrlich et al., 2013).

In *P.aeruginosa* infection and the basal pro-inflammatory state typical of CF patients, new evidence supports the role of Cer, which is catabolized from complex GSLs directly at the cell surface by different plasma membrane-associated glycohydrolases. In particular, a pivotal role is exerted by GBA2, a transmembrane beta-glucocerebrosidase involved in the production of Cer by the hydrolysis of GlcCer mainly at PM level. Treatment of CF epithelial bronchial cells with miglustat, an inhibitor of the cellular beta-glucocerebrosidases, has been found to reduce the immune response to *P. aeruginosa* infection (Loberto et al., 2014). In fact, treating CF epithelial bronchial cells with miglustat down-regulates the expression of the main genes up-regulated by *P. aeruginosa* in CF, such as chemokines (IL-8, growth-regulated Gro-$\alpha/\beta/\gamma$ proteins, and granulocyte chemotactic peptide-2 [GCP-2]), proinflammatory cytokines (IL-1 α/β, IL-6, and TNF-α), and the intercellular adhesion molecule–1, nuclear factor kB1, TLR2, and the human defensin B4 genes. Moreover, it has been demonstrated that GBA2 inhibition by miglustat results in a reduction of Cer formation at the PM of CF human bronchial cells infected by *P. aeruginosa*. Consequently, a possible relationship between GBA2 and inflammatory response has been proposed. Similar effects of

those obtained by the pharmacological inhibition of GBA2 were also observed in CF human bronchial epithelial cells exposed to *P. aeruginosa* by lowering GBA2 expression with siRNA oligonucleotides (Loberto et al., 2014).

These findings parallel the attenuation of systemic inflammation with the deletion of the GBA2 gene in a mouse model of Gaucher disease 1. In particular, a very impressive reduction of IL-2, IL-6, GM-CSF and the murine IL-8 homologue, keratinocyte-derived chemokine KC was found (Mistry et al., 2014). Therefore, GBA2 may determine the susceptibility to inflammatory stimuli due to its ability to modify the lipid organization at the plasma membrane level.

The contribution of PM glycohydrolases in the production of Cer in response to bacterial infections, and the involvement of these enzymes in determining the high concentrations of Cer found in the airway of the CF patients, could be higher than expected. This possibility is related to the fact that, besides the up-regulation of these enzymes in CF, the possibility of an acidification of the extracellular milieu of the CF-related organs, including the lungs (Machen, 2006), could further up-regulate glycohydrolases. In fact, most of these enzymes are most effective in low pH values; therefore, an acidic extracellular environment could in turn induce their activation and increase the *in-situ* hydrolysis of complex GSLs with production of Cer. The main open question is to understand if Cer works directly as a signaling molecule or if the increased activity of PM glycohydrolases is responsible for a general reorganization of the cell plasma membrane with the formation of a specific signaling platform. Cer is a very hydrophobic compound and, as a pure component, it is insoluble in aqueous solution. Within a membrane, it can be considered an amphiphilic molecule due to the primary hydroxyl group and the amide planar linkage located at the water–lipid interface. On the other hand, the specific interaction between SLs and protein is widely described. We argue that for these reasons, it is not surprising that when Cer is produced from complex SLs, the membrane undergoes a deep reorganization that in turn is responsible for the modifications of the cellular signaling.

Another actor in CF is represented by the sphingoid long chain base, sphingosine, in determining susceptibility to lung infection caused by *P. aeruginosa*. Tracheal and bronchial sphingosine levels were significantly reduced in tissues from CF patients and from CF mouse models due to a reduction in the activity of acid ceramidase, which generates sphingosine from Cer, thus leading to Cer accumulation. Therefore, in CF cells, the distribution of sphingosine and Cer is inversely related. Furthermore, the inhalation with sphingosine, the sphingosine analog FTY720, or with acid ceramidase induces a rescued in susceptible mice from *P. aeruginosa* infection, suggesting that sphingosine plays an important role in antibacterial defense in healthy lungs (Pewzner-Jung et al., 2014). In summary, the balance between Cer and sphingosine concentrations seems to determine the susceptibility to *P. aeruginosa* infection, although they are not the only molecules controlling infections.

5. Relationship between CFTR and sphingolipids

Since the discovery of the CFTR gene, there has been worldwide interest in pharmacologic approaches to identify a single agent capable of restoring mutant CFTR to therapeutically relevant levels (Ramsey et al., 2011; Boyle et al., 2014; Solomon et al., 2015).

Most of studies on CFTR interactome have focused on protein-protein interactions without considering that CFTR is a transmembrane protein associated with lipid rafts (Kowalski and Pier, 2004; Abu-Arish et al., 2015). Given CFTR localization within plasma membrane regions enriched in SLs, we speculate the existence of interactions between SLs and proteins that participate in the stabilization or to the function of CFTR. Relevantly, data emerging from literature indicate that CFTR is both regulated by SLs and/or is involved in the regulation of SL metabolism. For this latter point, a regulatory relationship between expression of CFTR and SL synthesis has been reported (Hamai et al., 2009). In particular, expression of defective CFTR, *i.e.*, the F508del mutation of CFTR or decreased CFTR expression, increases SL synthesis, resulting in a significantly increased mass of SM, sphingosine, and sphinganine. Moreover, the mass of four saturated long-chain Cer species, such as C16-di-hydroCer and C22- C24- C26-Cer, proportionally increased up two fold for C22–C24 and 1.5 fold for C16-di-hydroCer and C26. On the other hand, Cer species containing C18:0 and C18:1 fatty acids decreased. These data suggest that failure to express CFTR has distinct effects on Cer species composition, although exact mechanisms are currently unknown. The significance of altered Cer composition, specifically of increased saturated long-chain Cer, may be a cellular response to the lack of CFTR, which consequently leads to plasma membrane destabilization. The expression of defective CFTR, previously reported to coincide with characteristics of a destabilized membrane, may trigger the synthesis of a class of lipids that promotes membrane stabilization (Thelin et al., 2007). If membrane stability is indeed affected by the expression of defective CFTR, then an increase in SL synthesis, including that of SM, could be a compensatory mechanism directed to increase membrane stability or embedment of CFTR into the SL and cholesterol-rich membrane regions.

For instance, Itokazu and colleagues describe a direct correlation between ganglioside GM1 PM levels and CFTR expression (Itokazu et al., 2014). In particular, they found that CFTR-silenced human airway cells had a 60% decrease in GM1, reduced β1-integrin activation, decreased phosphorylated tyrosine 576 of focal adhesion kinase (pFAK), and phosphorylation of Crk-associated substrate (pCAS), which resulted in a reduction of cell motility. The addition of GM1 (but not GM3) ganglioside to CFTR-silenced cells restored activated β1-integrin, pFAK, and pCAS to near control levels and partially restored (40%) cell migration. The decreased GM1 content in CFTR-silenced cells is consistent with studies demonstrating lower GM1 in CFTR-deficient cells compared to normal cells. They also demonstrated that reduced GM1 in CFTR-silenced cells was a result of loss of CFTR function, since the expression of GFP-CFTR in CFTR-silenced cells significantly increased GM1 levels, whereas the pharmacologic inhibition of CFTR in control cells decreased GM1. However, the mechanisms underlying this alteration in gangliosides have never been identified even if preliminary data seem to suggest an involvement of the lysosomal compartment. These results suggest that decreased GM1 in CFTR-silenced cells depresses β1-integrin signaling, which contributes to the delayed wound repair observed in these cells. These findings have implications for CF pathology, where altered SL levels in airway epithelial cells might result in a diminished capacity for wound repair after injury.

For the regulation of the CFTR function by the PM microenvironment, it has been reported that the clusterization of CFTR in membrane areas rich in Cer causes an inhibition in its chloride channel function (Ramu et al., 2007). In particular, the authors describe that inhibition of the CFTR current is the result of the enzymatic hydrolysis of SM, leading to the formation of Cer, which makes it more difficult to activate CFTR by phosphorylation of the R domain. This would then imply that SM, complexed to CFTR, promotes the channel gating in a way that cannot be duplicated by Cer or Cer-1-phosphate. On the other hand, recent investigation demonstrates that S1P inhibits CFTR *via* adenosine monophosphate-activated kinase (AMPK) (Malik et al., 2015).

Many respiratory pathogens have SMase activity and *P. aeruginosa,* one of the major offenders, which permanently

colonizes the airways of virtually all of CF patients and secretes a phospholipase (termed PLC-H) working as a virulence factor that can hydrolyze SM *in situ*, leading to the formation of Cer (Stonehouse et al., 2002). In this frame, *P. aeruginosa* infection reducing CFTR chloride channel activity produces a negative impact on mucociliary clearance and consequently on the ability of airways to clear bacteria, resulting in an aggravation of the CF phenotype. The molecular mechanism linking the CFTR loss of function to Cer clusterization is still unknown.

Many studies report that *P. aeruginosa* reduces apical membrane expression and the chloride secretion of both wild-type (wt) and rescued F508del CFTR in respiratory epithelia by inhibiting apical endocytic recycling (Swiatecka-Urban et al., 2006; MacEachran et al., 2007; Bomberger et al., 2011). Recently, it has been observed that *P. aeruginosa* infection initially results in the post-translational modification of Na^+/H^+ exchange regulatory factor1 (NHERF1), causing an almost complete removal of CFTR from the apical membrane both *in vitro* and *in vivo* (Rubino et al., 2014). An important function of NHERF1 is the binding of its carboxy-terminal domain to ezrin. This scaffolding protein is a member of the ezrin/radixin/moesin (ERM) protein family comprising critical regulators of cytoskeletal-plasma membrane interactions (Pearson et al., 1977). Indeed, the NHERF1-dependent increase of apical membrane wt and F508del CFTR occurs *via* the re-organization of the actin cytoskeleton by inducing the formation of the multi-protein complex NHERF1-RhoA-ROCK-ezrin-actin (Favia et al., 2010; Monterisi et al., 2012).

Furthermore, ezrin is a PKA-anchoring protein and, in its opened form, may recruit the regulatory subunit of PKA to the proximity of CFTR, leading to its activation (Sun, 2000). Recently, it has been demonstrated that ERM proteins are strongly regulated by SLs. Indeed, the hydrolysis of plasma membrane SM, induced by bacterial sphingomyelinases, produces Cer that promotes ERM dephosphorylation through the activation of protein phosphatases (Hannun and Obeid, 2008; Canals et al., 2010). On the other hand, due to the physico-chemical properties of Cer, its increase on the plasma membrane could also sequester the CFTR in a rigid region that does not allow phosphorylation of the R domain. In fact, the rapid increase of Cer exerted by the *in-situ* hydrolysis of SM is followed by its spontaneous segregation. This process requires total rearrangement of the membrane, obtained by excluding some components and sorting others. In this context, it is not difficult to imagine that the original lipid-protein interactions undergo modifications and that the forces exerted by the lipid environment on the protein conformation could result in changes of the biological properties of proteins and of protein-protein interactions.

6. Conclusions and perspectives

Despite the availability of a wide spectrum of therapeutic options, the onset of lung disease characteristic of Cystic Fibrosis represents the highest challenge to overcome in order to reduce CF mortality. Although several findings clearly indicate altered SL metabolism in CF pulmonary disease, unfortunately, the direct involvement of SLs in the pathogenesis of CF lung disease is still unclear. In particular, it is not clear what the relationships between CFTR and SL metabolism and its dependence on membrane organization are. This opens the discussion, poorly addressed by the scientific community, on the importance of the lipid micro-environment in the regulation of the biological activity of plasma membrane-resident proteins, such as CFTR. For example, changes in SL composition associated with CF lung disease can directly determine a different membrane organization, which, in turn, could decrease CFTR stability and function. We think that it is fundamental to address this issue not only to define the molecular mechanisms involved in CF disease, but also in other pathologies caused by alteration in the activity of PM-associated proteins. Future progress in understanding the critical roles of SLs in these processes could open new perspectives for the development of alternative SL-based therapeutic strategies to reduce the inflammatory response as well as to rescue or increase CFTR function at the cell surface.

Conflict of interest

None.

Acknowledgement

The financial support of the Italian Cystic Fibrosis Research Foundation (FFC) (Grants #24/2014 to SS, #22/2015 to MCD,#9/2015 to AT, #18/2009, #19/2011, #17/2014 to GC) is gratefully acknowledged. Gina Jouè (University of Verona) is gratefully acknowledged for the English editing.

References

Abu-Arish, A., Pandzic, E., Goepp, J., Matthes, E., Hanrahan, J.W., Wiseman, P.W., 2015. Cholesterol modulates CFTR confinement in the plasma membrane of primary epithelial cells. Biophys. J. 109, 85–94.
Adamo, R., Sokol, S., Soong, G., Gomez, M.I., Prince, A., 2004. Pseudomonas aeruginosa flagella activate airway epithelial cells through asialoGM1 and toll-like receptor 2 as well as toll-like receptor 5. Am. J. Respir. Cell Mol. Biol. 30, 627–634.
Baker, N., Hansson, G.C., Leffler, H., Riise, G., Svanborg-Eden, C., 1990. Glycosphingolipid receptors for Pseudomonas aeruginosa. Infect. Immun. 58, 2361–2366.
Becker, K.A., Riethmuller, J., Luth, A., Doring, G., Kleuser, B., Gulbins, E., 2010. Acid sphingomyelinase inhibitors normalize pulmonary ceramide and inflammation in cystic fibrosis. Am. J. Respir. Cell Mol. Biol. 42, 716–724.
Bezzerri, V., Borgatti, M., Finotti, A., Tamanini, A., Gambari, R., Cabrini, G., 2011. Mapping the transcriptional machinery of the IL-8 gene in human bronchial epithelial cells. J. Immunol. 187, 6069–6081.
Billet, A., Jia, Y., Jensen, T., Riordan, J.R., Hanrahan, J.W., 2015. Regulation of the cystic fibrosis transmembrane conductance regulator anion channel by tyrosine phosphorylation. FASEB J. 29, 3945–3953.
Bodas, M., Min, T., Mazur, S., Vij, N., 2010. Critical modifier role of membrane-cystic fibrosis transmembrane conductance regulator-dependent ceramide signaling in lung injury and emphysema. J. Immunol. 186, 602–613.
Bodas, M., Min, T., Vij, N., 2011. Critical role of CFTR-dependent lipid rafts in cigarette smoke-induced lung epithelial injury. Am. J. Physiol. Lung Cell. Mol. Physiol. 300, L811–820.
Bomberger, J.M., Guggino, W.B., Stanton, B.A., 2011. Methods to monitor cell surface expression and endocytic trafficking of CFTR in polarized epithelial cells. Methods Mol. Biol. 741, 271–283.
Boyle, M.P., Bell, S.C., Konstan, M.W., McColley, S.A., Rowe, S.M., Rietschel, E., Huang, X., Waltz, D., Patel, N.R., Rodman, D., 2014. A CFTR corrector (lumacaftor) and a CFTR potentiator (ivacaftor) for treatment of patients with cystic fibrosis who have a phe508del CFTR mutation: a phase 2 randomised controlled trial. Lancet Respir. Med. 2, 527–538.
Brodlie, M., McKean, M.C., Johnson, G.E., Gray, J., Fisher, A.J., Corris, P.A., Lordan, J.L., Ward, C., 2010. Ceramide is increased in the lower airway epithelium of people with advanced cystic fibrosis lung disease. Am. J. Respir. Crit. Care Med. 182, 369–375.
Canals, D., Jenkins, R.W., Roddy, P., Hernandez-Corbacho, M.J., Obeid, L.M., Hannun, Y.A., 2010. Differential effects of ceramide and sphingosine 1-phosphate on ERM phosphorylation: probing sphingolipid signaling at the outer plasma membrane. J. Biol. Chem. 285, 32476–32485.
Cheng, S.H., Gregory, R.J., Marshall, J., Paul, S., Souza, D.W., White, G.A., O'Riordan, C. R., Smith, A.E., 1990. Defective intracellular transport and processing of CFTR is the molecular basis of most cystic fibrosis. Cell 63, 827–834.
Cohen, T.S., Prince, A., 2012. Cystic fibrosis: a mucosal immunodeficiency syndrome. Nat. Med. 18, 509–519.
Cutting, G.R., 2010. Modifier genes in Mendelian disorders: the example of cystic fibrosis. Ann. N.Y. Acad. Sci. 1214, 57–69.
Cutting, G.R., 2015. Cystic fibrosis genetics: from molecular understanding to clinical application. Nat. Rev. Genet. 16, 45–56.
de Bentzmann, S., Roger, P., Dupuit, F., Bajolet-Laudinat, O., Fuchey, C., Plotkowski, M.C., Puchelle, E., 1996. Asialo GM1 is a receptor for Pseudomonas aeruginosa adherence to regenerating respiratory epithelial cells. Infect. Immun. 64, 1582–1588.
Dechecchi, M.C., Nicolis, E., Mazzi, P., Cioffi, F., Bezzerri, V., Lampronti, I., Huang, S., Wiszniewski, L., Gambari, R., Scupoli, M.T., Berton, G., Cabrini, G., 2011.

Modulators of sphingolipid metabolism reduce lung inflammation. Am. J. Respir. Cell Mol. Biol. 45, 825–833.
Du, K., Sharma, M., Lukacs, G.L., 2005. The DeltaF508 cystic fibrosis mutation impairs domain-domain interactions and arrests post-translational folding of CFTR. Nat. Struct. Mol. Biol. 12, 17–25.
Favia, M., Guerra, L., Fanelli, T., Cardone, R.A., Monterisi, S., Di Sole, F., Castellani, S., Chen, M., Seidler, U., Reshkin, S.J., Conese, M., Casavola, V., 2010. Na+/H+ exchanger regulatory factor 1 overexpression-dependent increase of cytoskeleton organization is fundamental in the rescue of F508del cystic fibrosis transmembrane conductance regulator in human airway CFBE41o- cells. Mol. Biol. Cell 21, 73–86.
Feizi, T., 1985. Demonstration by monoclonal antibodies that carbohydrate structures of glycoproteins and glycolipids are onco-developmental antigens. Nature 314, 53–57.
Geeraert, L., Mannaerts, G.P., van Veldhoven, P.P., 1997. Conversion of dihydroceramide into ceramide: involvement of a desaturase. Biochem. J. 327 (Pt 1), 125–132.
Grassmé, H., Jekle, A., Riehle, A., Schwarz, H., Berger, J., Sandhoff, K., Kolesnick, R., Gulbins, E., 2001. CD95 signaling via ceramide-rich membrane rafts. J. Biol. Chem. 276, 20589–20596.
Grassme, H., Cremesti, A., Kolesnick, R., Gulbins, E., 2003a. Ceramide-mediated clustering is required for CD95-DISC formation. Oncogene 22, 5457–5470.
Grassme, H., Jendrossek, V., Riehle, A., von Kurthy, G., Berger, J., Schwarz, H., Weller, M., Kolesnick, R., Gulbins, E., 2003b. Host defense against Pseudomonas aeruginosa requires ceramide-rich membrane rafts. Nat. Med. 9, 322–330.
Guilbault, C., De Sanctis, J.B., Wojewodka, G., Saeed, Z., Lachance, C., Skinner, T.A., Vilela, R.M., Kubow, S., Lands, L.C., Hajduch, M., Matouk, E., Radzioch, D., 2008. Fenretinide corrects newly found ceramide deficiency in cystic fibrosis. Am. J. Respir. Cell Mol. Biol. 38, 47–56.
Guilbault, C., Wojewodka, G., Saeed, Z., Hajduch, M., Matouk, E., De Sanctis, J.B., Radzioch, D., 2009. Cystic fibrosis fatty acid imbalance is linked to ceramide deficiency and corrected by fenretinide. Am. J. Respir. Cell Mol. Biol. 41, 100–106.
Hamai, H., Keyserman, F., Quittell, L.M., Worgall, T.S., 2009. Defective CFTR increases synthesis and mass of sphingolipids that modulate membrane composition and lipid signaling. J. Lipid Res. 50, 1101–1108.
Hannun, Y.A., Obeid, L.M., 2008. Principles of bioactive lipid signalling: lessons from sphingolipids. Nat. Rev. Mol. Cell Biol. 9, 139–150.
Hanqing, M., Avrova, N., Mansson, J.E., Molin, K., Svennerholm, L., 1986. Gangliosides and neutral glycosphingolipids of normal tissue and oat cell carcinoma of human lung. Biochim. Biophys. Acta 878, 360–370.
Itokazu, Y., Pagano, R.E., Schroeder, A.S., O'Grady, S.M., Limper, A.H., Marks, D.L., 2014. Reduced GM1 ganglioside in CFTR-deficient human airway cells results in decreased beta1-integrin signaling and delayed wound repair. Am. J. Physiol. Cell Physiol. 306, C819–830.
Jeckel, D., Karrenbauer, A., Burger, K.N., van Meer, G., Wieland, F., 1992. Glucosylceramide is synthesized at the cytosolic surface of various Golgi subfractions. J. Cell Biol. 117, 259–267.
Kabayama, K., Sato, T., Saito, K., Loberto, N., Prinetti, A., Sonnino, S., Kinjo, M., Igarashi, Y., Inokuchi, J., 2007. Dissociation of the insulin receptor and caveolin-1 complex by ganglioside GM3 in the state of insulin resistance. Proc. Natl. Acad. Sci. U.S.A. 104, 13678–13683.
Kawai, T., Akira, S., 2010. The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. Nat. Immunol. 11, 373–384.
Kowalski, M.P., Pier, G.B., 2004. Localization of cystic fibrosis transmembrane conductance regulator to lipid rafts of epithelial cells is required for Pseudomonas aeruginosa-induced cellular activation. J. Immunol. 172, 418–425.
Krivan, H.C., Ginsburg, V., Roberts, D.D., 1988a. Pseudomonas aeruginosa and Pseudomonas cepacia isolated from cystic fibrosis patients bind specifically to gangliotetraosylceramide (asialo GM1) and gangliotriaosylceramide (asialo GM2). Arch. Biochem. Biophys. 260, 493–496.
Krivan, H.C., Roberts, D.D., Ginsburg, V., 1988b. Many pulmonary pathogenic bacteria bind specifically to the carbohydrate sequence GalNAc beta 1-4Gal found in some glycolipids. Proc. Natl. Acad. Sci. U. S. A. 85, 6157–6161.
Lahiri, S., Futerman, A.H., 2007. The metabolism and function of sphingolipids and glycosphingolipids. Cell. Mol. Life Sci. 64, 2270–2284.
Lavrentiadou, S.N., Chan, C., Kawcak, T., Ravid, T., Tsaba, A., van der Vliet, A., Rasooly, R., Goldkorn, T., 2001. Ceramide-mediated apoptosis in lung epithelial cells is regulated by glutathione. Am. J. Respir. Cell Mol. Biol. 25, 676–684.
Lee, J., Yeganeh, B., Ermini, L., Post, M., 2015. Sphingolipids as cell fate regulators in lung development and disease. Apoptosis 20, 740–757.
Loberto, N., Tebon, M., Lampronti, I., Marchetti, N., Aureli, M., Bassi, R., Giri, M.G., Bezzerri, V., Lovato, V., Cantu, C., Munari, S., Cheng, S.H., Cavazzini, A., Gambari, R., Sonnino, S., Cabrini, G., Dechecchi, M.C., 2014. GBA2-encoded beta-glucosidase activity is involved in the inflammatory response to Pseudomonas aeruginosa. PLoS One 9, e104763.
MacEachran, D.P., Ye, S., Bomberger, J.M., Hogan, D.A., Swiatecka-Urban, A., Stanton, B.A., O'Toole, G.A., 2007. The Pseudomonas aeruginosa secreted protein PA2934 decreases apical membrane expression of the cystic fibrosis transmembrane conductance regulator. Infect. Immun. 75, 3902–3912.
Machen, T.E., 2006. Innate immune response in CF airway epithelia: hyperinflammatory? Am. J. Physiol. Cell Physiol. 291, C218–C230.
Malik, F.A., Meissner, A., Semenkov, I., Molinski, S., Pasyk, S., Ahmadi, S., Bui, H.H., Bear, C.E., Lidington, D., Bolz, S.S., 2015. Sphingosine-1-Phosphate is a novel regulator of cystic fibrosis transmembrane conductance regulator (CFTR) activity. PLoS One 10, e0130313.
Mandon, E.C., van Echten, G., Birk, R., Schmidt, R.R., Sandhoff, K., 1991. Sphingolipid biosynthesis in cultured neurons: down-regulation of serine palmitoyltransferase by sphingoid bases. Eur. J. Biochem. 198, 667–674.
Mansson, J.E., Mo, H.Q., Egge, H., Svennerholm, L., 1986. Trisialosyllactosylceramide (GT3) is a ganglioside of human lung. FEBS Lett. 196, 259–262.
McNamara, N., Gallup, M., Sucher, A., Maltseva, I., McKemy, D., Basbaum, C., 2006. AsialoGM1 and TLR5 cooperate in flagellin-induced nucleotide signaling to activate Erk1/2. Am. J. Respir. Cell Mol. Biol. 34, 653–660.
Medler, T.R., Petrusca, D.N., Lee, P.J., Hubbard, W.C., Berdyshev, E.V., Skirball, J., Kamocki, K., Schuchman, E., Tuder, R.M., Petrache, I., 2008. Apoptotic sphingolipid signaling by ceramides in lung endothelial cells. Am. J. Respir. Cell Mol. Biol. 38, 639–646.
Wang, A.H., Wang Jr., E., 1986. Biosynthesis of long-chain (sphingoid) bases from serine by LM cells. Evidence for introduction of the 4-trans-double bond after de novo biosynthesis of N-acylsphinganine(s). J. Biol. Chem. 261, 3764–3769.
Michel, C., van Echten-Deckert, G., Rother, J., Sandhoff, K., Wang, E., Merrill Jr., A.H., 1997. Characterization of ceramide synthesis. A dihydroceramide desaturase introduces the 4: 5-trans-double bond of sphingosine at the level of dihydroceramide. J. Biol. Chem. 272, 22432–22437.
Mikami, T., Kashiwagi, M., Tsuchihashi, K., Akino, T., Gasa, S., 1998. Substrate specificity and some other enzymatic properties of dihydroceramide desaturase (ceramide synthase) in fetal rat skin. J. Biochem. (Tokyo) 123, 906–911.
Mistry, P.K., Liu, J., Sun, L., Chuang, W.L., Yuen, T., Yang, R., Lu, P., Zhang, K., Li, J., Keutzer, J., Stachnik, A., Mennone, A., Boyer, J.L., Jain, D., Brady, R.O., New, M.I., Zaidi, M., 2014. Glucocerebrosidase 2 gene deletion rescues type 1 Gaucher disease. Proc. Natl. Acad. Sci. U.S.A. 111, 4934–4939.
Mogayzel, P.J., Flume Jr., P.A., 2010. Update in cystic fibrosis 2009. Am. J. Respir. Crit. Care Med. 181, 539–544.
Mol, M.J., Erkelens, D.W., Leuven, J.A., Schouten, J.A., Stalenhoef, A.F., 1986. Effects of synvinolin (MK-733) on plasma lipids in familial hypercholesterolaemia. Lancet 2, 936–939.
Monterisi, S., Favia, M., Guerra, L., Cardone, R.A., Marzulli, D., Reshkin, S.J., Casavola, V., Zaccolo, M., 2012. CFTR regulation in human airway epithelial cells requires integrity of the actin cytoskeleton and compartmentalized cAMP and PKA activity. J. Cell Sci. 125, 1106–1117.
Nagiec, M.M., Baltisberger, J.A., Wells, G.B., Lester, R.L., Dickson, R.C., 1994. The LCB2 gene of saccharomyces and the related LCB1 gene encode subunits of serine palmitoyltransferase, the initial enzyme in sphingolipid synthesis. Proc. Natl. Acad. Sci. U.S.A. 91, 7899–7902.
Nahrlich, L., Mainz, J.G., Adams, C., Engel, C., Herrmann, G., Icheva, V., Lauer, J., Deppisch, C., Wirth, A., Unger, K., Graepler-Mainka, U., Hector, A., Heyder, S., Stern, M., Doring, G., Gulbins, E., Riethmuller, J., 2013. Therapy of CF-patients with amitriptyline and placebo–a randomised, double-blind, placebo-controlled phase IIb multicenter, cohort-study. Cell. Physiol. Biochem. 31, 505–512.
Pearson, A.D., Ricciardi, I.D., Benjamin, E., Mammen, K.E., 1977. Pasteurella multocida infection: a cause of abdominal pain? Lancet 2, 145.
Petrache, I., Natarajan, V., Zhen, L., Medler, T.R., Richter, A.T., Cho, C., Hubbard, W.C., Berdyshev, E.V., Tuder, R.M., 2005. Ceramide upregulation causes pulmonary cell apoptosis and emphysema-like disease in mice. Nat. Med. 11, 491–498.
Petrache, I., Medler, T.R., Richter, A.T., Kamocki, K., Chukwueke, U., Zhen, L., Gu, Y., Adamowicz, J., Schweitzer, K.S., Hubbard, W.C., Berdyshev, E.V., Lungarella, G., Tuder, R.M., 2008. Superoxide dismutase protects against apoptosis and alveolar enlargement induced by ceramide. Am. J. Physiol. Lung Cell. Mol. Physiol. 295, L44–L53.
Pewzner-Jung, Y., Tavakoli Tabazavareh, S., Grassme, H., Becker, K.A., Japtok, L., Steinmann, J., Joseph, T., Lang, S., Tuemmler, B., Schuchman, E.H., Lentsch, A.B., Kleuser, B., Edwards, M.J., Futerman, A.H., Gulbins, E., 2014. Sphingoid long chain bases prevent lung infection by Pseudomonas aeruginosa. EMBO Mol. Med. 6, 1205–1214.
Prinetti, A., Loberto, N., Chigorno, V., Sonnino, S., 2009. Glycosphingolipid behaviour in complex membranes. Biochim. Biophys. Acta 1788, 184–193.
Ramsey, B.W., Davies, J., McElvaney, N.G., Tullis, E., Bell, S.C., Drevinek, P., Griese, M., McKone, E.F., Wainwright, C.E., Konstan, M.W., Moss, R., Ratjen, F., Sermet-Gaudelus, I., Rowe, S.M., Dong, Q., Rodriguez, S., Yen, K., Ordonez, C., Elborn, J.S., 2011. A CFTR potentiator in patients with cystic fibrosis and the G551D mutation. N. Engl. J. Med. 365, 1663–1672.
Ramu, Y., Xu, Y., Lu, Z., 2007. Inhibition of CFTR Cl- channel function caused by enzymatic hydrolysis of sphingomyelin. Proc. Natl. Acad. Sci. U.S.A. 104, 6448–6453.
Riordan, J.R., Rommens, J.M., Kerem, B., Alon, N., Rozmahel, R., Grzelczak, Z., Zielenski, J., Lok, S., Plavsic, N., Chou, J.L., et al., 1989. Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. Science 245, 1066–1073.
Riordan, J.R., 2008. CFTR function and prospects for therapy. Annu. Rev. Biochem. 77, 701–726.
Rother, J., van Echten, G., Schwarzmann, G., Sandhoff, K., 1992. Biosynthesis of sphingolipids: dihydroceramide and not sphinganine is desaturated by cultured cells. Biochem. Biophys. Res. Commun. 189, 14–20.
Rubino, R., Bezzerri, V., Favia, M., Facchini, M., Tebon, M., Singh, A.K., Riederer, B., Seidler, U., Iannucci, A., Bragonzi, A., Cabrini, G., Reshkin, S.J., Tamanini, A., 2014. Pseudomonas aeruginosa reduces the expression of CFTR via post-translational modification of NHERF1. Pflugers Arch. 466, 2269–2278.
Saiman, L., Prince, A., 1993. Pseudomonas aeruginosa pili bind to asialoGM1 which is increased on the surface of cystic fibrosis epithelial cells. J. Clin. Invest. 92, 1875–1880.

Seitz, A.P., Grassme, H., Edwards, M.J., Pewzner-Jung, Y., Gulbins, E., 2015. Ceramide and sphingosine in pulmonary infections. Biol. Chem. 396, 611–620.
Shimeno, H., Soeda, S., Sakamoto, M., Kouchi, T., Kowakame, T., Kihara, T., 1998. Partial purification and characterization of sphingosine N-acyltransferase (ceramide synthase) from bovine liver mitochondrion-rich fraction. Lipids 33, 601–605.
Solomon, G.M., Marshall, S.G., Ramsey, B.W., Rowe, S.M., 2015. Breakthrough therapies: cystic fibrosis (CF) potentiators and correctors. Pediatr. Pulmonol. 50 (Suppl. 40), S3–S13.
Sonnino, S., Prinetti, A., Mauri, L., Chigorno, V., Tettamanti, G., 2006. Dynamic and structural properties of sphingolipids as driving forces for the formation of membrane domains. Chem. Rev. 106, 2111–2125.
Sonnino, S., Prinetti, A., Nakayama, H., Yangida, M., Ogawa, H., Iwabuchi, K., 2009. Role of very long fatty acid-containing glycosphingolipids in membrane organization and cell signaling: the model of lactosylceramide in neutrophils. Glycoconjugate J. 26, 615–621.
Stoffel, W., Assmann, G., Binczek, E., 1970. Metabolism of sphingosine bases. 13. Enzymatic synthesis of 1-phosphate esters of 4t-sphingenine (sphingosine) sphinganine (dihydrosphingosine), 4-hydroxysphinganine (phytosphingosine) and 3-dehydrosphinganine by erythrocytes. Hoppe Seylers Z. Physiol. Chem. 351, 635–642.
Stonehouse, M.J., Cota-Gomez, A., Parker, S.K., Martin, W.E., Hankin, J.A., Murphy, R. C., Chen, W., Lim, K.B., Hackett, M., Vasil, A.I., Vasil, M.L., 2002. A novel class of microbial phosphocholine-specific phospholipases C. Mol. Microbiol. 46, 661–676.
Sun, F., Hug, M.J., Bradbury, N.A., Frizzell, R.A., 2000. Protein kinase A associates with cystic fibrosis transmembrane conductance regulator via an interaction with ezrin. J. Biol. Chem. 275, 14360–14366.
Swiatecka-Urban, A., Moreau-Marquis, S., Maceachran, D.P., Connolly, J.P., Stanton, C.R., Su, J.R., Barnaby, R., O'Toole G, A., Stanton, B.A., 2006. Pseudomonas aeruginosa inhibits endocytic recycling of CFTR in polarized human airway epithelial cells. Am. J. Physiol. Cell Physiol. 290, C862–872.
Teichgraber, V., Ulrich, M., Endlich, N., Riethmuller, J., Wilker, B., De Oliveira-Munding, C.C., van Heeckeren, A.M., Barr, M.L., von Kurthy, G., Schmid, K.W., Weller, M., Tummler, B., Lang, F., Grassme, H., Doring, G., Gulbins, E., 2008. Ceramide accumulation mediates inflammation, cell death and infection susceptibility in cystic fibrosis. Nat. Med. 14, 382–391.
Thelin, W.R., Chen, Y., Gentzsch, M., Kreda, S.M., Sallee, J.L., Scarlett, C.O., Borchers, C. H., Jacobson, K., Stutts, M.J., Milgram, S.L., 2007. Direct interaction with filamins modulates the stability and plasma membrane expression of CFTR. J. Clin. Invest. 117, 364–374.
Uhlig, S., Gulbins, E., 2008. Sphingolipids in the lungs. Am. J. Respir. Crit. Care Med. 178, 1100–1114.
Vilela, R.M., Lands, L.C., Meehan, B., Kubow, S., 2006. Inhibition of IL-8 release from CFTR-deficient lung epithelial cells following pre-treatment with fenretinide. Int. Immunopharmacol. 6, 1651–1664.
Warnock, D.E., Lutz, M.S., Blackburn, W.A., Young, W.W., Baenziger Jr., J.U., 1994. Transport of newly synthesized glucosylceramide to the plasma membrane by a non-Golgi pathway. Proc. Natl. Acad. Sci. U.S.A. 91, 2708–2712.
Weiss, B., Stoffel, W., 1997. Human and murine serine-palmitoyl-CoA transferase–cloning, expression and characterization of the key enzyme in sphingolipid synthesis. Eur. J. Biochem. 249, 239–247.
Welsh, M.J., Smith, J.J., 2001. cAMP stimulation of HCO3- secretion across airway epithelia. JOP 2, 291–293.
Wright, J.M., Merlo, C.A., Reynolds, J.B., Zeitlin, P.L., Garcia, J.G., Guggino, W.B., Boyle, M.P., 2006. Respiratory epithelial gene expression in patients with mild and severe cystic fibrosis lung disease. Am. J. Respir. Cell Mol. Biol. 35, 327–336.
Yang, Y., Uhlig, S., 2011. The role of sphingolipids in respiratory disease. Ther. Adv. Respir. Dis. 5, 325–344.
Yu, H., Zeidan, Y.H., Wu, B.X., Jenkins, R.W., Flotte, T.R., Hannun, Y.A., Virella-Lowell, I., 2009. Defective acid sphingomyelinase pathway with Pseudomonas aeruginosa infection in cystic fibrosis. Am. J. Respir. Cell Mol. Biol. 41, 367–375.
Zhang, Y., Li, X., Grassme, H., Doring, G., Gulbins, E., 2010. Alterations in ceramide concentration and pH determine the release of reactive oxygen species by Cftr-deficient macrophages on infection. J. Immunol. 184, 5104–5111.