Chapter 10

Gangliosides and Cell Surface Ganglioside Metabolic Enzymes in the Nervous System

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Abstract

Gangliosides are a large group of complex lipids found predominantly in the outer layer of the plasma membrane of cells, particularly abundant in nerve endings. Their half-life in the nervous system is short, and their membrane composition and content are strictly connected to their metabolism. The neobiosynthesis of gangliosides starts in the endoplasmic reticulum and is completed in the Golgi apparatus, whereas catabolism occurs primarily in lysosomes. However, the final content of gangliosides in the plasma membrane is defined by other cellular processes.

This chapter will discuss structural changes in the oligosaccharide chains of gangliosides, induced by the activity of plasma membrane-associated glycohydrolases and glycosyltransferases. Some of the plasma membrane enzymes originate from fusion processes between intracellular fractions and the plasma membrane, while, others display a different structure. Several of these plasma membrane enzymes have been characterized and some of them seem to have a specific role in the nervous system.

Keywords

Gangliosides; Glycosphingolipids; Glycohydrolases; Sphingolipid Metabolism; Central Nervous System; Neuronal Differentiation; Neurodegeneration.

10.1. Gangliosides

Gangliosides are glycosphingolipids (GSLs) containing one or more sialic acid residues. They are components of the external layer of the plasma membrane of all mammalian cells and are particularly abundant in the brain, where they display tenfold higher levels with respect to extra-nervous tissues, representing one-twelfth of the outer layer of

glycerophospholipids. Neuronal gangliosides are lipids with strong amphiphilic properties due to their acidic and, in general, large saccharide head group and double-tailed hydrophobic moiety.

10.1.1 Ceramide: the lipid portion of gangliosides

The lipid backbone of gangliosides, as well as the one of all sphingolipids, is ceramide (Fig. 10.1.1 A), which consists of a long-chain amino alcohol connected to a fatty acid by an amide linkage. As a peculiar feature of the nervous system, the amino alcohol can either be 2*S*,3*R*,*4E*,2-amino-1,3-dihydroxy-octadecene, known as sphingosine and C18-sphingosine or 2*S*,3*R*,*4E*,2-amino-1,3-dihydroxy-eicosene, known as eicosasphingosine and C20-sphingosine. The ratio between the two long-chain alcohols is variable: eicosasphingosine is barely detectable in fetal brain and increases gradually with age until it becomes the main component during old age. A small percentage of saturated compounds, sphinganine and eicosasphinganine, has also been identified (Valsecchi et al. 1996; Valsecchi et al. 1993; Chiricozzi et al. 2021a).

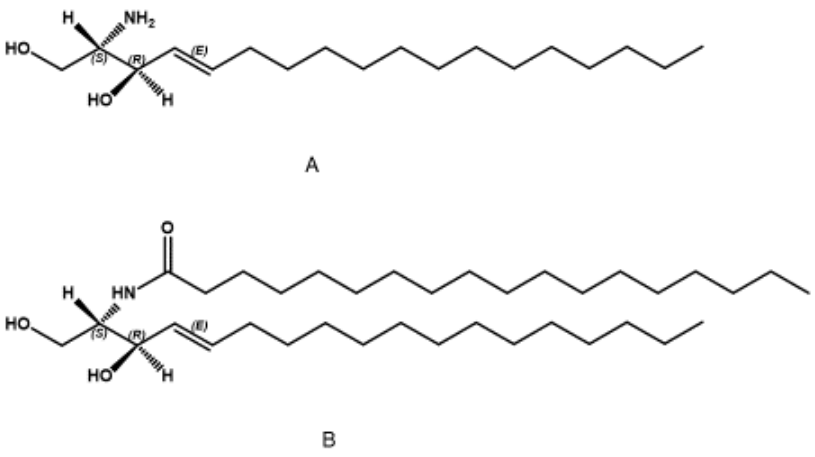

Fig. 10.1.1 Chemical structures of C18-sphingosine (A) and ceramide containing C18-sphingosine and stearic acid (B).)

In extra-nervous tissues, gangliosides exhibit heterogeneity in their acyl chains, where often a very long fatty acid moiety becomes the major component of the ceramide structure. While in the nervous system, stearic acid (Fig. 10.1.1 B) is the most common fatty acid of the gangliosides accounting for 90–95 % of the total fatty acid content. This characteristic of neuronal gangliosides may be necessary for neuronal membrane plasticity.

10.1.2. The oligosaccharide portion of gangliosides.

The oligosaccharide portion of neuronal gangliosides widely varies due to the neutral sugar content and the number of sialic acid residues. This property, together with the heterogeneity of the ceramide moiety, makes gangliosides a very large family of compounds. Table 10.1.2 shows the main ganglioside structures found in the human

nervous system, together with trivial and commonly used abbreviations.

Table 10.1.2 The main oligosaccharide series in the human nervous system

Structure of the oligosaccharide chain	Series
β-Gal-	Gal
β-Gal-(1-4)-β-Glc-	Lac
β-GalNAc-(1-4)-β-Gal-(1-4)-β-Glc-	Gg_3
β-Gal-(1-3)-β-GalNAc-(1-4)-β-Gal-(1-4)-β-Glc-	Gg_4
β-GalNAc-(1-4)-β-Gal-(1-3)-β-GalNAc-(1-4)-β-Gal-(1-4)-β-Glc-	Gg_5
β-Gal-(1-4)-β-GlcNAc-(1-3)-β-Gal-(1-4)-β-Glc-	nLc_4
The main gangliosides from the human nervous system	
Svennerholm nomenclature	IUPAC-IUB nomenclature
GM4	I^3Neu5AcGalCer
GM3	II^3Neu5AcLacCer
GD3	$II^3(Neu5Ac)_2$LacCer
GM2	II^3Neu5AcGg$_3$Cer
GD2	$II^3(Neu5Ac)_2Gg_3$Cer
GM1	II^3Neu5AcGg$_4$Cer
3′-LM1	IV^3nLc_4Cer
GD1a	IV^3Neu5AcII^3Neu5AcGg$_4$Cer
GalNAc-GD1a	IV^3Neu5AcII^3Neu5AcGg$_5$Cer
GD1b	$II^3(Neu5Ac)_2Gg_4$Cer
GD1b-lactone	II^3[Neu5Ac-(2-8,1-9)-Neu5Ac]Gg$_4$Cer
GT1b	IV^3Neu5Ac$II^3(Neu5Ac)_2Gg_4$Cer
O-Acetyl-GT1b	IV^3Neu5AcII^3[Neu5,9Ac_2-(2-8)-Neu5Ac]Gg$_4$Cer
GQ1b	$IV^3(Neu5Ac)_2II^3(Neu5Ac)_2Gg_4$Cer
O-Acetyl-GQ1b	$IV^3(Neu5Ac)_2II^3$[Neu5,9Ac_2-(2-8)-Neu5Ac]Gg$_4$Cer

Sialic acid is a name that identifies all the derivatives of 5-amino-3,5-dideoxy-d-*glycero*-D-*galacto*-non-2-ulopyranosonic acid, or neuraminic acid. Among the sialic acids, the 5-*N*-acetyl (Fig. 10.1.2a), the 5-*N*-acetyl-9-*O*-acetyl (Fig. 10.12b), and the 5-*N*-glycolyl derivatives (Fig. 10.1.2c) are the most common ones. Importantly, healthy humans present only the first two sialic acids, in a ratio of 9:1 (Kamerling and Vliegenthart 1975). Also ganglioside lactones, containing polysialyl chains where the sialic acids are linked together with ketosidic and ester linkages (Fig. 10.1.2d), have been found in human brains (Riboni et al. 1986).

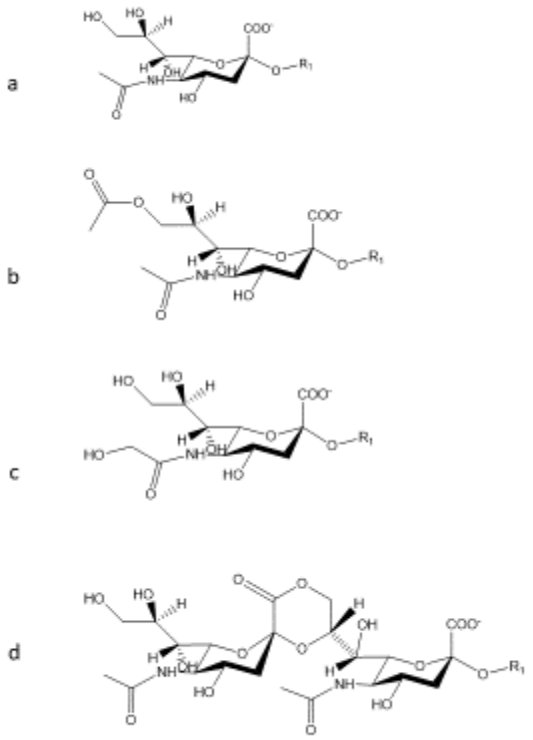

Figure 10.1.2 Sialic acid structures. (**a**) 5-acetamido-3,5-dideoxy-D-*glycero*-D-*galacto*-non-2-ulopyranosonic acid, Neu5Ac. (**b**) 5-acetamido-9-*O*-acetyl-3,5-dideoxy-D-*glycero*-D-*galacto*-non-2-ulopyranosonic acid, Neu5,9Ac$_2$. (**c**) 3,5 dideoxy-5-glycolamido-D-*glycero*-D-*galacto*-non-2-ulopyranosonic acid, Neu5Gc. (**d**) Sialic acids linked together with ketosidic and ester linkages. R1: saccharides or oligosaccharide chain

10.2. Gangliosides and membrane organization

Major part of the gangliosides are located in confined areas of the plasma membrane known as “lipid rafts” (Sonnino et al. 2006). Lipid rafts are specific membrain domains enriched in sphingolipids and cholesterol, with respect to glycerophospholipids, and contain about 1–4 % of the total protein content of the cell (Sonnino et al. 2006). Many lipid raft proteins are involved in cell signaling events, and this led to the concept that ganglioside–protein interactions are instrumental in signal transduction and cell function (Lunghi et al. 2021; Chiricozzi et al. 2019). Protein properties might be affected by specific interactions, however, could also be modified by the physicochemical properties of the membrane, which are determined by the lipid pattern, lipid amphiphilic and geometric properties and lipid organization. Gangliosides, with their complex and expanded oligosaccharide portion, need a larger interfacial area than glycerolipids. Phase separation by clustering GSLs in a phospholipid bilayer is a spontaneous process driven by minimization of the interfacial free energy. Segregation of amphiphilic molecules with a large hydrophilic head group implies the acquisition of a positive membrane curvature. The interfacial area increases along with the size of the oligosaccharide chain, confering a more positive membrane curvature and a

more pronounced segregation. The geometric properties of each ganglioside inserted into the membrane, depend primarily on the structure of the oligosaccharide portion (Sonnino et al. 1994) and, to a lesser extent, on those of ceramide.

Any variation in either the head group or the ceramide portion can have marked effects on the membrane surface, particularly on lipid rafts, where gangliosides are highly enriched. For instance, in an artificial membrane model, a dramatic effect on membrane curvature and organization, has been observed when sialidase is used to catabolize the disialoganglioside GD1a into monosialoganglioside GM1(Del Favero et al. 2011). Interestingly, this process has also been described in senescent neurons and during neurodegeneration, where the sequential hydrolysis of ganglioside to ceramide mediated by plasma membrane-associated GSLs hydrolases is described (Aureli et al. 2011c).

Ceramide is very hydrophobic and almost insoluble inaqueous solutions, because of the presence of the two hydrocarbon chains. However, in a membrane, it can be considered amphiphilic due to the primary hydroxyl group and the amide planar linkage located at the water–lipid interface. It is claimed that when a large amount of ceramide is produced from complex sphingolipids, it rapidly segregates forming microdomains necessary for cell signaling (Gulbins and Grassme 2002). Ceramide has a very high packing parameter suitable for negative curvature. The removal of the head group from glycosphingolipids and sphingomyelin leads to ceramide-enriched endocytic vesicles as demonstrated in artificial membranes (Holopainen et al. 2000). On the other hand, in a natural membrane this process would require rearrangement of the membrane with the exclusion of some components and the inclusion of others. In this context, the original lipid–protein interactions or the forces exerted by the lipid environment on the protein conformation would change with concomitant modifications of the biological properties of proteins.

10.3. Metabolic pathways of gangliosides

The plasma membrane ganglioside content and pattern is due to a dynamic process determined by the balance of neobiosynthesis, catabolism, and complex trafficking in- and outside the cell. Changes in any of these pathways may result in alterations of the plasma membrane ganglioside content that can affect neuronal differentiation and cause neurodegeneration. A general scheme for GSLs metabolism is shown in Fig. 10.3a.

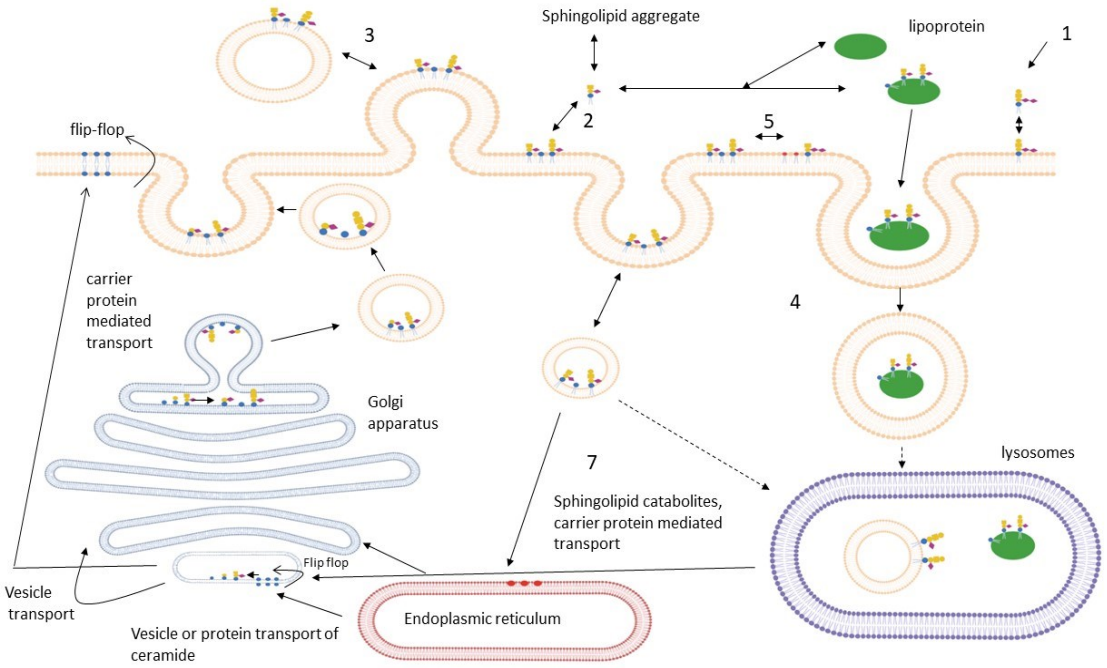

Fig. 10.3a Scheme depicting glycosphingolipid metabolism. Different metabolic pathways involved in changing plasma membrane glycosphingolipids composition. 1—plasma membrane uptake of extracellular glycolipids shed by neighbour cells; 2—shedding of glycolipid monomers, which can either directly fuse with the membrane , or interact with the extracellular proteins or lipoproteins and are subsequently taken up by the cells and catabolized into lysosomes; 3—release of glycolipid-containing vesicles from the plasma membrane; 4—membrane endocytosis followed by sorting to lysosomes and lysosomal catabolism; 5—biosynthetic modifications by plasma membrane-associated glycosyltransferases and glycosidases; 6—neobiosynthesis of glycosphingolipids and their transport to the cell surface; 7—recycle in the biosynthetic pathway of partially catabolized molecules.

De novo biosynthesis of GSLs requires ceramide, which is synthesized in the endoplasmic reticulum (ER). In contrast to other cells, neurons have two different serine acyl-CoA acyltransferases: one specific for palmitoyl-CoA and the other for stearoyl-CoA. They are expressed in different proportions and in a spatiotemporal dependent manner during the development of neurons (Chigorno et al. 1997a) and are necessary for the first step in the synthesis of sphinganine and eicosasphinganine, respectively. The biosynthetic process leading to the production of ceramide is shown in Fig. 10.3b. Ceramide can be transported to the Golgi apparatus, the site of ganglioside synthesis, by either vesicular or non-vesicular (protein mediated) trafficking (Olayioye and Hausser 2012). From the Golgi apparatus, neobiosynthesized gangliosides are transferred to the plasma membrane by a vesicle-mediated transport system, becoming components of the external plasma membrane leaflet.

On the other hand, catabolism of gangliosides takes place in lysosomes, where complex products are degraded into less complex ones and can be released in the cytosol (Kolter and Sandhoff 2005).

Among the more hydrophilic gangliosides, a minor portion is released from the plasma membranes into the extracellular environment (Chigorno et al. 2006). Nevertheless, sphingolipids present in the extracellular milieu are, at least in part, taken up by other cells,

becoming either components of their membranes, where they may modify the composition, or be directly sorted to the lysosomes (Saqr et al. 1993).

In addition, enzymes involved in catabolism (hydrolases) and biosynthesis (transferases) of sphingolipids are found to be associated with the plasma membrane where they may act on membrane components (Aureli et al. 2011b).

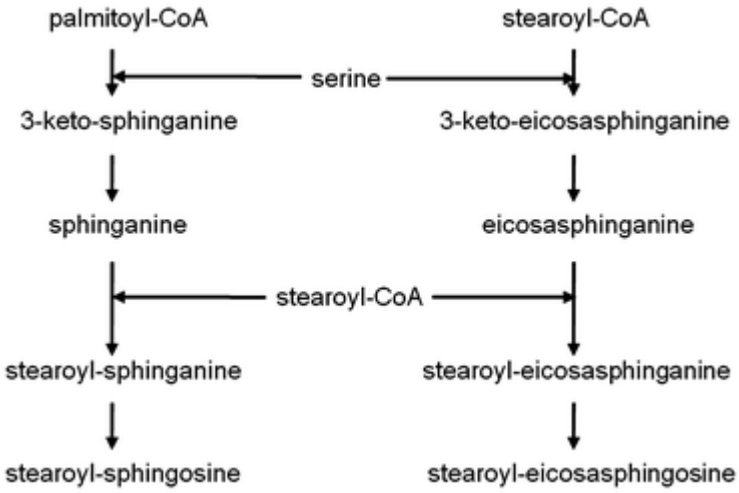

Fig. 10.3b Biosynthetic pathway of ceramide in the brain. Ceramide is synthesized in the endoplasmic reticulum. Neurons have two different serine acyl-CoA acyltransferases: one specific for palmitoyl-CoA and and the other for stearoyl-CoA. They are expressed in different proportions and in spatiotemporal- dependent manner during neuronal development, and are necessary for the first step in the synthesis of sphinganine and eicosasphinganine, respectively

While the steps of the biosynthesis of GSLs are already well defined, little is known about its regulation. According to a general knowledge, the biosynthesis is primarily regulated at transcriptional level controlling the expression of glycosyltransferases and/or transporter proteins. Indeed, changes in the cellular GSL pattern, as those observed along neuronal development, upon oncogenic transformation, and upon the development of drug resistance of tumor cells, are paralleled by changes in the expression of the corresponding glycosyltransferases. However, it is also possible that alterations in the intracellular metabolism and/or in the half-life of each GSL, influence their pattern (Veldman et al. 2002). Indeed, regulation of intracellular trafficking may be as important as the regulation of enzyme expression, in determining the final GSLs composition of the plasma membrane. Dependence of cells on neobiosynthesis and subsequent transport of the GSLs to the plasma membrane could require too much time to modify the plasma membrane GSLs composition in response to extracellular signals. In addition, even though the half-life of GSLs may be short in neurons (Prinetti et al. 2001), it is long or very long in the majority of the other cells. In fibroblasts, where the half-life of gangliosides is long (Chigorno et al. 1997b), their major loss is due to shedding into the extracellular environment (Chigorno et al. 2005), rather than to catabolism. Indeed, it has been determined that up to 7–8 % of the total cell sphingolipids are shed every day by cells in culture (Chigorno et al. 2006), making neobiosynthesis of GSLs a necessity.

The fact that some enzymes involved in GSLs metabolism have been found also in the

plasma membrane, modifies the general concept that glycohydrolases are located only in lysosomes and glycosyltransferases only in the Golgi apparatus. The association of the plasma membrane with enzymes, involved in GSLs metabolism, allowed the gain of information about their activity on natural substrates in living cells, along with the identification of the presence of enzyme pairs, such as the sialidase and the sialyltransferase, able to catalyze opposite reactions. This suggests that alterations in plasma membrane GSLs could occur rapidly in response to different stimuli. These changes would only depend on the kinetic properties of the enzymes, which can change very rapidly in response to ligand-triggered stimuli.

10.4. Plasma membrane-associated enzymes and ganglioside pattern

The concept that GSL biosynthesis and catabolism are specifically associated with the Golgi apparatus and the lysosomes respectively, has been partially overcome by emerging information related to the presence of enzymes involved in GSLs metabolism on the plasma membrane. These enzymes include the sialidases and sialyltransferases, β-hexosaminidases and β-*N*-acetyl-galactosyltransferases, β-galactosidases, and the β-glucocerebrosidase GCase and the non lysosomal β-glucosylceramidase (NL-Gase). Description of the synaptosomal membrane association of sialidases and sialyltransferases goes back to the 70s (Schengrund and Rosenberg 1970; Tettamanti et al. 1972; Tettamanti et al. 1973; Tettamanti et al. 1975; Preti et al. 1980), and leads to the hypothesis that a sialylation–desialylation cycle exists for gangliosides at plasma membrane level and might be involved in defining the functional role of gangliosides in neurons. The sialylation–desialylation cycle anticipates the elucidation of the "sphingomyelin cycle" of 20 years (Venable et al. 1995). In this cycle, the two enzymes, sphingomyelinase and sphingomyelin synthase, modulate cell proliferation and apoptosis playing on the levels of ceramide content. Recent information on the existence of a similar cycle for *N*-acetylgalactosamine, confirms the possibility to drastically modify the sphingolipid composition of the plasma membrane. The head group of an amphiphilic compound, together with the volume of the hydrophobic backbone, determines the packing parameter of the monomer inserted into the membrane. In GSLs, this parameter is determined by the ratio between the volume occupied by the oligosaccharide chain and by the ceramide. The enzymatic removal of sugars from the GSL leads to a higher packing parameter resulting in a less curved surface. On the other hand, the enzymatic addition of sugars reduces this packaging parameter and promotes an enhanced curvature. (Sonnino et al. 1994; Brocca P. and Sonnino S. 1997). All these aspects allow the formation of "lipid rafts" with specific size and dynamics. In addition, drastic changes in the amphiphilic properties of GSLs, such as the sequential action of hydrolytic enzymes able to produce ceramide from gangliosides, favorites the translocation of ceramide from the outer to the inner membrane layer, or the formation of endocytic vesicles. These modifications explain the first step in a process, that causes the shift of ceramide from the plasma membrane to internal cell membranes. Fig. 10.4 is a schematic representation showing how the membrane curvature changes in function of enzymatic activities.

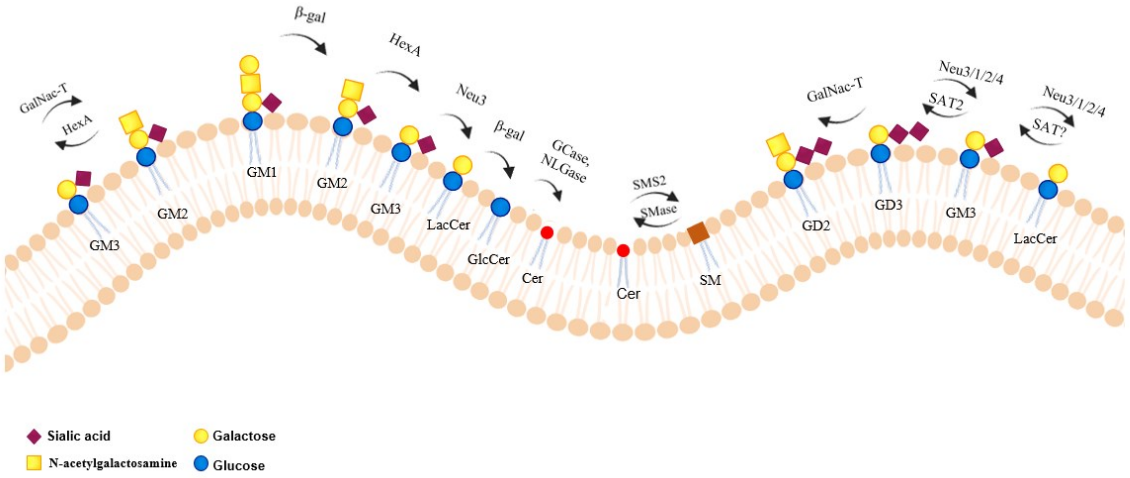

Fig. 10.4 Schematic representation of the glycosyltransferases and glycohydrolases associated with the cell surface. The presence of these different enzymes at this level, allows the in situ modification of the cell surface glycolipid composition. The availability of a series of enzyme couples catalyzing the same reaction in opposite directions extends the concept of cycle, originally reported for the sphingomyelin, to several other sphingolipids.

β-hexosaminidase A (Hex A); UDP-N-UDP-*N*-acetylgalactosaminyltransferase (GalNAc-T); β-galactosidase Smase(β-Gal); sphingomyelinase (SMase); sphyngomyelinsinthase2 (SMS2) SAT1

10.5. Sialidases, sialyltransferases and plasma membrane sialidase Neu3

In gangliosides, sialic acid is usually linked to the C3 position of galactose or to the C8 position of another sialic acid residue, by an α-linkage. Four different sialidases capable of catalyzing the release of ganglioside sialic acid moieties have been identified. Neu1 is located in lysosomes and it is known from long time to be needed for the catabolism of sialo-compounds. Neu2 is a cytosolic enzyme that seems to be involved in differentiation processes, and is capable of removing also the sialic acid linked to the inner galactose of GM1, under specif experimental conditions (Tringali et al. 2004). Neu3 is associated with the plasma membrane and has been described to be involved in several processes.

Neu4, which is present in humans in two different forms, a short and a long one, has been found associated with mitochondria, ER and lysosomes.(Glanz Victor Yu, European Journal of Pharmacology 2018).

Interestingly, it has been demonstrated that the localization of the sialidases can vary according to specific stimuli. Although Neu3 is considered the plasma membrane specific enzyme isoform, small amounts of the other sialidases have also been found to be associated with the plasma membrane or intracellular membranes (Wang et al. 2009; Akita et al. 1997; Lukong et al. 2001; Vinogradova et al. 1998).

Monocyte differentiation up-regulates the expression of the lysosomal sialidase Neu1, and triggers its targeting to the plasma membrane via major histocompatibility complex class II-positive compartments. (Liang et al. 2006; Nath et al. 2018)

Neu3 (NEU3, EC 3.2.1.18) is the first sialidase to be distinguished from lysosomal isoforms and to be identified at plasma membrane level. This finding has been suggested by enzymatic and immunological studies (Schengrund et al. 1976; Miyagi et al. 1990a; Miyagi et al. 1990b; Schneider-Jakob and Cantz 1991; Kopitz et al. 1994), as well as by

metabolic studies (Riboni et al. 1991; Kopitz et al. 1997a), where a membrane-bound sialidase was purified from human brain grey matter (Kopitz et al. 1997b) and from bovine brain (Hata et al. 1998; Oehler et al. 2002). In 1999, the existence of a specific membrane associated sialidase (coded as Neu3), different from other known sialidases, was proven cloning its cDNA sequence from human (Wada et al. 1999), bovine (Miyagi et al. 1999) and mouse (Hasegawa et al. 2000). It can be considered an ubiquitous enzyme since it is expressed, even thought at different levels, in the plasma membrane of the majority of healthy and pathological human tissues, such as the human brain (Kopitz et al. 1994), healthy colon, as well as colorectal carcinoma tissues, hepatic tumors, and kidney carcinomas (Monti et al. 2002; Miyagi et al. 2008b; Miyagi et al. 2008°; Ueno et al. 2006; Kakugawa et al. 2002). In addition, its expression and activity were also assayed in healthy and pathological cell lines, for example in erythroid and erythroleukemic cell lines (Venerando et al. 2002; Tringali et al. 2007b; Tringali et al. 2007°), fibroblasts (Chigorno et al. 1986), neurons, neuroblastoma cells (Schengrund and Repman 1982), breast ductal cancer T47D cells, colon carcinoma CaCo2 cells, colorectal adenocarcinoma HT29 cells, different types of ovarian cancer cells and cervix adenocarcinoma HeLa cells (Kakugawa et al. 2002).
The partial association of lysosomal Neu1 with the plasma membrane is in some cases the result of the fusion processes required to repair plasma membrane damages (Reddy et al. 2001). Nevertheless, other pathways, such as the lysosomal exocytosis due to an excess of intracelluar lysosomes, (Samarani et al. 2018) as well as the fusion of mithocondria and ER with the plasma membrane, (Annunziata et al. 2018) are involved in the trafficking of Neu1 towards the cell surface. On the other hand, even if some evidence suggest its occurrence, it is more difficult to imagine Neu2 traslocation from the cytosol to the plasma membrane (Nath et al. 2018).

It is well-known that in the plasma membrane Neu3 and gangliosides co-localize in Triton X-100 insoluble “lipid rafts” (Kalka et al. 2001). The non random distribution of Neu3 at the cell surface could suggest that the biological effects of this enzyme can be attributed to the local reorganization of GSLs-based signaling units. Remarkably, Neu3 modulates cell surface GSLs composition by *trans* interactions, hydrolyzing substrates on the surface of neighbour cells (Papini et al. 2004). The optimal pH of Neu3, Neu1, and Neu4, ranges between 4.4 and 4.8. This raises the question of how such an acidic pH can be achieved at the cell surface. The proton pumps co-colocalize with Neu3 and gangliosides and allow the to achieve in a small environment, the proper pH required for enzyme function and ganglioside hydrolysis. This has been demonstrated in *in vitro* experiments by the inhibition or activation of the cell surface proton pump system (Aureli et al. 2012b).
Neu3 was originally described as an enzyme that specifically hydrolyzes gangliosides (Miyagi et al. 2018). Later, thanks to the metabolic *in vitro* labelling of glycoproteins with [^{3}H]*N*-acetylmannosamine, hydrolysis was also observed to occur on sialic acid residues linked to glycoproteins(Valaperta et al., 2006). It is difficult to compare the kinetic properties of glycohydrolases on different substrates, such as glycolipids, oligosaccharides, and glycoproteins. The enzyme K_m and V_{max} strictly depend on how the substrate gets presented to the enzyme. Kinetic properties of hydrolases working on gangliosides have been studied in detail. These parameters can vary in several orders of magnitude, from aggregates to free oligosaccharides, due to the different interaction with the substrate and

the substrate recognition. Similarly, the two parameters are completely different on the same substrate presented as homogeneous micelle, micelle containing a detergent, or lyposomess with different content of substrate (Masserini et al. 1982; Venerando et al. 1982)

Since Neu3 is of great interest, several studies have been conducted to understand its role in cellular processes. In colon and renal cancer this sialidase appears to be responsible for acting on the monosialo-ganglioside GM3, maintaining high cellular levels of lactosylceramide (LacCer), that can exert a Bcl-2-dependent antiapoptotic effect, contributing in this way to the survival of cancer cells and subsequent tumor progression (Kakugawa et al. 2002; Ueno et al. 2006). Neu3, together with plasma membrane associated β-galactosidase and β-glucosidase (see the following paragraphes), can act on gangliosides to produce bioactive ceramide at cell surface level of cultured human fibroblasts (Valaperta et al. 2006). Expression of any of these three enzymes can affect the expression/activity of the others, for instance overexpression of Neu3 results in an increased expression/activity of both β-galactosidase and β-glucosidase. The concomitant increase of their enzymatic activity catalyzes the conversion of GM3, a substrate for Neu3, to ceramide leading to higher ceramide levels and to a switch from a cell proliferative state to apoptosis.

Nevertheless, the increase of cell surface ceramide is paralleled by slight reduction in ganglioside GM3, the main ganglioside in fibroblasts. In fact, the overexpression of Neu3 leads to an increased expression of GM3 synthase, the sialyltransferase (SAT1) responsible for GM3 biosinthesis, allowing to compensate the reduction of GM3 mediated by Neu3. The augmented expression of SAT1 depletes LacCer, reducing its availability for the biosynthesis of globotrihexosylceramide.

Neu3 catalyzes the hydrolysis of sialic acid acting both on α2–8 and α2–3 external ketosidic linkages, but it is ineffective on inner α2–3 sialyl residues. An increase in Neu3 activity modifies the cell surface ganglioside composition by catalyzing the progressive conversion of GD1a to GM1 and of GM3 to LacCer. These changes have significant effects on neuronal differentiation and apoptosis, both in normal and pathological cells (Kakugawa et al. 2002; Valaperta et al. 2007; Valaperta et al. 2006). In mouse and human neuroblastoma cells, the pharmacologically induced neuronal differentiation is paralleled by a higher Neu3 expression and activity (Proshin et al. 2002). Neurite outgrowth can also be induced overexpressing Neu3. In addition, an increase in Neu3 activity, enhances the extension and/or branching of neurites as demonstrated by the exposure of neuroblastoma cells to 5-bromodeoxyuridine (Hasegawa et al. 2000). Conversely, in SK-N-MC neuroblastoma cells, inhibition of Neu3 activity results in the loss of neuronal differentiation markers (Kopitz et al. 1994; von Reitzenstein et al. 2001).

In cultured rat granule cells, expression of Neu3 increases during differentiation and is maintained constant during aging (Aureli et al. 2011c). In cultured hippocampal neurons, Neu3 activity regulates the plasma membrane content of GM1 ganglioside, essential for axonal growth and regeneration upon axotomy (Rodriguez et al. 2001; Kappagantula et al. 2014; Chiricozzi et al. 2021b; Chiricozzi et al. 2020). In these neurons, Neu3 activity is asymmetrically concentrated at the end of neurites, determining their fate by a local increase in TrkA activity (Da Silva et al. 2005).

However, decrease of Neu3 expression does not prevent induction of neuroblastoma cell differentiation (Valaperta et al. 2006).

Several evidences are available regarding in situ sialylation of cell surface gangliosides. The original report on the presence of a synaptosomal membrane sialyltransferase in calf brain (Preti et al. 1980) was confirmed by metabolic studies in chicken embryos (Matsui et al. 1986) and rat brain (Durrie et al. 1988; Durrie and Rosenberg 1989). More recently, it has been shown that exposure to dexamethasone markedly increases GM3 ganglioside synthesis by enhancing gene expression and enzyme activity of SAT1. Metabolic studies indicate that this event is localized at the plasma membrane level (Iwamori and Iwamori 2005), thus confirming that glycolipid sialylation might not be confined to the Golgi apparatus, contributing to the local modulation of the cell surface GSLpattern.

Direct evidence of expression and activity of plasma membrane- associated sialyltransferases has been demonstrated in epithelial and melanoma cells, where GD3 is produced from endogenous GM3 ganglioside, as well as from exogenously incorporated substrate. GD3 synthesis occurs starting from CMP-NeuAc synthesized inside the cell and transferred outside the cell through an unknown process Crespo et al. 2010). In addition, it is important to recall that this sialyltransferase displayes both *cis* and *trans* activity (Vilcaes et al. 2011).

Given the high concentration of gangliosides in the nervous system, and the very high and progressive increase of Neu3 and total sialyltransferases during neuronal differentiation, it is possible to hypothesize the existence of a sialylation–desialylation cycle on the plasma membrane. This could have a specific role during neuronal cell specialization, especially during cell stages in which it is necessary to build specialized membranes, such as axonal protrusion and elongation, dendritic arborization, and synaptogenesis. These enzymes could also be essential for axon repair and/or synaptic function.

A further modification of the oligosaccharide structure of gangliosides, that involves sialic acid, is represented by the lactonization of molecules containing a disialosyl residue, such as GD1b ganglioside. Ganglioside lactones are present as minor components in the brain of vertebrates (Sonnino et al. 1983; Riboni et al. 1986). GD1b monolactone formation has been studied *in vitro* in the presence of catalytic protons (Bassi et al. 1989) (Fig. 10.5), and it has been demonstrated that lactonization influences the conformation and both the aggregative (Acquotti et al. 1987) and biological properties of GD1b (Bassi et al. 1991). Indeed, GD1b is able to directly interact with several proteins (Prinetti et al. 2000b) and to modulate the activity of several plasma membrane associated protein kinases (Bassi et al. 1991), while GD1b-lactone does not, or does it in a reduced way (Bassi et al. 1991; Sonnino et al. 1990). This suggests that lactonization/delactonization of gangliosides might represent a localized event able to trigger specific ganglioside-mediated cellular events. *In vivo*, GD1b lactonization occurs in neurons through a process that involves the presence of a specific enzyme associated with the plasma membrane (Bassi et al. 1994).

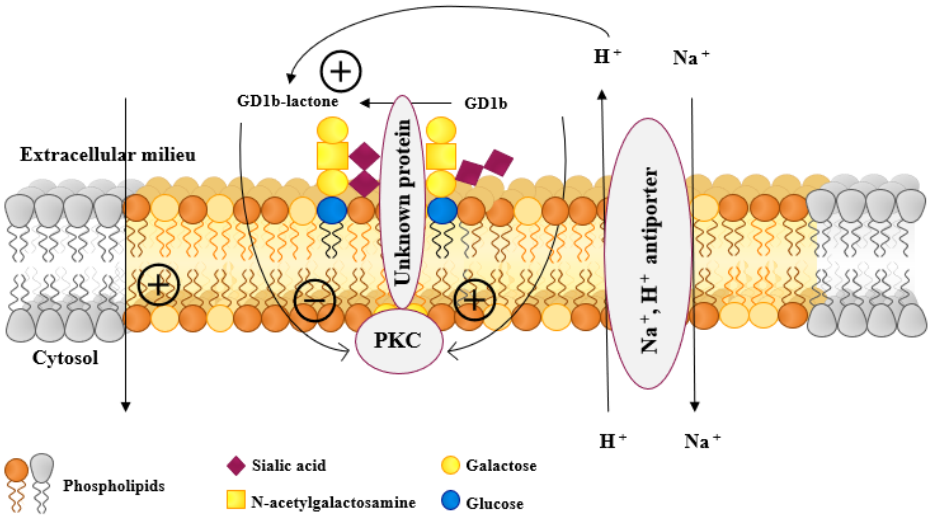

Fig. 10.5 Monolactone formation due to the acidic plasma membrane microenvironment. The catalytic protons are generated by the Na^+/H^+ pump located at the cell surface and activated through the action of protein kinase C (PKC). The local acidic microenvironment is directly responsible for lactone formation.

10.6. β-Hexosaminidase and *N*-acetylgalactosaminyltransferase

In neurons, β-hexosaminidase (Hex) is the enzyme that removes *N*-acetylgalactosamine from GM2 and GalNAc-GD1a forming GM3 and GD1a, respectively. Both GM2 and GalNac-GD1a are minor compounds at the cell surface. GalNAc-GD1a is difficult to be recognized within a ganglioside mixture separated by HPTLC since it has a similar chromatographic behavior as GD1a (Svennerholm et al. 1973; Acquotti et al. 1994). Indeed, β-hexosaminidase is worldwide associated with the lysosomal hydrolysis of the ganglioside GM2.

β-Hexosaminidase is dimeric enzyme that exists in three different isoforms. It has two diverse subunits, α (528 residues) and β (556 residues), encoded by two different but evolutionarily related genes, HEX A and HEX B (Triggs-Raine et al. 2001). Each subunit has its own active site; the β-subunit hydrolyzes uncharged substrates, whereas the α-subunit catalyzes cleavage of GalNAc from negatively charged ones (Bearpark and Stirling 1978; Kytzia and Sandhoff 1985). However, dimerization is necessary for the enzyme in order to become fully functional. Thus, the α- and β-subunits can form three different β-hexosaminidase isoenzymes: Hex A (αβ), Hex B (ββ), and Hex S (αα). Only the αβ heterodimer, Hex A, is able to remove β-linked GalNAc at the non-reducing end of ganglioside GM2 and GalNAc-GD1a, in the presence of the GM2 activator protein, a specific cofactor of Hex A (Kolter and Sandhoff 2006).

Genetic defects, either in the genes encoding the α- and β-subunits of Hex A, or in the GM2 activator protein, can result in GM2 accumulation in neural tissues leading to the onset of GM2-gangliosidosis. GM2-gangliosidosis manifests in three forms; Tay–Sachs disease, due to defects in the α-subunit (TSD, OMIN 2728800), Sandhoff disease, characterized by defects in the β-subunit (SD, OMIN 268800), and the AB variant of Sandhoff disease (OMIN 272750), in which both subunits are affected (Bateman et al.

2011). The massive neuronal accumulation of GM2 is accompanied by progressive neurological deterioration affecting motor, cerebral and spinocerebellar functions.

The presence of active Hex A in the external leaflet of the plasma membrane was discovered in cultured fibroblasts (Mencarelli et al. 2005). Immunological and biochemical characterization of the membrane associated Hex A shares the same structure as the lysosomal isoform . This suggests that a regulated fusion process between lysosomes and the external leaflet of the plasma membrane (Reddy et al. 2001) might be responsible for the transport of lysosomal enzymes to the cell surface, where the enzymes could exert their activity, remodeling the glycolipid content and pattern. It has been demonstrated that during differentiation of murine neuronal stem cells, plasma membrane Hex increases its activity, reaching a peak in fully differentiated cells (Aureli et al. 2011a). On the other hand, analysis of the plasma membrane-associated glycohydrolases in fibroblasts derived from patients affected by different variants of Gaucher disease (GD) demonstrates an increased activity only in cells derived from patients affected by the most severe neuronopathic form of Gaucher disease (GD2) (Aureli et al. 2012a).

Analysis of epithelial cells indicate the presence on the cell surface of an UDP-GalNAc: LacCer/GM3/GD3 *N*-acetylgalactosaminyl transferase able to act on exogenous GM3 (Crespo et al. 2010). Currently, no data are available regarding its activity on endogenously synthesized GSLs. However, its ability to act on exogenous substrates could be important in the plasma membrane remodeling process. In fact, the glycolipid composition of the plasma membrane could also be remodeled by the uptake of glycolipids from the extracellular environment (e.g., from other cells, lipoproteins, or molecules shed by other cells). These compounds, depending on the "cellular request" and on their aggregation, could be endocytosed or could become components of the cell surface, either directly, or after modification by the action of plasma membrane-associated enzymes. The coexistence of Hex and β-hexosaminyl transferase on the cell surface supports the hypothesis of the existence of a glycolipid cycle at plasma membrane level, which can have important biophysical effects on the membrane itself, and can affects events that regulate the "cell social life."

10.7. β-Glucocerebrosidase

β-glucocerebrosidase is an hydrolase responsible for the catabolism of glucosylceramide to glucose and ceramide. At least three different β-glucocerebrosidase enzymes have been described; β-glucocerebrosidase (GCase, EC 3.2.1.45) sensitive to inhibition by conduritol B epoxide (CBE) and primarily associated with lysosomes (Neufeld et al. 1996), a cytosolic β-glucosidase (GBA3, EC 3.2.1.21) (Daniels et al. 1981), and a non-lysosomal β-glucosylceramidase (NLGase, EC 3.2.1.45) (Van Weely et al. 1993).

NLGase is able to catalyze the hydrolysis of glucosylceramide both at the cell surface and in the ER (Van Weely et al. 1993; Korschen et al. 2012). In fact, the cellular localization of NLGase is still controversial, since it has been described to be associated with endosomal vesicles, plasma membrane, ER, and the Golgi apparatus. Analysis of data describing its localization, indicate that it depends on the cell type and stage of development. Until now, most of the pieces of information concerning NLGase localization come from studies performed on cells overexpressing NLGase, as a chimeric protein fused

to the green fluorescence protein (GFP), therefore, further investigations are needed to define its localization. Database searching of NLGase cDNA sequences revealed apparent orthologs of this enzyme in species ranging from Drosophila to Arabidopsis, and to vertebrates, indicating that the protein is highly conserved among species and suggesting its functional importance. Study of NLGase expression and activity showed that in humans this enzyme is abundant in the brain, heart, skeletal muscle, and kidney (Matern et al. 2001), whereas in mouse it is mostly represented in the testis and brain (Yildiz et al. 2006).

Interestingly, a study on NLGase knockout (KO) mice presented an abnormal accumulation of glucosylceramide in multiple tissues, including brain, liver, and testis. The KO mice show normal bile acid metabolism and, apparently, no impairment in the central nervous system (CNS), however, the accumulated glucosylceramide leads to decreased fertility due to the formation of misshapen spermatozoa (Yildiz et al. 2006). More recent studies indicated that mutations in NLGase cause autosomal recessive cerebellar ataxia with spasticity and spastic paraplegia in humans (Martin et al. 2013; Hammer et al. 2013; Malekkou et al. 2018; Gatti et al. 2021). Moreover, antisense morpholino oligonucleotides targeting the NLGase orthologous gene in a zebrafish model lead to abnormal motor behaviour, axonal shortening, and branching of motoneurons (Martin et al. 2013). This condition can be rescued transfecting cells with human wild-type mRNA for NLGase, but not with mRNA containing the missense mutation found inpatients affected by autosomal recessive cerebellar ataxia with spasticity. These data suggest a specific role of NLGase in the control of the cellular glucosylceramide–ceramide balance that could be responsible for the onset of motoneuron defects (Martin et al. 2013). As mentioned before, homozygous NLGase KO mice at 4 months of age, do not show apparent neurological signs, liver dysfunction, or reduced viability, even if the accumulation of glycolipid species has been detected by mass spectrometry analyses in the brain, liver, and testis (Yildiz et al. 2006). It may take longer to observe the neurological phenotype in these mice, as it has been observed in other mouse models of hereditary spastic paraplegia. The latter may reflect the fact that the neurological signs are very slight during the first months of life (Ferreirinha et al. 2004; Soderblom et al. 2010). Impairment of their nervous system could also be masked by the the fact that the structure of the corticospinal tract of mice is different from that of humans or zebrafish, or by a compensatory mechanism mediated by other enzyme isoforms during early stages of development (Martin et al. 2013). Of note, NLGase of KO mice lacks only exons 5 to 10, and retains 50 % of normal glucosidase activity. Therefore, it has been hypothesized that the accumulation of glucosylceramide in the ER and/or at plasma membrane level, does not reach the threshold necessary for the development of neurological symptoms.

It has been shown that NLGase activity increases during neuronal differentiation (Aureli et al. 2011a). Indeed, in primary neuronal cultures, cell surface β-glucosidase activity is mainly due to the NLGase enzyme, whose activity increases more than threefold during neuronal differentiation (Aureli et al. 2011c). On the contrary, in murine neuronal stem cells, plasma membrane β-glucosidase activity is largely due to the CBE-sensitive β-glucosidase enzyme, GCase, with a GCase/NLGase ratio of 0.75 in precursors and 2.3 in differentiated cells.

The different behaviors of these enzymes in the two different cell models could be due to the serum-induced murine neuronal stem cell differentiation, which allows to obtain only about 10 % of neurons and about 70–80 % of glial cells, whose contribution in terms of the

two different β-glucosidase enzymes is very different than that of neurons. Indeed, in non-neuronal cell lines, such as human fibroblasts, a GCase/NLGase ratio around 7 has been observed (Aureli et al. 2009). The lack of data on the activity of plasma membrane-associated glycohydrolases in cultures of astrocytes and oligodendrocytes, indicates that further studies are needed to be perfomed. Interestingly, studies on fibroblasts derived from patients affected by different variants of Gaucher's disease (GD), show that the reduction of total GCase activity is paralleled by an increase in NLGase activity and expression. This is particularly evident in fibroblasts derived from patients affected by GD2, the most severe form of the neuronopathic pathology (Aureli et al. 2012a). Despite all these observations, the link between NLGase and neuronal differentiation, as well as neurodegeneration, are still unclear and requires further studies.

Several years ago the presence of a cell surface GCase activity has been described (Aureli et al. 2009). Studies on human fibroblasts derived from patients affected by GD showed that GCase plasma membrane-associated activity is significantly reduced with respect to fibroblasts derived from healthy donors, ascribing this reduction to a deficiency in GCase enzyme (Aureli et al. 2012a).

The increase in GCase and NLGase activities on the plasma membrane of human fibroblasts and subsequent increase in ceramide levels are responsible for cell cycle arrest and apoptosis (Valaperta et al. 2006). To date, a multicentre study has demonstrated that GCase mutations represent the most common genetic risk factor for the development of Parkinson's disease (PD) (Sidransky et al. 2009). Importantly, Mazzulli et al. has demonstrated that in the brain and neurons derived from PD patients, the lysosomal accumulation of glucosylceramide, the substrate of GCase, directly influences the abnormal lysosomal storage of α-synuclein oligomers resulting in a further inhibition of GCase activity (Mazzulli et al. 2011). These findings suggest, for the first time, that the bidirectional effect of decreased GCase activity and α-synuclein accumulation forms a positive feedback loop that may lead to a self-propagation of the disease (Mazzulli et al. 2011). To date, this process has been described for the lysosomal function of GCase, but due to its involvement in the production of pro-apoptotic ceramide at the plasma membrane level, GCase and NLGase associated with the cell surface could contribute to neuronal impairment in neurodegenerative diseases.

In mammalian cells, GCase and NLGase are not just involved in the catabolism of glucosylceramide, but show also transferase activity catalyzing the transglucosylation reaction of glucose from glucosylceramide to cholesterol, forming, glucosylcholesterol, *in vitro* (Akiyama et al. 2016). On the other hand, both GCase and NLGase are able to catalyze the opposing reaction, sintesizing again glucosylceramide starting from glucosylcholesterol (Marques et al. 2016). Interestingly, it has been demonstrated that the increased content of glucosylcholesterol is paralled by the accumulation of its substrates, glucosylceramide or cholesterol, suggesting that the equilibrium between the synthesis and the catabolism of glucosylcholesterol is strongly dependent on the concentration of the substrates (Marques et al. 2016). The transglucosylation activity of GCase and NLGase represents an interesting intersection between the two major lipid metabolic pathways of sphingolipids and sterols, which could be fundamental in maintaing cell homeostasis. Since GCase and NLGase are widely expressed in the brain, their altered expression does lead to changes in glucosylceramide and glucosylcholesterol metabolism, opening a new scenario on the study of the related pathologies.

10.8. β-Galactosidase

Two different β-galactosidase enzymes, involved in GSLs metabolism, have been described: β-galactocerebrosidase (β-Gal-ase, GALC EC 3.2.1.46), which catalyzes the hydrolysis of galactose from lactosylceramide, LacCer, and galactosylsphingosine, and the β-galactosidase (β-Gal, EC 3.2.1.23), which catalyzes the hydrolysis of the terminal galactose from GM1 (Li and Li 1999). As it is well known, the loss of function of β-Gal-ase, is responsible for the development of globoid leukodystrophy (Krabbe disease), while deficiency of β-Gal causes GM1 gangliosidosis (Xu et al. 2010). Both sphingolipidoses are characterized by an impairment of the central nervous system, even if the molecular bases are still unclear.

In addition to the other lysosomal enzymes, plasma membrane-associated β-galactosidase activity has been demonstrated in several cell lines (Aureli et al. 2011b). The identity of the proteins responsible for the β-galactosidase activity present at the cell surface is still unknown. However, in living human fibroblasts, the presence of a β-galactosidase which displays a *trans* activity on neighbour cells has been verified. The enzyme is active in the absence of detergents or activator proteins, suggesting that on the cell surface there is at least one enzyme with a β-galactosidase-like activity (Aureli et al. 2009). In the same cell model, it has been shown, that the expression of β-galactosidase is upregulated by Neu3 overexpression and correlates with the onset of ceramide-mediated apoptosis (Valaperta et al. 2006). β-galactosidase activity was measured during neuronal differentiation and aging, in both the total cell lysate and the plasma membrane fraction derived from rat cerebellar granule cells. Both activities were upregulated during cell differentiation. As expected, β-galactosidase activity associated with the plasma membrane is lower than the one present in the total cell lysates. Total cell activity is maintained constant during differentiation, however, it increases of about four fold during aging. In contrast, cell surface activity increases of ten fold during differentiation and then is doubled during neuronal senescence (Aureli et al. 2011c). A similar behavior has been described for plasma membrane-associated β-galactosidase activity during neuronal differentiation of neural stem cells (Aureli et al. 2011a). To this purpose, β-galactosidase activity has been proposed as a marker of aging and senescence (Coates 2002; Dimri et al. 1995; Severino et al. 2000; Geng et al. 2010). The behavior of the plasma membrane-associated enzyme in rat cerebellar granule cells suggests that β-galactosidase activity could be used as a hallmark of both neuronal differentiation and aging, as well as of apoptosis, in fibroblasts. On the other hand, little is known regarding the functional role of plasma membrane-associated β-galactosidases. It has been hypothesized that they may act as cell surface receptors mediating various cell–cell and cell–matrix interactions which are responsible for cell migration, differentiation, and axonal branching (Evans et al. 1993; Huang et al. 1995). No data are available on their enzymatic properties at plasma membrane level.

10.9 Membrane dinamics: a non-canonical pathway involved in the establishment of the GSL profile at the plasma membrane

Recent evidence suggests that the complex network represented by the membrane trafficking both inside and outside the cell is involved in defining the final cell GSL

composition.
The most common event is the fusion of intracellular vesicles with the plasma membrane. A clear example is given by the pre-synapsis, where the fusion of thousand of neurotransmitters-containing microvesicles, induces direct important local changes in the plasma membrane composition. A similar effect is induced by the fusion of lysosomes with the plasma membrane (Blott et al. 2002; Baron et al. 1985; Blott et al. 2002; Chambers et al. 1917; Reddy et al. 2001; Rothman et al. 1994; Rao et al. 2004; Raiborg et al. 2015; Arantes et al. 2006). This process is an evolutionary conserved mechanism, called lysosomal exocytosis, that has important implications in the cell homeostasis. Indeed, it is a mechanism that allows to drive the glycohydrolitic enzymes associated with the luminal side of lysosomal membranes, directly to the external leaflet of the cell plasma membrane. Here, thanks to the presence of vacuolar H^+-ATPase proton pumps and Na^+/H^+ exchangers, they can exert their activity directly *in-situ* as decribed above (Aureli et al. 2012b) On the other hand, recent evidence concerning lysosomal storage disorders, suggests that cells activate the lysosomal exocytosis upon aberrant accumulation of uncatabolized molecules in the lysosomes, in order to release their content in the extracellular milieu. Despite this process may be beneficial for the cells, a recent paper shows that it is responsible for a dramatic increase of the activity of lysosomal glycosphingolipid hydrolases at the cell surface. These enzymes promote a remodeling of the plasma membrane with the aberrant ectopic catabolism of GSLs and production of ceramide, event responsible for the induction of cell cycle arrest (Samarani et al. 2018).
Another intersting mechanism that could determine changes in the GSL composition is represented by the shedding. In particular, GSL shedding could be considered an active and controlled process able to modify the sphingolipid composition of neighbour cells in order to condition their fate. The amphiphilic nature of the GSL raises the possibility that multiple equilibriums exist between the lipids inserted in the plasma membrane (where they are present in different grade of segregation) and those present in the aqueous extracellular milieu (Riboni et al. 1997; Sonnino et al. 1994; Koynova et al. 1995). Despite the shedding process was considered only a minor event, further studies demonstrate that especially tumor cells can shed up to 0.5% of their membrane gangliosides per hour (Li RX et al. 1991). At the same time acceptor membranes could receive exogenous gangliosides up to 3% of their total membrane lipid content. Interestingly, the aberrant shedding of gangliosides from the neurons may be responsible for the glia activation, a common feature of several neurodegenerative disorders (Jou et al. 2006).
New findings suggest also that the membrane contact sites (MCS) are intracellular components capable of changing the GSL composition of the cells. In particular, these specific areas of the membranes seem to facilitate the lipid transport among the different organells. In these regions, the membrane of two organelles reach a distance of 30nm and lipids are transposted from one organell to the other by specific proteins.
ER is considered the hub of these MCS-net since it is widespread into the cell allowing the recruitment of ER membrane in forming multiple contact sites with other organellar membranes, such as those of mitochondria, lysosomes, Golgi apparatus, endosome, and plasma membrane (Levine et al. 2016; Prinz et al. 2014; Levine et al. 2006; Gatta et al. 2017).
In the scenario of the MCS it is reasonable to imagine that ER is the highway for the lipids trafficking. Through the ER the cell shares sphingolipids among all intracellular

membranes, organelles, and the plasma membrane through a mechanism coordinated by the MCS.

10.10 Conclusions

During neuronal development dramatic changes occur in GSL cell content, along with the reorganization of plasma membrane lipid domains enriched in GSLs (Yu 1994; Prinetti et al. 2001; Yu et al. 2004). While the driving forces of these modifications are not completely understood, some information exists regarding the possibility of a fine-tuning of the cell plasma membrane GSL composition, mediated by the synergic activity of the different plasma membrane- associated glycohydrolases (Valaperta et al. 2006; Aureli et al. 2009).

In cultured rat cerebellar granule cells, the increase in total ceramide content (eight fold from the 2^{nd} to the 17^{th} day in culture) and in that belonging to the sphingolipid-enriched domains (ten fold from the 2^{nd} to the 17^{th} day in culture) has been observed REF. A parallel reduction in the endogenous content of both sphingomyelin and gangliosides is present in sphingolipid-enriched domains of senescent cells with respect to fully differentiated neurons (Prinetti et al. 2000a). The increase in ceramide content could be explained by the well-known ceramide–sphingomyelin cycle that is known to correlate with apoptotic phenomena (Venable et al. 1995). As found in human fibroblasts, the increased activity of the plasma membrane-associated glycohydrolases during cell aging, supports the speculation that the augmented in the plasma membrane could be derived from cell surface GSL catabolism (Valaperta et al. 2006). As reported (Rodriguez et al. 2001; Da Silva et al. 2005), the sialidase Neu3 is able to influence extension and symmetry of axons in neuronal cells, possibly by inducing a local change in plasma membrane sphingolipid composition at the axonal cones. All these data support the idea that modulations of the activities of other plasma membrane -associated glycohydrolases during neuronal differentiation could affect the differentiation itself. In addition, they participate in defining the curvature of specific plasma membrane areas, such as synapses or the negative curvature of the membrane near the axon protrusion, by a rapid *in situ* modification of GSL components. An example of the latter is provided by the geometry of synapses. It is typically characterized by an alternative presence of plasma membrane regions with negative or positive curvature, that correlate respectively to an enrichment of simple sphingolipids or of more complex GSLs (Sonnino et al. 1994; Brocca and Sonnino 1997). On the other hand, an aberrant increase in cell surface glycohydrolases can promote the formation of apoptotic ceramide, leading to the onset of neuronal impairment. For these reasons, the balance between glycosylation and de-glycosylation at the cell surface could be a very important mechanism for maintaining the appropriate neuronal physiology.

Conflict of Interest

All the authors declare that they have no conflict of interest.

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