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Review

Ceramide-enriched membrane domains—Structure and function

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ABSTRACT

Membrane lipids seem to be organized and not randomly distributed in the cell membrane. In particular, sphingolipids seem to interact with cholesterol in the outer leaflet of the cell membrane resulting in the formation of distinct membrane domains, i.e. rafts. The generation of ceramide within rafts alters their biophysical properties and results in the formation of large ceramide-enriched membrane platforms. These platforms serve to cluster receptor molecules and to organize intracellular signalling molecules to facilitate signal transduction via a receptor upon stimulation. Thus, ceramide-enriched membrane domains amplify not only receptor-, but also stress-mediated signalling events. Although many receptors cluster, the molecular mechanisms mediating this important and general event in signal transduction need to be identified.

Contents

1. Lipid interactions and domain formation

Sphingolipids, (glycerol-)phospholipids and cholesterol are the major components of biological membranes in eukaryotic cells [1]. Sphingolipids consist of an sphingoid base, i.e. a 1,3-dihydroxy-2-aminoalken backbone. Sphingosine, which is (2S, 3R, 4E)-2-amino-4-octadecene-1,3-diol, is the most prevalent backbone of mammalian sphingolipids. Sphingoid bases vary in length of the alkyl chain and position and number of the double bonds. Ceramide is generated from sphingosine by attachment of a fatty acid via an amide ester bond [1,2]. The fatty acids in ceramides vary between 2 and 28 carbon atoms in the acyl chain and saturation. Ceramides are further modified by attachments of headgroups to form, for instance, sphingomyelin, gangliosides, sulfatides, globosides or cerebrosides [1–3].

Studies in the last years revealed that lipids in cell membranes do not form a homogenous liquid phase [4], but are ordered into domains mediated by interactions of, for instance, sphingolipids and cholesterol [5]. Sphingomyelin is the most prevalent cellular sphingolipid, which is synthesized on the luminal side of the Golgi apparatus or the plasma membrane, and, thus, localizes predominantly to anti-cytoplasmic leaflets of the cell membrane and intracellular vesicles [6–8]. This distribution pattern of sphingomyelin results in lipid bilayer asymmetry, which is critical for the genesis of distinct membrane domains and, as discussed below, signal transduction. Sphingomyelin is comprised of a highly hydrophobic ceramide moiety and a hydrophilic phosphorylcholine headgroup [2,3]. The hydrophilic phosphorylcholine groups tightly interact with each other, other hydrophilic headgroups of glycosphingolipids and the hydrophilic parts of cholesterol [3,9,10]. The ceramide moiety of sphingomyelin binds to cholesterol via hydrophobic van der Waal interactions [3,9,10]. The tight hydrophilic and hydrophobic interactions and the high local concentration of sphingolipids and cholesterol mediate a lateral association of these lipids in the cell membrane, separation from other phospholipids within the bilayer and the spontaneous formation of distinct domains [3,9,10]. These very small, tightly packed sphingolipid- and cholesterol-enriched membrane domains are named rafts that seem to exist in a more ordered liquid phase than

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other parts of the cell membrane [3,9,10]. Cholesterol and some cholesterol precursors do not only interact with sphingolipids in these rafts, but also stabilize the structure of rafts by filling void spaces between the bulky sphingolipids [9,11]. This notion is consistent with the finding that extraction of cholesterol from membranes using drugs as beta-cyclodextrin, nystatin or filipin destroys membrane rafts [12]. Many studies characterized rafts by their resistance to detergents, which are caused by the high biophysical order in this phase, although the use of detergents might be problematic to investigate the physiology of lipids and detergent-resistant membrane domains are not the same as membrane rafts [13,14].

2. Ceramide-enriched membrane domains

Hydrolysis of sphingomyelin results in the release of ceramide, which is catalyzed by acid, neutral and alkaline sphingomyelinases with the respective pH optimum of activity [3,15]. Ceramide is also formed in cellular membranes by de novo synthesis via a pathway involving the serine-palmitoyl-CoA transferase as the key enzyme.

Recent studies further revealed three additional pathways for the formation of ceramide, i.e. by the reverse activity of the acid ceramidase catalyzing synthesis of ceramide from sphingosine [16], by hydrolysis of complex-glycosylated lipids [17] and by hydrolysis of ceramide-1-phosphate [18].

Acid sphingomyelinase is the best-characterized sphingomyelinase and the enzyme was shown to be critically involved in many forms of cell activation [19]. The acid sphingomyelinase exists in two forms, a lysosomal acid sphingomyelinase and a secretory acid sphingomyelinase that are both derived from the same gene, but differ in their glycosylation pattern and are also differentially processed at the NH_2-terminus [20].

Acid sphingomyelinase was in addition shown to bind to the extracellular leaflet of the cell membrane [21,22], however, at present it is unknown whether this form is a third isoform or a specialized form of the secretory or lysosomal acid sphingomyelinase.

The in vivo mechanisms that mediate activation of the acid sphingomyelinase are still unknown. It was shown that CD95 mediates activation of the acid sphingomyelinase by a caspase 8 dependent pathway, since Crm A or Ac-YVAD-cmk, viral or pharmacological blocker, respectively, of some caspases prevent stimulation of the acid sphingomyelinase [23,24]. Likewise, overexpression of caspase 8, or constitutively active caspase 8 mutants resulted in increased ceramide release [25].

The Tumor Necrosis Factor Receptor (TNF-R), another receptor well known to stimulate the acid sphingomyelinase [26], seems to mediate acid sphingomyelinase activation independent of caspase 8 via a yet unknown initiator caspase [27]. Acid sphingomyelinase was also shown to associate with phosphatidylinositol-3-kinase (PI-3-K) and the CD161 molecule [28,29]. While it is unknown how CD161 regulates acid sphingomyelinase, activation of PI-3-K by nerve growth factor (NGF) triggering resulted in an approximately 50% reduction of acid sphingomyelinase activity suggesting that phoshatidylinositol-phosphates are able to regulate acid sphingomyelinase [28,30].

An additional study demonstrated that oxidation of cysteine 629 of acid sphingomyelinase mediates activation of the enzyme, although it has to be determined whether this amino acid residue is oxidized in vivo to regulate the enzyme [31]. Recent studies have supported the view that reactive oxygen species (ROS) regulate sphingomyelinase-mediated formation of signalling platforms in mammalian cells induced by various ligands or stimuli. Charruyer et al. described that UV-C irradiation resulted in redox-dependent activation and translocalization of acid sphingomyelinase to the external surface of membrane microdomains, hydrolysis of sphingomyelin associated with the plasma membrane outer leaflet, ceramide generation and apoptosis [32]. Further, TNF-related apoptosis-inducing ligand (TRAIL) induced a rapid release of ROS and the antioxidants Tiron or *N*-acetylcysteine blocked TRAIL-induced formation of ceramide-enriched membrane lipid raft platforms and apoptosis in BJAB or splenocytes [33]. Likewise, γ-irradiation of a radiosensitive human head and neck squamous carcinoma cell line (SCC61) results in the formation of raft platforms associated with the externalization of an acid sphingomyelinase, leading to ceramide release and apoptosis and these events were abolished by ROS scavengers [34].

Finally, recent in vitro studies suggest that the concentration of ceramide itself influences the activity of the enzyme, at least in model membranes [35].

The release of ceramide within the cell membrane alters the biophysical characteristics of membranes, which was first demonstrated by in vitro studies showing that addition of ceramide to dipalmitoylphosphatidylcholine bilayers induced lateral phase separation into domains with gel and liquid crystalline phases, respectively [36]. Ceramide molecules spontaneously associate and tightly bind with other ceramide molecules resulting in the formation of small ceramide-enriched membrane microdomains [3,21,37,38]. The accumulation of ceramide very likely occurs in the above-described membrane rafts, although this has not been formally proven. Since ceramide molecules displace cholesterol from rafts, at least in artificial membranes [37], the generation of ceramide within rafts may also completely alter the composition of rafts. Ceramide-enriched microdomains have the tendency to spontaneously fuse and to form ceramide-enriched macrodomains, also named ceramide-enriched membrane platforms, with a diameter between a few hundred nanometers up to several micrometers [21,22] (Fig. 1). Relative low concentrations, i.e. as little as 5 mol% ceramide in model membranes, are required for spontaneous formation of ceramide-enriched membrane domains [38].

Ceramide-enriched membrane domains seem to be tightly packed and, thus, rather stabile membrane domains, consistent with the observation that ceramide promotes the formation of membrane domains into a liquid ordered state [3,37,38].

Ceramide-enriched membrane domains were demonstrated in living cells by fluorescence microscopy [21,39]. Studies on phosphatidylcholine/sphingomyelin-composed unilamellar vesicle that were treated with sphingomyelinase immobilized onto a microbead confirmed the formation of ceramide-enriched membrane macrodomains [40].

The formation of distinct membrane domains by ceramide is also indicated by magnetic resonance spectroscopy and atomic force microscopy studies that revealed laminar phase separation of long chain ceramides in glycerophospholipid/cholesterol bilayers and formation of stable, distinct domains that correspond with a transition of fluid phospholipid bilayers into a gel-like phase [38,41]. Finally, biophysical studies demonstrated that small amounts of ceramide significantly increased the gel-to-fluid transition temperature of sphingomyelin suggesting the formation and stabilization of a liquid ordered state [41].

3. Function of ceramide-enriched membrane platforms and receptor clustering

The accumulation of ceramide within cellular membranes results in the formation of membrane domains with altered biophysical properties. Thus, ceramide-enriched membrane domains are perfect structures to sort proteins in cells and to provide a mean for the spatial re-organization of receptors and intracellular signalling molecules upon cellular stimulation. Ceramide-enriched membrane platforms were shown to trap and, thus, cluster receptor molecules [21–23,40,42]. Clustering of receptor molecules results in a very high receptor density within a small area of the cell membrane, a phenomenon that is required for the transmission of signals via many receptor molecules. Receptor aggregation and trapping in ceramide-enriched membrane domains may limit lateral diffusion and, thus, stabilize the interaction of a ligand with its receptor, in particular if the ligand is also trapped in distinct membrane domains.

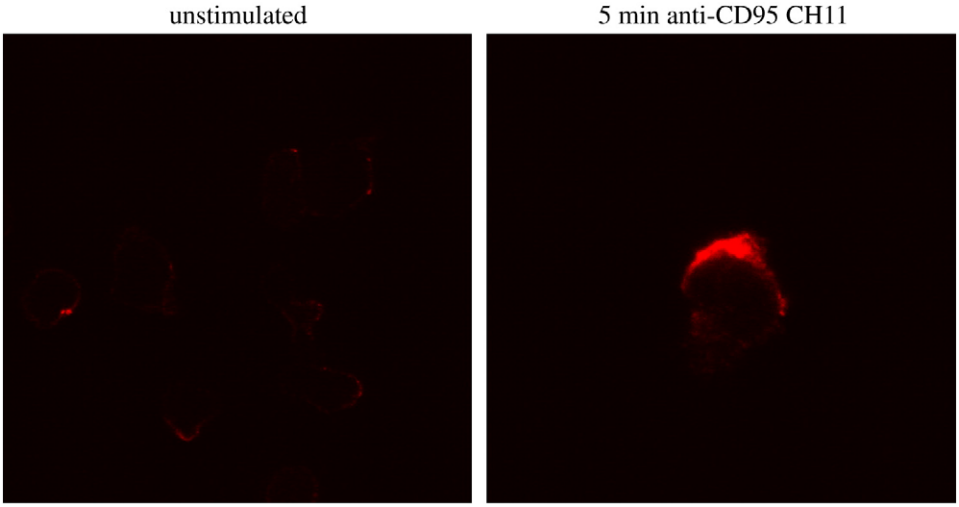

Fig. 1. CD95 stimulation induces ceramide-enriched membrane platforms. Stimulation of Jurkat T-lymphocytes via CD95 results in the formation of ceramide-enriched membrane platforms on the cell surface. Cells were stimulated, fixed, stained with Cy3-coupled anti-ceramide antibodies and analyzed by confocal microscopy.

The phenomenon that both, receptor and ligand, are immobilized in rafts or ceramide-enriched membrane domains has been shown for the CD95/CD95 ligand and CD40/CD40 ligand pairs [21,39,43,44]. The interaction of ligand-bound receptors with the very hydrophobic ceramide-enriched membrane platform and/or individual ceramide molecules may in addition stabilize conformational changes that may occur upon activation of a receptor by its ligand. Clustering of receptors also results in clustering of receptor-associated signalling molecules within or at ceramide-enriched domains. Furthermore, ceramide-enriched membrane domains, although present in the outer leaflet of the cell membrane, may sort intracellular signalling molecules, for instance via farnesyl- or geranyl-moieties. This sorting function may result in the spatial association of activated receptors with signalling molecules that transmit the signal from the receptor into the cell, while at the same time inhibitory molecules are excluded from this area of the cells. In addition, a high concentration and close vicinity of enzymes may permit enhanced transactivation of enzymes associating with the activated receptor, for instance of caspases associating with death receptor.

These considerations suggest that ceramide-enriched membrane platforms have a general function in signal transduction, i.e. they may primarily function to spatially organize receptors and the intracellular signalosome in and at the cell membrane to facilitate and amplify signalling processes. This general function of ceramide-enriched membrane domains is also consistent with the finding that many diverse receptors and stimuli trigger the release of ceramide and the formation of ceramide-enriched membrane domains, including CD95 [21–23,42], CD40 [39], DR5 [34], FcγRII [45], the PAF-receptor [46], CD14 [47], infection with *P. aeruginosa* [48], *S. aureus* [49], *N. gonorrhoeae* [50,51], Rhinovirus [52], application of stress stimuli such as γ-irradiation [53], UV-light [32,54–56], cisplatin [57], gemcitabine [58], Cu^{2+}-treatment [59] and in some conditions of developmental death [60].

The function of ceramide-enriched membrane domains is best studied for CD95. CD95 ligation results in activation of the acid sphingomyelinase, mobilization of intracellular vesicles that contain the acid sphingomyelinase and, thus, translocation of the enzyme onto the extracellular leaflet of the cell membrane and the release of extracellularly-oriented ceramide [21,22] (Figs. 1 and 2). Ceramide molecules form ceramide-enriched membrane platforms that serve to cluster CD95 resulting in a very high density of CD95 molecules within a small and described area of the membrane [21,22,42] (Fig. 1). This

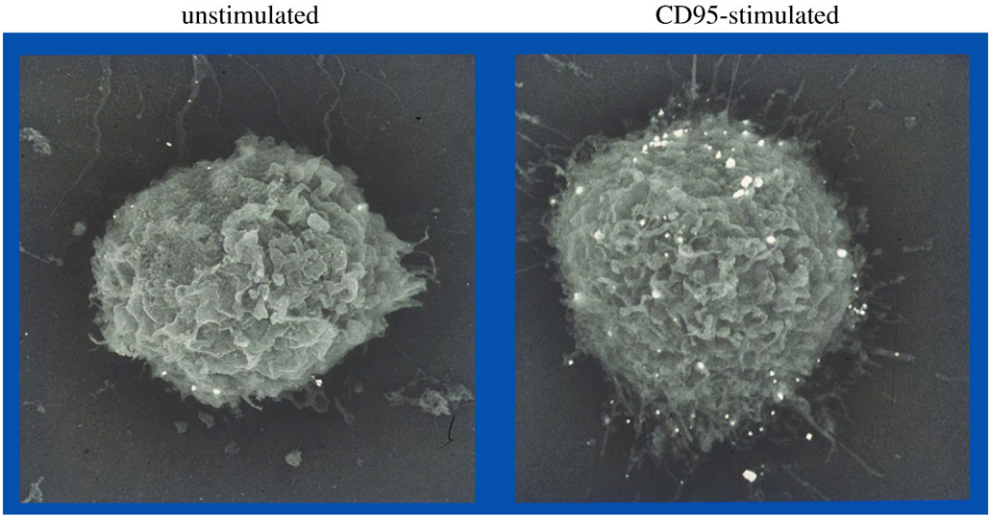

Fig. 2. CD95 induces surface exposure of the acid sphingomyelinase. Stimulation of Jurkat T-lymphocytes results in surface exposure of acid sphingomyelinase on the cell surface. Cells were fixed, stained with gold-coupled anti-acid sphingomyelinase antibodies and analyzed by scanning electron microscopy. The gold particles are visualized as small white dots. Printed with permission of the JBC.

mechanism was shown to be operative in many cells including Jurkat cells, SKW 6.4 and JY B cell lymphoma, H9 T cell lymphoma, epithelial and mouse granulosa cells, lung epithelial cells and in primary murine splenocytes and hepatocytes [61].

Destruction of rafts also abrogated the assembly of CD95 with downstream signalling molecules such as FADD or caspase 8 indicating that ceramide-enriched membrane domains do not only serve the clustering of receptors [62].

The amplification function of ceramide-enriched membrane platforms for CD95 signalling was shown on B-lymphocytes lacking functional acid sphingomyelinase expression [21,22,42]. These cells fail to release ceramide upon CD95 stimulation. Binding of CD95 ligands to CD95 only resulted in a very weak activation in these cells as indicated by levels of recruitment of FADD and stimulation of caspase 8 that only reached approximately 1% of the levels achieved after maximal activation of CD95 in wild type, acid sphingomyelinase-positive cells. Formation of ceramide-enriched membrane platforms by addition of C_{16}-ceramide restored CD95 clustering and CD95-initiated signalling in acid sphingomyelinase-deficient cells.

These studies demonstrate that ceramide-enriched membrane platforms serve to cluster activated receptor molecules and provide a feed-forward mechanism resulting in recruitment of signalling molecules and marked amplification of a primary weak signal.

4. Mechanisms of receptor clustering in ceramide-enriched membrane domains

Although receptor clustering has been shown for many receptor molecules including CD95, DR5, the T cell receptor/CD3 complex [63,64], B cell receptor [65,66], CD40 [39,67–69], Fcε receptor 1 [70], CD28 [71], TNF-R [72], platelet-derived growth factor receptor [73] to name a few, the mechanisms, mediating the clustering of receptors, particular in rafts and ceramide-enriched membrane domains, are only poorly characterized.

First insights into the molecular mechanisms of receptor clustering were provided by studies on the CD40 receptor [74]. CD40 is recruited to and clusters in sphingolipid-enriched rafts of dendritic cells and B-lymphocytes [39,67–69]. Furthermore, it was shown that CD40 clusters in ceramide-enriched membrane domains after stimulation, an event that was required for the initiation of CD40 signalling [39].

In contrast, the tyrosine phosphatase CD45 was shown to be excluded from lipid rafts [66,75,76], at least in many cells. This difference in spatial distribution was employed to test the role of the transmembranous role of CD40 for a preferential partitioning into ceramide-enriched membrane domains. In these studies, the membrane-spanning region of CD40 was exchanged with the transmembranous domain of the protein tyrosine phosphatase CD45 [74]. These studies revealed clustering of CD40 in ceramide-enriched membrane platforms, which was not observed with the CD40/45 chimera after stimulation. In fact the CD40/45 chimera was excluded from lipid rafts and platforms very similar to CD45. The mutant also failed to initiate signals in cells upon stimulation that were observed after stimulation via CD40, for instance phosphorylation of p38MAPK. However, forced cross-linking of the CD40/CD45 mutant was sufficient to trigger phosphorylation of p38MAPK [74].

These data support the notion that clustering of CD40 in membrane platforms serves to initiate a high local concentration of receptor molecules, which initiates and amplifies signal transduction. However, since artificial clustering is not sufficient to fully restore CD40 signalling, clustering of CD40 in rafts and ceramide-enriched membrane domains, respectively, might also have additional functions for the transmission of signals via CD40. Those functions could be exclusion of inhibitory signalling molecules, e.g. CD45, direct modification of CD40 signalling by ceramide and/or stabilization of the receptor–ligand interaction by a limitation of lateral diffusion.

The transmembranous domains of CD40 and CD45 are both composed of 22 amino acids suggesting that preferential partitioning of a receptor is mediated by a preferred interaction of individual amino acid residues with rafts and ceramide-enriched membrane platforms, while the length of the transmembranous domain might be less important. The transmembranous domain of CD40 contains three phenylalanine residues and otherwise only uncharged amino acids, which might mediate preferential integration of the receptor in hydrophobic, ceramide-enriched membrane platforms. In contrast, the transmembranous domain of CD45 is less hydrophobic with central threonine and serine residues containing aliphatic hydroxyl side chains and a tyrosine residue at the interface to the cytosol, a pattern that may exclude the protein from ceramide-enriched membrane platforms. In addition, binding of the ligand to its receptor may alter the conformation of the transmembranous domain in the receptor molecule. Since ceramide-enriched membrane domains may have a different diameter than other domains of the plasma membrane, an alteration of the length of the transmembranous domain of a receptor upon ligand binding may facilitate integration into ceramide-enriched membrane domains. However, at present the molecular and structural determinants that finally mediate clustering of a certain receptor molecule in rafts remain to be elucidated.

5. Conclusions

Many studies in the last ten years demonstrated the central role of rafts and ceramide-enriched membrane domains for the initiation and amplification of receptor and stress-mediated signalling in almost all cell types. Although these studies provided novel concepts for the understanding of signalling networks within cells, in particular the spatial organization of signalling networks, at present no drugs exist that specifically target rafts or ceramide-enriched membrane domains to either prevent or amplify their function. The development of those drugs might open novel opportunities to treat infectious diseases, degenerative disorders and malignant tumors. Thus, it could be envisioned that local generation or application of ceramide to tumors prevents tumor growth or acts synergistically with γ-irradiation or chemotherapy, respectively. This concept has been recently proved in animal experiments [77]. Further, since many drugs seem to kill tumor cells via ceramide an inhibition of the acid ceramidase to prevent ceramide degradation might promote the effects of chemotherapy and γ-irradiation on the tumor, although the problem of a local inhibition of the acid ceramidase remains to be solved. Diseases with an increased formation of ceramide for instance cystic fibrosis [78] or chronic obstructive pulmonary disorder [79] might be treated with inhibitors of the acid sphingomyelinase and first clinical trials are already ongoing to treat cystic fibrosis with drugs that inhibit the acid sphingomyelinase. Other disorders such as Alzheimer's disease that has been described to be linked to increased concentrations of ceramide [80] might also be treated by inhibition of ceramide generation. The inhibition of infectious diseases by a manipulation of the ceramide-metabolism is certainly much more difficult, since ceramide seems to have multiple and, most importantly, not yet completely defined functions in infectious biology.

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References

[1] S. Hakomori, Chemistry of glycosphingolipids, in: J.N. Kanfer, S. Hakomori (Eds.), Sphingolipid Biochemistry, Plenum Press, New York and London, 1983, pp. 1–165.

[2] Y. Barenholz, T.E. Thompson, Sphingomyelins in bilayers and biological membranes, Biochim. Biophys. Acta 604 (1980) 129–158.
[3] R.N. Kolesnick, F.M. Goni, A. Alonso, Compartmentalization of ceramide signaling: physical foundations and biological effects, J. Cell. Physiol. 184 (2000) 285–300.
[4] S.J. Singer, G.L. Nicolson, The fluid mosaic model of the structure of cell membranes, Science 175 (1972) 720–731.
[5] K. Simons, E. Ikonen, Functional rafts in cell membranes, Nature 387 (1997) 569–572.
[6] D. Jeckel, A. Karrenbauer, R. Birk, R.R. Schmidt, F. Wieland, Sphingomyelin is synthesized in the cis Golgi, FEBS Lett. 261 (1990) 155–157.
[7] A.H. Futerman, B. Stieger, A.L. Hubbard, R.E. Pagano, Sphingomyelin synthesis in rat liver occurs predominantly at the cis and medial cisternae of the Golgi apparatus, J. Biol. Chem. 265 (1990) 8650–8657.
[8] P. Emmelot, R.P. Van Hoeven, Phospholipid unsaturation and plasma membrane organization, Chem. Phys. Lipids 14 (1975) 236–246.
[9] T. Harder, K. Simons, Caveolae, DIGs, and the dynamics of sphingolipid–cholesterol microdomains, Curr. Opin. Cell Biol. 9 (1997) 534–542.
[10] D.A. Brown, E. London, Functions of lipid rafts in biological membranes, Annu. Rev. Cell Dev. Biol. 14 (1998) 111–136.
[11] X. Xu, R. Bittman, G. Duportail, D. Heissler, C. Vilcheze, E. London, Effect of the structure of natural sterols and sphingolipids on the formation of ordered sphingolipid/sterol domains (rafts). Comparison of cholesterol to plant, fungal, and disease-associated sterols and comparison of sphingomyelin, cerebrosides, and ceramide, J. Biol. Chem. 276 (2001) 33540–33546.
[12] P. Keller, K. Simons, Cholesterol is required for surface transport of influenza virus hemagglutinin, J. Cell. Biol. 140 (1998) 1357–1367.
[13] S. Munro, Lipid rafts: elusive or illusive? Cell 115 (2003) 377–388.
[14] D. Lichtenberg, F.M. Goni, H. Heerklotz, Detergent resistant membranes should not be identified with membrane rafts, Trends Biochem. Sci. 30 (2005) 430–436.
[15] Y.A. Hannun, L.M. Obeid, Principles of bioactive lipid signalling: lessons from sphingolipids, Nat. Rev. Mol. Cell Biol. 9 (2008) 139–150.
[16] N. Okino, X. He, S. Gatt, K. Sandhoff, M. Ito, E.H. Schuchman, The reverse activity of human acid ceramidase, J. Biol. Chem. 278 (2003) 29948–29953.
[17] Y. Ishibashi, T. Nakasone, M. Kiyohara, Y. Horibata, K. Sakaguchi, A. Hijikata, S. Ichinose, A. Omori, Y. Yasui, H. Ishida, M. Kiso, N. Okino, M. Ito, A novel endoglycoceramidase hydrolyzes oligogalactosylceramides to produce galactooligosaccharides and ceramides, J. Biol. Chem. 282 (2007) 11386–11396.
[18] P. Mitra, M. Maceyka, S.G. Payne, N. Lamour, S. Milstien, C.E. Chalfant, S. Spiegel, Ceramide kinase regulates growth and survival of A549 human lung adrenocarcinoma cells, FEBS Lett. 581 (2007) 735–740.
[19] E. Gulbins, R.N. Kolesnick, Raft ceramide in molecular medicine, Oncogene 22 (2003) 7070–7077.
[20] S.L. Schissel, X. Jiang, J. Tweedie-Hardman, T. Jeong, E.H. Camejo, J. Najib, J.H. Rapp, K.J. Williams, I. Tabas, Secretory sphingomyelinase, a product of the acid sphingomyelinase gene, can hydrolyze atherogenic lipoproteins at neutral pH, Implications for atherosclerotic lesion development, J. Biol. Chem. 273 (1998) 2738–2746.
[21] H. Grassmé, A. Jekle, A. Riehle, H. Schwarz, J. Berger, K. Sandhoff, R. Kolesnick, E. Gulbins, CD95 signaling via ceramide-rich membrane rafts, J. Biol. Chem. 276 (2001) 20589–20596.
[22] H. Grassmé, H. Schwarz, E. Gulbins, Molecular mechanisms of ceramide-mediated CD95 clustering, Biochem. Biophys. Res. Commun. 284 (2001) 1016–1030.
[23] H. Grassmé, A. Cremesti, R. Kolesnick, E. Gulbins, Ceramide-mediated clustering is required for CD95-DISC formation, Oncogene 22 (2003) 5457–5470.
[24] B. Brenner, K. Ferlinz, H. Grassmé, M. Weller, U. Koppenhoefer, J. Dichgans, K. Sandhoff, F. Lang, E. Gulbins, Fas/CD95/Apo-I activates the acidic sphingomyelinase via caspases, Cell Death Differ. 5 (1998) 29–37.
[25] C. Grullich, M.C. Sullards, Z. Fuks, A.H. Merrill Jr., R. Kolesnick, CD95(Fas/APO-1) signals ceramide generation independent of the effector stage of apoptosis, J. Biol. Chem. 275 (2000) 8650–8656.
[26] S. Schutze, K. Potthoff, T. Machleidt, D. Berkovic, K. Wiegmann, M. Kronke, TNF activates NF-kappa B by phosphatidylcholine-specific phospholipase C-induced "acidic" sphingomyelin breakdown, Cell 71 (1992) 765–776.
[27] R. Schwandner, K. Wiegmann, K. Bernardo, D. Kreder, M. Kronke, TNF receptor death domain-associated proteins TRADD and FADD signal activation of acid sphingomyelinase, J. Biol. Chem. 273 (1998) 5916–5922.
[28] T.R. Bilderback, V.R. Gazula, R.T. Dobrowsky, Phosphoinositide 3-kinase regulates crosstalk between Trk A tyrosine kinase and p75(NTR)-dependent sphingolipid signaling pathways, J. Neurochem. 76 (2001) 1540–1551.
[29] D. Pozo, M. Vales-Gomez, Mavaddat, S.C. Williamson, S.E. Chisholm, H. Reyborn, CD161 (human NKR-P1A) signalling in NK cells involves the activation of acid sphingomyelinase, J. Immunol. 176 (2006) 2397–2406.
[30] M. Kolzer, C. Arenz, K. Ferlinz, N. Werth, H. Schulze, R. Klingenstein, K. Sandhoff, Phosphatidylinositol-3,5-bisphosphate is a potent and selective inhibitor of acid sphingomyelinase, Biol. Chem. 384 (2003) 1293–1298.
[31] H. Qiu, T. Edmunds, J. Baker-Malcolm, K.P. Karey, S. Estes, C. Schwarz, H. Hughes, S.M. Van Patten, Activation of human acid sphingomyelinase through modification or deletion of C-terminal cysteine, J. Biol. Chem. 278 (2003) 32744–32752.
[32] A. Charruyer, S. Gradize, C. Bezombes, S. Muller, G. Laurent, J.P. Jaffrezou, UV-C induces raft-associated acid sphingomyelinase and JNK activation and translocation independently on a nuclear signal, J. Biol. Chem. 280 (2005) 19196–19204.
[33] C.A. Dumitru, E. Gulbins, TRAIL activates acid sphingomyelinase via a redox mechanism and releases ceramide to trigger apoptosis, Oncogene 25 (2006) 5612–5625.
[34] C. Bionda, E. Hadchity, G. Alphonse, O. Chapet, R. Rousson, C. Rodriguez-Lafrasse, D. Ardail, Radioresistance of human carcinoma cells is correlated to a defect in raft membrane clustering, Free Radic. Biol. Med. 43 (2007) 681–694.
[35] L. De Tullio, B. Maggio, M.L. Fanani, Sphingomyelinase acts by an area-activated mechanism on the liquid-expanded phase of sphingomyelin monolayers, J. Lipid Res. (2008) PMID: 18509194.
[36] H.W. Huang, E.M. Goldberg, R. Zidovetzki, Ceramide induces structural defects into phosphatidylcholine bilayers and activates phospholipase A_2, Biochem. Biophys. Res. Comm. 220 (1996) 834–838.
[37] Megha, E. London, Ceramide selectively displaces cholesterol from ordered lipid domains (rafts): implications for lipid raft structure and function, J. Biol. Chem. 279 (2004) 9997–10004.
[38] M.P. Veiga, J.L. Arrondo, F.M. Goni, A. Alonso, Ceramides in phospholipid membranes: effects on bilayer stability and transition to nonlamellar phases, Biophys. J. 76 (1999) 342–350.
[39] H. Grassmé, V. Jendrossek, J. Bock, A. Riehle, E. Gulbins, Ceramide-rich membrane rafts mediate CD40 clustering, J. Immunol. 168 (2002) 298–307.
[40] T.A. Nurminen, J.M. Holopainen, H. Zhao, P.K. Kinnunen, Observation of topical catalysis by sphingomyelinase coupled to microspheres, J. Am. Chem. Soc. 124 (2002) 12129–12134.
[41] H.W. Huang, E.M. Goldberg, R. Zidovetzki, Ceramides modulate protein kinase C activity and perturb the structure of phosphatidylcholine/phosphatidylserine bilayers, Biophys. J. 77 (1999) 1489–1497.
[42] Cremesti, F. Paris, H. Grassmé, N. Holler, J. Tschopp, Z. Fuks, E. Gulbins, R. Kolesnick, Ceramide enables fas to cap and kill, J. Biol. Chem. 276 (2001) 23954–23961.
[43] N. Cahuzac, W. Braun, V. Kirkin, F. Conchonaud, L. Wawrezinieck, D. Marguet, O. Janssen, M. Zörnig, A.-O. Hueber, Fas ligand is localized to membrane rafts, where it displays increased cell death-inducing activity, Blood 107 (2006) 2384–2391.
[44] H. Grassmé, J. Bock, J. Kun, E. Gulbins, Clustering of CD40 ligand is required to form a functional contact with CD40, J. Biol. Chem. 277 (2002) 30289–30399.
[45] A.B. Abdel Shakor, K. Kwiatkowska, A. Sobota, Cell surface ceramide generation precedes and controls FcgammaRII clustering and phosphorylation in rafts, J. Biol. Chem. 279 (2004) 36778–36787.
[46] R. Goggel, S. Winoto-Morbach, G. Vielhaber, Y. Imai, K. Lindner, L. Brade, H. Brade, S. Ehlers, A.S. Slutsky, S. Schütze, E. Gulbins, S. Uhlig, PAF-mediated pulmonary edema: a new role for acid sphingomyelinase and ceramide, Nat. Med. 10 (2004) 155–160.
[47] A. Pfeiffer, A. Bottcher, E. Orso, M. Kapinsky, P. Nagy, A. Bodnar, I. Spreitzer, G. Liebisch, W. Drobnik, K. Gempel, M. Horn, S. Hollmer, T. Hartung, G. Multhoff, G. Schütz, A.J. Ulmer, H. Heine, F. Stelter, C. Schütt, G. Rothe, J. Szöllosi, S. Damjanovich, G. Schmitz, Lipopolysaccharide and ceramide docking to CD14 provokes ligand-specific receptor clustering in rafts, Eur. J. Immunol. 31 (2001) 3153–3164.
[48] H. Grassmé, V. Jendrossek, A. Riehle, G. von Kurthy, J. Berger, H. Schwarz, M. Weller, R. Kolesnick, E. Gulbins, Host defense against *Pseudomonas aeruginosa* requires ceramide-rich membrane rafts, Nat. Med. 9 (2003) 322–330.
[49] M. Esen, B. Schreiner, V. Jendrossek, F. Lang, K. Fassbender, H. Grassme, E. Gulbins, Mechanisms of *Staphylococcus aureus* induced apoptosis of human endothelial cells, Apoptosis 6 (2001) 431–439.
[50] H. Grassmé, E. Gulbins, B. Brenner, K. Ferlinz, K. Sandhoff, K. Harzer, F. Lang, T.F. Meyer, Acidic sphingomyelinase mediates entry of *N. gonorrhoeae* into nonphagocytic cells, Cell 91 (1997) 605–615.
[51] C.R. Hauck, H. Grassmé, J. Bock, V. Jendrossek, K. Ferlinz, T.F. Meyer, E. Gulbins, Acid sphingomyelinase is involved in CEACAM receptor-mediated phagocytosis of *Neisseria gonorrhoeae*, FEBS Lett. 478 (2000) 260–266.
[52] H. Grassmé, A. Riehle, B. Wilker, S. Dreschers, E. Gulbins, Rhinoviruses infect human epithelial cells via ceramide-enriched membrane platforms, submitted for publication, J. Biol. Chem. 280 (2005) 26256–26262.
[53] P. Santana, L.A. Pena, A. Haimovitz-Friedman, S. Martin, D. Green, M. McLoughlin, C. Cordon-Cardo, E.H. Schuchman, Z. Fuks, R. Kolesnick, Acid sphingomyelinase-deficient human lymphoblasts and mice are defective in radiation-induced apoptosis, Cell 86 (1996) 189–199.
[54] Y. Zhang, P. Mattjus, P.C. Schmid, Z. Dong, S. Zhong, W.Y. Ma, R.E. Brown, A.M. Bode, H.H. Schmid, Involvement of the acid sphingomyelinase pathway in UVA-induced apoptosis, J. Biol. Chem. 276 (2001) 11775–11782.
[55] J.A. Rotolo, J. Zhang, M. Donepudi, H. Lee, Z. Fuks, R. Kolesnick, Caspase-dependent and -independent activation of acid sphingomyelinase signaling, J. Biol. Chem. 280 (2005) 26425–26434.
[56] H. Kashkar, K. Wiegmann, B. Yazdanpanah, D. Haubert, M. Kronke, Acid sphingomyelinase is indispensable for UV light-induced Bax conformational change at the mitochondrial membrane, J. Biol. Chem. 280 (2005) 20804–20813.
[57] S. Lacour, A. Hammann, S. Grazide, D. Lagadic-Gossmann, A. Athias, O. Sergent, G. Laurent, P. Gambert, E. Solary, M.T. Dimanche-Boitrel, Cisplatin-induced CD95 redistribution into membrane lipid rafts of HT29 human colon cancer cells, Cancer. Res. 64 (2004) 3593–3598.
[58] G. Grammatikos, V. Teichgräber, A. Carpinteiro, T. Trarbach, M. Weller, U.R. Hengge, E. Gulbins, Over-expression of the acid sphingomyelinase sensitizes glioma cells to chemotherapy, Antioxid. Redox Signal. 9 (2007) 1449–1456.
[59] P.A. Lang, M. Schenck, J.P. Nicolay, J.U. Becker, D.S. Kempe, A. Lupescu, S. Koka, K. Eisele, B.A. Klarl, H. Rübben, K.W. Schmid, K. Mann, S. Hildenbrand, H. Hefter, S.M. Huber, T. Wieder, A. Erhardt, D. Häussinger, E. Gulbins, F. Lang, Liver cell death and anemia in Wilson disease involve acid sphingomyelinase and ceramide, Nat. Med. 13 (2007) 164–170.

[60] D. Scheel-Toellner, K. Wang, R. Craddock, P.R. Webb, H.M. McGettrick, L.K. Assi, N. Parkes, L.E. Clough, E. Gulbins, M. Salmon, J.M. Lord, Reactive oxygen species limit neutrophil life span by activating death receptor signaling, Blood 104 (2004) 2557–2564.
[61] J.C. Fanzo, M.P. Lynch, H. Phee, M. Hyer, A. Cremesti, H. Grassmé, J.S. Norris, K.M. Coggeshall, B.R. Rueda, A.B. Pernis, R. Kolesnick, E. Gulbins, CD95 rapidly clusters in cells of diverse origins, Cancer Biol. Ther. 2 (2003) 392–395.
[62] A.O. Hueber, A.M. Bernard, Z. Herincs, A. Couzinet, H.T. He, An essential role for membrane rafts in the initiation of Fas/CD95-triggered cell death in mouse thymocytes, EMBO Rep. 3 (2002) 190–196.
[63] J.J. Boniface, J.D. Rabinowitz, C. Wülfing, J. Hampl, Z. Reich, J.D. Altan, R.M. Kantor, C. Beeson, H.M. McConnell, M.M. Davis, Initiation of signal transduction through the t cell receptor requires the multivalent engagement of peptide/MHC ligands, Immunity 9 (1998) 459–466.
[64] C.R. Monks, B.A. Freiberg, H. Kupfer, N. Sciaky, A. Kupfer, Three-dimensional segregation of supramolecular activation clusters in T cells, Nature 395 (1998) 82–86.
[65] L. Graziadei, K. Riabowol, D. Bar-Sagi, Co-capping of ras proteins with surface immunoglobulins in B lymphocytes, Nature 347 (1990) 396–400.
[66] P.C. Cheng, M.L. Dykstra, R.N. Mitchell, S.K. Pierce, A role for lipid rafts in B cell antigen receptor signaling and antigen targeting, J. Exp. Med. 190 (1999) 1549–1605.
[67] P.O. Vidalain, O. Azocar, C. Servet-Delprat, C. Rabourdin-Combe, D. Gerlier, S. Manie, CD40 signaling in human dendritic cells is initiated within membrane rafts, EMBO J. 19 (2000) 3304–3313.
[68] B.S. Hostager, I.M. Catlett, G.A. Bishop, Recruitment of CD40 and tumor necrosis factor receptor-associated factors 2 and 3 to membrane microdomains during CD40 signaling, J. Biol. Chem. 275 (2000) 15392–15398.
[69] A. Kaykas, K. Worringer, B. Sugden, CD40 and LMP-1 both signal from lipid rafts but LMP-1 assembles a distinct, more efficient signaling complex, Embo J. 20 (2001) 2641–2654.
[70] K.A. Field, D. Holowka, B. Baird, FcεRI-mediated recruitment of p53/56(lyn) to detergent-resistant membrane domains accompanies cellular signaling, Proc. Natl. Acad. Sci. USA 92 (1995) 9201–9205.
[71] A. Viola, S. Schroeder, Y. Sakakibara, A. Lanzavecchia, T lymphocyte costimulation mediated by reorganization of membrane microdomains, Science 283 (1999) 680–682.
[72] G. Natoli, A. Costanzo, F. Guido, F. Moretti, M. Levrero, Apoptotic, non-apoptotic, and anti-apoptotic pathways of tumor necrosis factor signalling, Biochem. Pharmacol. 56 (1998) 915–920.
[73] P. Liu, Y. Ying, Y.G. Ko, R.G. Anderson, Localization of platelet-derived growth factor-stimulated phosphorylation cascade to caveolae, J. Biol. Chem. 271 (1996) 10299–10303.
[74] J. Bock, E. Gulbins, The transmembranous domain of CD40 determines CD40 partitioning into lipid rafts, FEBS-Lett. 534 (2002) 169–174.
[75] P.W. Janes, S.C. Ley, A.I. Magee, Aggregation of lipid rafts accompanies signaling via the T cell antigen receptor, J. Cell Biol. 147 (1999) 447–461.
[76] M.W. Su, C.L. Yu, S.J. Burakoff, Y.J. Jun, Targeting Src homology 2 domain-containing tyrosine phosphatase (SHP-1) into lipid rafts inhibits CD3-induced T cell activation, J. Immunol. 166 (2001) 3975–3982.
[77] E.L. Smith, E.H. Schuchman, Acid sphingomyelinase overexpression enhances the antineoplastic effects of irradiation in vitro and in vivo, Mol. Ther. (2008) PMID 18628757.
[78] V. Teichgräber, M. Ulrich, N. Endlich, J. Riethmüller, B. Wilker, C. De Oliveira-Munding, A. van Heeckeren, M.L. Barr, G. von Kurthy, K.W. Schmid, M. Weller, B. Tümmler, F. Lang, H. Grassme, G. Döring, E. Gulbins, Ceramide accumulation mediates inflammation, cell death and infection susceptibility in cystic fibrosis, Nat. Med. 14 (2008) 382–391.
[79] I. Petrarche, V. Natarjan, L. Zhen, T.R. Medler, A.T. Richter, C. Cho, W.C. Hubbard, E.V. Berdyshev, R.M. Tuder, Ceramide upregulation causes pulmonary cell apoptosis and emphysema-like disease in mice, Nat. Med. 11 (2005) 491–498.
[80] X. He, Y. Huang, B. Li, X. Gong, E.H. Schuchman, Deregulation of sphingolipid metabolism in Alzheimer's disease, Neurobiol. Aging (2008) PMID 18547682.