Sphingolipids as modulators of cancer cell death: Potential therapeutic targets

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Received 8 February 2006; received in revised form 4 May 2006; accepted 6 May 2006
Available online 6 June 2006

Abstract

Through modifications in the fine membrane structure, cell–cell or cell–matrix interactions, and/or modulation of intracellular signaling pathways, sphingolipids can affect the tumorigenic potential of numerous cell types. Whereas ceramide and its metabolites have been described as regulators of cell growth and apoptosis, these lipids as well as other sphingolipid molecules can modulate the ability of malignant cells to grow and resist anticancer treatments, and their susceptibility to non-apoptotic cell deaths. This review summarizes our current knowledge on the properties of sphingolipids in the regulation of cancer cell death and tumor development. It also provides an update on the potential perspectives of manipulating sphingolipid metabolism and using sphingolipid analogues in anticancer therapy.

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Keywords: Ceramide; Apoptosis; Sphingolipid; Autophagy; Caspase; Lysosome

1. Several ways to die and many ways to resist cell death
2. Sphingolipids as regulators of cancer cell death
   2.1. Sphingolipids as tumorigenic molecules
      2.1.1. Tumorigenic effect of gangliosides
      2.1.2. Tumorigenic effect of sphingosine 1-phosphate
   2.2. Sphingolipids as tumor-suppressor molecules
      2.2.1. Induction of apoptosis
      2.2.2. Induction of lysosomal and autophagic cell death
      2.2.3. Induction of mitotic catastrophe
      2.2.4. Induction of other caspase-independent cell deaths
3. Changes in sphingolipid metabolism associated with resistance to anticancer treatments
   3.1. Alterations in the sphingomyelin to ceramide balance
   3.2. Alterations in the ceramide to sphingosine 1-phosphate balance
   3.3. Alterations in the ceramide to GlcCer/GalCer balance

Abbreviations: GalCer, galactosylceramide; GlcCer, glucosylceramide; GCS, glucosylceramide synthase; MDR, multidrug resistance; ROS, reactive oxygen species; SL, sphingolipid; SM, sphingomyelin; SMase, sphingomyelinase; SMS, sphingomyelin synthase; SIP, sphingosine 1-phosphate; TNF, tumor necrosis factor

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0005-2736/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbamem.2006.05.024
1. Several ways to die and many ways to resist cell death

During the last few years, a growing body of evidence has accumulated indicating that cancer cells can die by several ways as defined by distinct morphological and biochemical features [1,2]. Depending on the cell type and the death stimulus, multiple mechanisms leading to apoptosis, necrosis or intermediate cell deaths exhibiting some characteristics of apoptosis, necrosis or both have been described. Different names and definitions were given including apoptosis-like and necrosis-like cell deaths, parapaptosis, apoptonecrosis, caspase-dependent and -independent cell death, mitotic catastrophe as well as autophagic cell death [3]. However, since all these different types of cell death are not fully characterized at the morphological and biochemical levels, no precise or unequivocal definition can be delineated for all of them.

In a recent review, the nomenclature committee on cell death proposed some recommendations to unify criteria for the definition of the different types of cell death [4]. Apoptosis is mostly characterized by reduction of the cellular volume, chromatin condensation and nuclear fragmentation. In most (but not all) cases, apoptosis is associated with caspase activation and DNA fragmentation. Autophagic cell death (or preferably cell death associated with autophagy) occurs without chromatin condensation and is morphologically defined by the presence in the cytoplasm of two-membraned vacuoles containing degenerating organelles. Mitotic catastrophe is a form of cell death that occurs during the metaphase leading to aneuploidy and multinucleation and is most likely a consequence of abortive mitosis and/or cytokinesis. Necrosis is associated with plasma membrane disruption, swelling of organelles, oncosis and moderate chromatin condensation.

The molecular pathways leading to autophagic cell death, mitotic catastrophe and necrosis are largely unknown. Whereas recent data point to the putative involvement of cytotoxic sphingolipids (SLs) such as ceramide or its immediate metabolite derivatives not only in the induction of apoptosis but also in autophagic cell death and necrosis, some other SLs such as psychosine, a catabolite of galactosylceramide, were shown to trigger mitotic catastrophe (see below).

One of the characteristics of cancer cells is to resist cell death owing to the development of different resistance mechanisms including up-regulation of the expression of anti-apoptotic genes, inactivation of pro-apoptotic genes as well as immunosuppressive strategies to escape from cellular killers [5]. With regard to anti-apoptotic proteins, the expression of some Bcl-2 (B cell lymphoma 2) family members, including Bcl-2 and viral homologues, is frequently up-regulated in cancer cells. Those proteins confer resistance to chemotherapy, radiotherapy, as well as ceramide and its metabolites [6–8]. The anti-apoptotic mechanism relies mainly on the capacity of anti-apoptotic Bcl-2 family members to stabilize mitochondrial and endoplasmic reticulum membranes, blocking the re-localization of pro-apoptotic mitochondrial proteins (such as cytochrome c) [9] and calcium into the cytosol [10]. Other proteins such as FLIP (FADD-like interleukin-1 β-converting enzyme Inhibitory Protein) and IAPs (Inhibitor of Apoptosis Proteins), which are overexpressed in some cancer cells, interfere with caspase activation [5]. Treatment by siRNA for X-IAP overcomes the resistance of glioma cells in response to ceramide [11] indicating that IAP proteins can inhibit ceramide-induced cell death.

Concerning the pro-apoptotic genes, different mutations leading to the loss of protein expression or function have been reported in cancer cells. For instance, p53, BAX and APAF1 (Apoptotic Protease Activating Factor 1) mutations were reported in various cancer cells. Moreover, death receptors such as CD95 and TRAIL-R (Tumor Necrosis Factor-Related Apoptosis Inducing Ligand receptor) are frequently down-regulated or mutated in many solid tumors, myelomas and leukemia. These phenomena are part of the mechanisms developed by malignant cells to resist chemotherapy and radiotherapy and to escape from the immune system [5]. In some instances, ceramide analogues can overcome cell death resistance by activating caspase-dependent and -independent cell death (see below).

2. Sphingolipids as regulators of cancer cell death

The effects of SLs on the regulation of cell growth are remarkably complex. This complexity is exemplified by the influence that SLs exert on malignant transformation and tumor progression. Whereas some SLs tend to promote tumorigenesis, some others repress tumor extension, making these lipids viewed as double-edged swords with regard to their action on cancer cells (Fig. 1).

2.1. Sphingolipids as tumorigenic molecules

 Accumulating evidence indicates that some SLs can facilitate tumor growth, for instance by inhibition of tumor cell
death or, conversely, by enhancing tumorigenesis, metastatic abilities and tumoral angiogenesis, or by participating to tumor cell escape from the immune system. Among those SLs, sialic acid-bearing glycosphingolipids, i.e., gangliosides, and sphingosine 1-phosphate (S1P) are presently considered to play a major role in tumor biology.

2.1.1. Tumorigenic effect of gangliosides

The role of gangliosides in tumor development is complex, as these lipids can greatly influence cell death, invasion and metastasis [12,13]. One of the characteristics of tumor cells is an enhanced expression of various gangliosides. For instance, GD3 expression is markedly increased in melanoma cells as compared to human melanocytes. In addition, highly metastatic cells expressed more GD3 than poorly metastatic cells (see [12] and references therein). Consequently, this ganglioside has been proposed as a target for immunotherapy of cancer. For instance, anti-GD3 monoclonal antibodies can inhibit human melanoma cell growth in vitro and in vivo [14]. Recently, a chimeric anti-GD3 antibody (KM871) has been tested in phase I clinical trials in patients with metastatic melanoma [15], and another was evaluated in a phase III clinical trial against small cell lung cancers [16]. Furthermore, epigenetic modifications of ganglioside expression (using antisense vectors against GD3-synthase or glucosylceramide synthase) have demonstrated an inhibition of tumor incidence and metastatic potential of neuroblastoma [17,18] or melanoma [19]. Another putative pro-tumoral ganglioside is GM3. Indeed, the metastatic and invasive potential of mouse melanoma B16 seems to be correlated with the level of GM3 expression at the cell surface [20]. The presence of substantial amounts of GM3 on human melanomas and other tumors makes this glycolipid a possible target for cancer immunotherapy [21]. Consequently, GM3 has been used to develop vaccines and monoclonal antibody strategies. For instance, a phase I clinical trial using anti-GM3 human monoclonal antibody (L612) was recently performed in patients with metastatic melanoma [22]. Other gangliosides, often quantitatively minor in healthy cells, could be involved in promotion of tumor cell migration and invasiveness. For instance, the expression of disialyl-GaINAc-Le4Cer (RM2 antigen) or monosialyl-Gb5 has been associated with metastatic properties of renal carcinoma [23] or mammary carcinoma [24], respectively.

How can gangliosides promote tumor development? Gangliosides present at the tumor cell surface might regulate crosstalks with the cellular environment (healthy tissue cells, endothelial cells as well as immune cells), participating to the development of tumor and its invasive/metastatic properties. The communication between cells involves molecular interactions that have been mainly studied as protein–protein interactions, for instance in the immunological synapse. However, though this research area has received less attention, glycosyl epitopes can also act in cellular interactions, especially in cancer development modulation [25]. Thus, tumor cell gangliosides can interact with neighboring cells by acting as adhesion molecules, functional ligands or bioactive soluble factors. In glycosynapses, gangliosides may modulate not only cell adhesion but also signal transduction [26]. For instance, GM3 on B16 melanoma cell surface is strongly involved in adhesion to endothelial cells and induces complex signaling in these cells. Another possibility for ganglioside involvement in cellular adhesion is the interaction of the disialo epitope of certain gangliosides with sialic acid binding proteins named Siglecs (Sialic acid-binding Immunoglobulin-like Lectins). Because Siglecs are expressed on various types of blood cells, tumor cells expressing high levels of disialogangliosides can bind to Siglecs of blood cells, leading to the formation of cell clumps triggering microembolisms. Consequently, activated platelets release stimulating factors for endothelial cells, thereby inducing expression of adhesion molecules and thus allowing tumor cell adhesion and translocation out of the vessel to initiate metastases. Moreover, tumor cells shed gangliosides into the microenvironment, a phenomenon that can modulate angiogenesis and immunosurveillance. For instance, extracellular GD3 stimulates VEGF production whereas GM3 can inhibit EGF receptor tyrosine kinase activity [18,27].

Gangliosides in particular, but putatively other SLs as well, can suppress the antitumoral immune response [28]. The anti-tumor activity mediated by the immune system uses various mechanisms, such as cell-induced cytotoxicity. For instance, cytotoxic T lymphocytes (CTL) and NK cells, but also macrophages and granulocytes, can kill tumor cells. The interaction between killer cells and tumor cells and the regulation of killing involve adhesion molecules, activating and inhibiting receptors, co-stimulating molecules, death ligands and their receptors. The regulatory role of gangliosides in these complex molecular interactions is only partly elucidated. Thus, it has recently been demonstrated that Siglec-7 expressed by monocytes and NK lymphocytes induces an inhibitory effect on killer function and has a preferential binding for α 2,8-linked disialic acids which are displayed by gangliosides such as GD3, GD2 and GT1b [29,30]. As a matter of fact, GD3 present on tumor target cells can modulate NK-mediated cytotoxicity using Siglec-7 interaction. Further observations support the hypothesis that gangliosides may be indirectly active in the suppression of the anti-
<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Cell type/origin</th>
<th>Type of manipulation</th>
<th>Consequence on cell viability</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucosyl ceramide synthase</td>
<td>Human MCF-7 breast cancer</td>
<td>Overexpression</td>
<td>Resistance to adriamycin and ceramide</td>
<td>[180]</td>
</tr>
<tr>
<td></td>
<td>Human MCF-7 breast cancer</td>
<td>Knockdown (by antisense constructs or ODNs)</td>
<td>Restoration of anticancer drug-induced apoptosis in resistant cells</td>
<td>[181,197,198]</td>
</tr>
<tr>
<td></td>
<td>Human Jurkat T leukemia</td>
<td>Overexpression</td>
<td>No effect on stress-induced DNA fragmentation</td>
<td>[146]</td>
</tr>
<tr>
<td></td>
<td>Murine MEB4 melanoma</td>
<td>Knockdown (by an antisense construct)</td>
<td>Reduction in tumorigenicity</td>
<td>[19]</td>
</tr>
<tr>
<td></td>
<td>Murine B16 melanoma</td>
<td>Deficient (GM95) and corrected (GM95/GCS) cells</td>
<td>No difference in anticancer drug-induced apoptosis</td>
<td>[147]</td>
</tr>
<tr>
<td>GD3 synthase</td>
<td>Human HuT78 T cell lymphoma and U937 leukemia</td>
<td>Overexpression</td>
<td>Induction of apoptosis</td>
<td>[199]</td>
</tr>
<tr>
<td></td>
<td>Rat F-11 neuroblastoma</td>
<td>Knockdown (by an antisense construct)</td>
<td>Inhibition of Fas cross-linking-induced apoptosis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Human HT29 colon cancer</td>
<td>Knockdown (by an antisense construct)</td>
<td>Inhibition of TNF-induced cell death</td>
<td>[200]</td>
</tr>
<tr>
<td>GM3 synthase</td>
<td>Murine MBT-2 bladder carcinoma</td>
<td>Overexpression</td>
<td>Increase in apoptosis and reduction of tumor growth</td>
<td>[201]</td>
</tr>
<tr>
<td>Ganglioside sialidase</td>
<td>Murine B16 melanoma</td>
<td>Overexpression</td>
<td>Reduction in lung metastases</td>
<td>[202]</td>
</tr>
<tr>
<td></td>
<td>Human HCT-115, HCT116, DLD-1 colon cancer</td>
<td>Overexpression</td>
<td>Inhibition of sodium butyrate-induced apoptosis</td>
<td>[40]</td>
</tr>
<tr>
<td>Sphingomyelin synthase 1</td>
<td>Murine WR19L lymphoma</td>
<td>Deficient (WR/SM-)</td>
<td>Partial resistance to anti-Fas-induced apoptosis</td>
<td>[108]</td>
</tr>
<tr>
<td>Neutral SMase 2</td>
<td>Human MCF-7 breast cancer</td>
<td>Overexpression</td>
<td>Growth inhibition</td>
<td>[126]</td>
</tr>
<tr>
<td></td>
<td>Human MCF-7 breast cancer</td>
<td>Knockdown (by siRNA)</td>
<td>Increase in cell proliferation</td>
<td>[128]</td>
</tr>
<tr>
<td>Acidic SMase</td>
<td>Human EBV-transformed lymphoid cells</td>
<td>Deficient (Niemann–Pick)</td>
<td>Impairment in stress-induced apoptosis</td>
<td>see [72] for review</td>
</tr>
<tr>
<td></td>
<td>Human L929 fibrosarcoma</td>
<td>Overexpression</td>
<td>No defect in stress-induced apoptosis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Murine L929 fibrosarcoma</td>
<td>Overexpression</td>
<td>Inhibition of TNF-induced apoptosis</td>
<td>[140]</td>
</tr>
<tr>
<td></td>
<td>Human EBV-transformed lymphoid cells</td>
<td>Deficient (Farber)</td>
<td>Inhibition of TNF-induced caspase-independent cell death</td>
<td>[61]</td>
</tr>
<tr>
<td>C18-ceramide synthase (LASS1)</td>
<td>Human UM-SCC-22A squamous cell carcinoma of hypopharynx</td>
<td>Overexpression</td>
<td>Induction of apoptotic cell death</td>
<td>[204]</td>
</tr>
<tr>
<td>Sphingosine kinase 1</td>
<td>Human Jurkat T leukemia</td>
<td>Overexpression</td>
<td>Partial inhibition of ceramide, serum starvation and Fas-induced apoptosis</td>
<td>[205]</td>
</tr>
<tr>
<td></td>
<td>Rat PC12 pheochromocytoma</td>
<td>Overexpression</td>
<td>Inhibition of ceramide and serum starvation-induced apoptosis</td>
<td>[206]</td>
</tr>
<tr>
<td></td>
<td>Human PC-3 and LNCaP prostate cancer</td>
<td>Overexpression</td>
<td>Inhibition of anticancer drug-induced apoptosis</td>
<td>[139]</td>
</tr>
<tr>
<td></td>
<td>Human HL-60 leukemia</td>
<td>Knockdown (by siRNA)</td>
<td>Induction of apoptosis</td>
<td>[207]</td>
</tr>
<tr>
<td></td>
<td>Human A-375 and Mel-2a melanoma</td>
<td>Overexpression</td>
<td>Inhibition of Fas- and ceramide-induced apoptosis</td>
<td>[208]</td>
</tr>
<tr>
<td></td>
<td>Murine erythroleukemia</td>
<td>Knockdown (by siRNA)</td>
<td>Decreased resistance to apoptosis</td>
<td>[209]</td>
</tr>
<tr>
<td></td>
<td>Human MCF-7 breast cancer</td>
<td>Overexpression</td>
<td>Resistance to serum starvation-induced apoptosis</td>
<td>[90]</td>
</tr>
<tr>
<td></td>
<td>Human MCF-7 breast cancer</td>
<td>Overexpression</td>
<td>Sensitization to serum starvation-induced apoptosis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Human MCF-7 breast cancer</td>
<td>Knockdown (by siRNA)</td>
<td>Increase in autophagy and inhibition of serum starvation-induced apoptosis</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Overexpression of catalytically-inactive form</td>
<td>Decrease in autophagy and stimulation of serum starvation-induced apoptosis</td>
<td></td>
</tr>
<tr>
<td>Sphingosine kinase 2</td>
<td>Rat PC12 pheochromocytoma</td>
<td>Overexpression</td>
<td>Stimulation of serum starvation-induced apoptosis</td>
<td>[210]</td>
</tr>
<tr>
<td></td>
<td>Murine NIH3T3 fibroblasts</td>
<td>Overexpression</td>
<td>Inhibition of DNA synthesis</td>
<td>[211]</td>
</tr>
<tr>
<td></td>
<td>Murine NIH3T3 fibroblasts</td>
<td>Overexpression</td>
<td>Stimulation of anticancer drug and serum starvation-induced apoptosis</td>
<td>[134]</td>
</tr>
<tr>
<td>SIP phosphatase 1</td>
<td>Murine NIH3T3 fibroblasts</td>
<td>Overexpression</td>
<td>Increase in stress-induced-apoptosis</td>
<td>[212]</td>
</tr>
<tr>
<td></td>
<td>Human HEK293 embryonic kidney</td>
<td>Overexpression</td>
<td>Induction of apoptosis upon addition of SIP</td>
<td>[135]</td>
</tr>
<tr>
<td>SIP lyase</td>
<td>Human HEK293 embryonic kidney</td>
<td>Overexpression</td>
<td>Increase in serum starvation-induced apoptosis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Human A549 lung cancer</td>
<td>Overexpression</td>
<td>Increased sensitivity to cisplatin, carboplatin, doxorubicin</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviation: ODNs, oligodeoxynucleotides.
tumor killer responses, and more generally of antitumor immunity. For instance, exogenous and tumor-derived gangliosides can inhibit antigen presentation by monocytes, down-regulate MHC molecule expression by dendritic cells [31] and suppress the function of monocyte-derived dendritic cells [32]. Exogenous gangliosides can inhibit B and T cell activation and helper T cell functions [33]. Released tumor gangliosides can also bind to IL-2 and, consequently, inhibit the interaction of the cytokine with its receptor [34]. In addition, exogenous GM3 induces inhibition of CD4 molecule expression that could prevent T lymphocyte activation [35]. Finally, some exogenous gangliosides could reduce Th1 immune response and favor Th2 response. The mechanisms involved in these effects remain poorly understood, but may include inhibition of IFNγ production; enhancement of IL-4 dependent differentiation of Th2 cells, known to inhibit Th1 responses; alternation of Th1 cell differentiation through an impairment of dendritic cell development and antigen presentation; and a higher susceptibility of Th1 cells to ganglioside-induced apoptosis [28,36–38]. Thus, gangliosides participate to the suppression of Th1 response, considered to be essential for the immune defense against tumor cells.

2.1.2. Tumorigenic effect of sphingosine 1-phosphate

By regulating cell death signaling, SLs can largely contribute to tumor promotion. Some SL metabolites, such as sphingosine 1-phosphate (S1P), ceramide 1-phosphate and lactosylceramide negatively modulate cell death and/or promote cell proliferation. For instance, ceramide 1-phosphate stimulates cell survival and blocks apoptosis (see review by [39]). Recently, lactosylceramide, produced by the Neu3 sialidase, has also been proposed to inhibit apoptosis of colon cancer cells, mainly through an increased Bcl-2 expression and diminished caspase activation [40]. Expression of this ganglioside sialidase has been reported to be greatly enhanced in different aggressive tumors. It is likely that tumor cells use such anti-apoptotic mechanisms involving SLs to escape death induced by tumor immunosurveillance.

Among the anti-apoptotic SLs, S1P is clearly considered as a tumor promoter, favoring cellular transformation, tumor proliferation and cell survival (see articles by Obeid et al. [224] and Spiegel et al. [225] in this issue). Inside the tumor cell, S1P interferes with cell death signaling pathways, inhibiting cell death and favoring cell growth and cell movement. One of the putative anti-apoptotic mechanisms of S1P is to prevent ceramide-induced mitochondrial events such as cytochrome c release and caspase activation [41,42]. In addition to these direct effects on tumor cells, S1P alters several mechanisms that trigger cell survival and growth. Thus, S1P promotes tumor angiogenesis, a process vital for the development of aggressive tumors (for reviews, see [43,44]). For instance, S1P can act on cancer development by: (i) facilitation of tumor cell activation by VEGF, a growth factor which stimulates sphingosine kinase 1, (ii) stimulation of endothelial cell growth and migration, (iii) synergistic effects with various pro-angiogenic factors on the neovascularization process, and (iv) inhibition of endothelial

Table 2

Examples of in vitro or in vivo effects of sphingolipid analogues on cell death and tumorigenicity of cancer cells (short-chain ceramides and FTY720 are not presented in this table; the reader is referred to the text)

<table>
<thead>
<tr>
<th>SL analogue</th>
<th>Cell type</th>
<th>In vitro effect</th>
<th>In vivo effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>B13</td>
<td>Human SW403 and Lovo colon cancer cells</td>
<td>Apoptosis</td>
<td>Prevention of liver metastases</td>
<td>[159]</td>
</tr>
<tr>
<td>B13 and D-e-MAPP</td>
<td>Human melanoma cells</td>
<td>Reduction in cell proliferation and apoptosis</td>
<td></td>
<td>[214]</td>
</tr>
<tr>
<td>B13 (R and S)</td>
<td>Human prostate LNCaP and PC3 cancer cells</td>
<td>Apoptosis</td>
<td>Reduction of tumor volume after irradiation</td>
<td>[158]</td>
</tr>
<tr>
<td>Adamantyl-ceramide</td>
<td>Human MCF-7/Adr breast cancer cells</td>
<td>Cell death (IC50 ~ 25 μM)</td>
<td></td>
<td>[156]</td>
</tr>
<tr>
<td>Thiouracil-ceramide</td>
<td>Human CEM leukemia cells</td>
<td>Caspase-independent cell death (IC50 ~ 1.7 μM)</td>
<td>Reduction of tumor (human colon cancer) mass</td>
<td>[215]</td>
</tr>
<tr>
<td>S18</td>
<td>Murine and human neuroblastoma cells</td>
<td>Apoptosis</td>
<td>Prevention of teratoma formation</td>
<td>[216–218]</td>
</tr>
<tr>
<td>PEITC-Sa</td>
<td>Human HL-60 leukemia cells</td>
<td>Apoptosis</td>
<td></td>
<td>[161]</td>
</tr>
<tr>
<td>RZ-2</td>
<td>Human HL-60 leukemia cells</td>
<td>Apoptosis (IC50 ~ 6 μM)</td>
<td></td>
<td>[219]</td>
</tr>
<tr>
<td>4,6-diene-ceramide</td>
<td>Human MCF-7 breast cancer cells</td>
<td>Apoptosis (IC50 ~ 11 μM)</td>
<td></td>
<td>[157]</td>
</tr>
<tr>
<td>C6-pyridinium- ceramide bromide</td>
<td>Human HepG2 hepatoma, m</td>
<td>Reduction in cell survival (IC50 ~ 0.25–8 μM)</td>
<td></td>
<td>[220,221]</td>
</tr>
<tr>
<td>AD2646, AD2687</td>
<td>Human Jurkat and HL-60 leukemia cells</td>
<td>Caspase-dependent cell death (IC50 ~ 1 μM)</td>
<td>Cell death (IC50 &gt; 10 μM)</td>
<td>[66,222]</td>
</tr>
<tr>
<td>AD2765</td>
<td>Human M059J glioma cells</td>
<td></td>
<td></td>
<td>[223]</td>
</tr>
</tbody>
</table>

Chemical names of SL analogues: B13 (R): (1R,2R)-2-(N-myristoylamino)-1-(4′-nitrophenyl)-1,3-propanediol; D-e-MAPP: (1S,2R)-2-N-myristoylamino-1-phenyl-1-propanol; Adamantyl-ceramide: (25,3R)-2-(N-adamantamido)-(4E)-octadecen-1-3-diol; S18: N-oleoyl-(2-amino-1,3-propanediol); PEITC-Sa: phenethylisothiocyanate-substituted sphinganine; RZ-2: (2R,3S)-2-acetylamino-3-octadecen-1-ol; 4,6-diene-ceramide: (2S,3R)-(4E,6E)-2-octanoylamido-octadecadiene-1,3-diol; AD2646: (2R,3R)-2-(N-tetradecylamino)-1-(4-nitrophenyl)-1,3-propanediol; AD2687: (2R,3S)-2-(N,N,N-trimethylamino)-1-(N-tetradecanoyl-4-aminophenyl)-1,3-propanediol.
cell apoptosis [45]. In addition, autotaxin is a phospholipase exoenzyme that is up-regulated in various cancers and generates not only lysophosphatidic acid but also S1P [46], giving another possibility to produce tumorigenic lipid mediators.

By stimulating inflammatory responses S1P could also help to tumor development as inflammation is known to participate to cancer promotion [47]. For example, TNF-treatment of tumor cells activates sphingosine kinase 1, leading to an increased intracellular S1P concentration, subsequent stimulation of COX2 expression and synthesis of PGE2 [48], which is considered as a cancer-promoting eicosanoid.

Finally, through its binding to S1P receptors, S1P could modulate differentiation, survival and migration of several immune cell types, such as T and B lymphocytes (for reviews, see [49,50]). Indeed, S1P reduces proliferation and regulates cytokine secretion by T cells, increasing IL-10 and decreasing IFN-γ production [51]. These observations suggest that S1P could inhibit Th1 response. The consequences of this putative inhibition on Th1 anticancer immune responses are currently unknown.

In conclusion, tumorigenic effects of SLs largely exceed the sole modulation of cell death. Hence, growing evidence indicates that SLs exhibit mediator activity in immunity that could affect anti-cancer immune responses. For instance, as mentioned above, S1P and some gangliosides have immunosuppressive effects on several immune cell-mediated cytotoxic mechanisms. On the other hand, some SLs probably act as stimulators of immune cells. The ambivalent role of SLs on the immune system can be illustrated by the activating function of ceramides on NKT cells. CD1d-restricted NKT cells have been identified as an important component of the immune system, which has the capacity both to augment beneficial host immunity, such as tumor rejection, and to prevent harmful autoimmune [52]. Activated NKT lymphocytes can mediate tumor cell lysis. In addition these cells secrete large amounts of Th1 and Th2 cytokines, including both pro-inflammatory and anti-inflammatory cytokines. Consequently, they are considered as important regulators of immune responses, with pleiotropic effects. Unlike classical Tcβ lymphocytes that recognize peptides, NKT lymphocytes recognize glycolipid ligands such as α-galactosylceramide [53]. Recently, isoglobotrihexosylceramide (iGb3), a natural lysosomal SL, has been proposed as a natural ligand for NKT and could be involved in the control of NKT cell responses to tumor cells [54]. In addition, mice immunized with GD3-positive human melanoma cells, or with syngeneic antigen-presenting cells loaded with GD3, develop a specific GD3-reactive NKT population, suggesting that some gangliosides are possible ligands for NKT lymphocytes [55].

2.2. Sphingolipids as tumor-suppressor molecules

Numerous observations have shown that certain SLs are able to trigger caspase activation and apoptosis (recently reviewed in [56]). Despite intensive research, however, the molecular mechanisms that intimately mediate the action of SLs in apoptosis remain unclear. In addition, recent data indicate the possible contribution of ceramide to alternative types of cell death. Thus, ceramide and possibly some of its metabolites seem to be capable of activating programmed cell death not only through caspase activation but also by caspase-independent mechanisms [57–61]. Understanding how these programs are triggered and regulated is of utmost importance in human cell biology and pathology since it is now well established that their dysregulation leads to many diseases including cancers.

2.2.1. Induction of apoptosis

Different studies underscore the putative function of ceramide or its metabolites in stress-induced apoptosis in cancer cells. First, diverse apoptotic stimuli led to the accumulation of intracellular ceramide level in sensitive but not in resistant cancer cells [62–65]. Distinct mechanisms including de novo ceramide synthesis, sphingomyelin (SM) breakdown as well as inhibition of ceramide conversion to complex SLs might contribute to this phenomenon (see also Section 3). Second, incubation of cancer cells in the presence of SL analogues triggered caspase activation and apoptosis (see Section 4 and Table 2). The mechanisms by which SLs induce caspase activation likely involve mitochondrial events such as cytochrome c release since overexpression of Bcl-2 or Bcl-XL counteracts SL cytotoxic effects [6,8,66]. Third, modulation of ceramide metabolism either by pharmacological or epigenetic strategies leading to ceramide accumulation is accompanied by caspase activation and apoptosis of cancer cells (see Tables 1 and 2). As a matter of fact, reducing ceramide content can be associated with cancer cell resistance to apoptosis [65].

Whereas different SL metabolites including ceramide [56], sphingosine [67] and some gangliosides such as GD3 [68] have been proposed to promote caspase activation, S1P [42,69] and possibly GaCer [70] have an opposite effect.

2.2.2. Induction of lysosomal and autophagic cell death

While the existence of a ‘lysosomal pathway’ of cell death is now accepted, the molecular mechanisms that underlie the action of these organelles or components in the death cascades are still unclear. In response to a variety of death stimuli, partial lysosomal-membrane permeabilization (LMP) can occur, leading to the release of a class of proteases, cathepsins, that can activate apoptotic and non-apoptotic cell death [71,72]. SLs have been associated with LMP due to the fact that sphingosine could permeabilize lysosomal membranes and facilitate the relocation of some lysosomal proteases to the cytosol [73]. Exogenous sphingosine, but not ceramide, can also permeabilize in vitro isolated lysosome membranes [74]. In contrast, exogenous SM has been reported to prevent lysosomal disruption and the induction of apoptosis in photodynamic therapy-treated murine hepatoma cells [75]. The role of SLs in LMP is further supported by the finding that overexpression of a dominant-negative form of FAN (Factor Associated with Neutral sphingomyelinase activation), a protein known to associate with TNF receptor-1 and to mediate the activation of neutral sphingomyelinase (SMase), was accompanied by a decrease in sphingosine generation and suppression of LMP in rat hepatoma cells [76].

Even though LMP might be dependent on sphingosine, ceramide generated by the endolysosomal acid SMase has also
been shown to act on lysosomes. Indeed, the direct binding of ceramide to the endolysosomal aspartate protease cathepsin D results in the processing and the activation of the protease [77], which, in turn, has been reported to be implicated in TNF- [78] and UV light- [79] induced cell death. However, the role played by cathepsin D in apoptosis seems to be independent of its catalytic activity [80,81]. Interestingly, the interaction between a cathepsin and another SL has also been demonstrated with the observation that cathepsin B could reduce the levels of antiapoptotic S1P during stress-induced tumor cell death [82].

Additional lysosomal proteins might be related to ceramide in cell death signaling. Indeed, the product of the CLN3 gene encoding battenin, a lysosomal transmembrane protein with unknown function, which is mutated in some inherited neurodegenerative diseases, has been reported to prevent ceramide-induced apoptosis [83]. Similar findings were obtained by using CLN3 antisense constructs in human cancer cells [84].

Whereas lysosomal hydrolases are active components in cell death induction [85], the implication of lysosomes in cell dismantling has also been demonstrated through their role in macroautophagy or autophagy. This process, which is defined as a catabolic pathway for organelles and macromolecules, is also classified as a programmed cell death mechanism [86]. Autophagy is controlled by ATG genes whose products are essential in the formation of the autophagosome and in the signaling pathways related to autophagy [87]. SLs have been associated with autophagy due to the fact that ceramide could mediate the tamoxifen dependent-autophagic cell death in human breast cancer MCF-7 cells. Exogenous ceramide stimulates autophagy by relieving the class I PI3K/Akt/PKB pathway and provoking the accumulation of the tumour suppressor gene product Atg6/beclin 1 [88]. Similarly, a cell-permeable analogue of ceramide induces autophagic cell death in glioma cells by increasing the expression of the mitochondrial bound BH3-only BNIP3 protein [89]. In contrast, S1P could stimulate autophagy by inhibiting mTOR independently of the class I PI3K signaling and with a poor stimulatory effect on beclin 1, suggesting that both SLs are able to trigger autophagy, but with different outcomes on cell survival [90].

Altogether, the afore-mentioned studies emphasize the connection of SLs and lysosomes in the control of cell death. However, future studies are obviously required to determine precisely their contribution to LMP and the regulation of autophagy.

2.2.3. Induction of mitotic catastrophe

Currently, a very limited number of observations suggest that SLs could be involved in this yet poorly defined mode of cell death which is characterized by cell cycle defects, aborted mitosis and production of multinucleated cells. To our knowledge, only the lysosphingolipid psychosine (β-galactosylsphingosine), also known to trigger apoptotic cell death, has been reported to induce inhibition of cytokinesis and the formation of multinuclear cells [91]. This could account for the presence of the so-called globoid cells in the nervous system of patients affected with Krabbe disease, i.e., galactosylceramidase deficiency, where psychosine accumulates. This effect of psychosine has been described to be mediated by its binding to the orphan receptor, T-cell death associated gene 8 (TDAG8) [92]. However, recent data indicate that this receptor is dispensable for psychosine-induced inhibition of cytokinesis and glucocorticoid-induced cell death of thymocytes [93].

2.2.4. Induction of other caspase-independent cell deaths

A growing body of evidence supports the involvement of ceramide in caspase-independent cell death, that is, in cell death processes that remain insensitive to the broad caspase inhibitor zVAD-fmk. Exogenous ceramide analogues induced cancer cell death, which was not, or partly, inhibitable by zVAD-fmk [57,60,66,94–96]. In this context, ceramide stimulated p38 MAPK [60] and JNK activation [95]. Overexpression of Bcl-xL inhibited ceramide analogue-induced cell death suggesting the involvement of mitochondrial events [66]. In agreement with this hypothesis, exogenous ceramides were shown to trigger AIF (Apoptosis Inducing Factor) release from the mitochondria [97]. The role of AIF in ceramide-induced caspase-independent cell death remains to be established. In addition, ceramide has been recently proposed as a mediator in TNF-induced caspase-independent cell death in various cell lines [61]. In this last study, it was reported that ceramide production involved RIP1 (Receptor-Interacting Protein), a serine/threonine kinase required for death receptors-induced caspase-independent cell death [98]. In addition, TNF-induced ceramide production was impaired in fibroblasts genetically deficient for acid SMase as well as in L929 cells overexpressing acid ceramidase. Thus, TNF-induced caspase-independent ceramide accumulation and cell death likely involves RIP and acid SMase [61].

The type of caspase-independent cancer cell death induced by ceramide has not been always fully characterized and remains to be analysed in more details at the biochemical and morphological levels. Some studies reported the possibility that ceramide triggers essentially necrosis in certain cancer cell lines [96,99,100].

3. Changes in sphingolipid metabolism associated with resistance to anticancer treatments

A number of alterations in SL metabolism and individual SL levels have been reported to accompany resistance of malignant cells to anticancer regimens. These changes are described below.

3.1. Alterations in the sphingomyelin to ceramide balance

Two different types of enzymes, SM synthases and SMases can regulate the SM to ceramide balance. SM synthase transfers the phosphocholine moiety from phosphatidycholine to ceramide to generate diacylglycerol and SM. Also, SM synthase (SMS) may catalyze the reverse reaction [101]. Thus, it is important to note that SMS can regulate in opposite directions the levels of two bioactive molecules, the diacylglycerol and ceramide [102]. Two different genes encoding SMS have been cloned so far. The corresponding proteins, SMS1 and SMS2, are mainly localized at the Golgi and at the plasma membrane, respectively [103,104]. In SV40-transformed cells [105] as well...
as in human chemo-resistant leukemia cells [65], SMS activity is increased, this phenomenon being a putative mechanism to up-regulate the level of the pro-proliferative diacylglycerol and to decrease that of the anti-proliferative ceramide. Also, TNF and FasL-induced cell death is accompanied by the decrease of SMS activity, the inhibition of which might contribute to the pro-apoptotic signaling notably by increasing ceramide cellular content [106,107]. On the other hand, anti-Fas-induced apoptosis is partly impaired in a murine leukemia cell line having deficient SMS activity, whereas overexpression of SMS1 is accompanied by an increase of caspase activation and cell death [108]. These conflicting observations might be explained by (i) the structural function of SM in the constitution of cellular membranes, (ii) the fact that SM can be a source of stress-induced ceramide generation either by SMase activation or, possibly, by stimulation of the reverse activity of SMS (a pathway that awaits confirmation). Thus, a sustained inhibition of SMS might alter the physicochemical properties of membranes by SM depletion and confer cell death resistance, whereas a transient inhibition (or stimulation of the reverse activity) of SMS could be involved in ceramide generation and apoptosis signaling.

SMase catalyzes SM leading to the generation of ceramide and phosphocholine. Two types of SMases have been characterized: acidic and neutral SMases. Acidic SMase, encoded by the SMPD1 (sphingomyelin phosphodiesterase 1) gene located on human chromosome 11, is mainly localized in the endolysosomal compartment and can be secreted into the cellular environment. Partial or total deficiency of acidic SMase activity is responsible for Niemann–Pick disease, a lysosomal storage disorder characterized by SM accumulation. Different studies have highlighted the putative function of acidic SMase in apoptosis [109,110] including in cancer cells [78]. One of the putative mechanism by which acidic (secreted?) SMase is supposed to promote cell death is by generating ceramide on the outer leaflet of the plasma membrane enabling the oligomerization of CD95 [111–113], CD40 [114] or CD20 [115]. The formation of ceramide-enriched domains has also been reported in cancer cells and in response to UV-light, cisplatin and other inducers (for a recent review, see [116]). However, controversy exists as to the ability of acidic SMase to be part of the cell death signaling machinery in response to various agents including agonists of CD95, CD40 and chemotherapeutic drugs [72,117–119]. Acidic SMase has been proposed to be a component of the TNF-R1 receptosome [120].

Three neutral SMases, namely neutral SMase 1, 2 and 3 have been cloned. Neutral SMase 1, encoded by SMPD2 gene located on human chromosome 6, localizes at the endoplasmic reticulum and the function of this enzyme in signaling and cell death is controversial [121,122]. While neutral SMase 1 has been claimed to mediate TCR-induced programmed cell death of a T cell hybridoma [123], overexpression of this SMase had no consequence on the viability of Jurkat cells and their sensitivity to Fas-induced apoptosis [124]. Neutral SMase 2, encoded by SMPD3 gene located on human chromosome 16, appears to localize mostly at the Golgi but also at the plasma membrane, and can be activated by TNF [125–127]. Whereas enforced expression of neutral SMase 2 in MCF-7 breast cancer cells induced cell growth inhibition [126], neutral SMase 2 knockdown was accompanied by an increase in cell proliferation [128]. Previous reports showed the involvement of neutral SMase in the pro-apoptotic signaling activated by TNF, chemoradiotherapy [129,130]. Accordingly, we, and others, have previously reported the role of FAN (Factor associated with neutral sphingomyelinase activation) in TNF-induced caspase activation and apoptosis in SV40-transformed fibroblasts [131] and in hepatoma cells [76]. Similarly, FAN was shown to be involved in CD40L- and hypoxia/reoxygenation-induced cell death in SV40-transformed fibroblasts and cardiomyocytes, respectively [118,132]. Very recently, a third neutral SMase, encoded by SMPD4 gene located on human chromosome 2q21, colocalizes with endoplasmic reticulum and Golgi markers, and can be activated by TNF through the FAN adapter protein [133]. The role of this novel enzyme in cell death signaling remains to be elucidated.

3.2. Alterations in the ceramide to sphingosine 1-phosphate balance

Spiegel and co-workers have proposed that ceramide and S1P exert opposite effects on cancer cell proliferation and death. In this paradigm, while ceramide inhibits cell growth and promotes apoptosis, S1P counteracts these effects. Thus, the ratio between the two lipids would determine cell fate. Interestingly, recent reports have highlighted alterations in the balance between ceramide and S1P in cancer cells (see also articles by Spiegel et al. [225] and Obeid et al. [224] in this issue). The levels of ceramide and S1P can be directly regulated not only by sphingosine kinases [134], but also by S1P phosphohydrolases [135] and S1P lyase [136]. These enzymes regulate in opposite directions the intracellular content of sphingosine that can be converted to ceramide by ceramide synthase. Thus, overexpression of sphingosine kinase 1 was accompanied by S1P accumulation and concomitant ceramide decrease [134]. In contrast, overexpression of S1P phosphohydrolase 1 in NIH3T3 led to S1P decrease and ceramide increase [135]. In addition, accumulation of long-chain ceramide occurred in S1P lyase-overexpressing HEK293 cells under serum starvation [136]. Of note, sphingosine kinase 1 is overexpressed in certain tumor tissues [137–139] whereas the human 10q21 chromosomal region carrying the S1P lyase gene is deleted in a variety of cancers [136]. The balance between ceramide and S1P is also likely influenced by the rate of conversion of ceramide to sphingosine, i.e., by the activity of ceramidases, which can affect induction of cancer cell death [61,140].

3.3. Alterations in the ceramide to GlcCer/GalCer balance

In vitro selection of mammalian cells for resistance to a specific chemotherapeutic drug frequently leads to cross-resistance to a wide variety of cytotoxic agents, i.e., multidrug resistance [MDR] (for review see [141]). In these cells, overexpression of an integral membrane protein termed P-glycoprotein (P-gp), which is the gene product of MDR1, is responsible for active efflux of a number of drugs thereby preventing them
from accumulating within the tumor cell. MDR cells have also been described as presenting altered lipid compositions such as elevated cholesterol, SM and various glycolipids (for review see [142]). Among the latter, glucosylceramide (GlcCer) has been proposed to play a significant role in MDR and P-gp regulation. Indeed, in 1996, Cabot’s group first demonstrated that drug-resistant MCF-7/AdR breast cancer cells accumulated GlcCer in comparison to wild-type MCF-7 cells. This study was followed by a series of studies which clearly demonstrated that inhibition of GlcCer synthesis could sensitize MDR cells to chemotherapeutic drugs whereas stimulation of GlcCer formation by glucosylceramide synthase (GCS) overexpression led to increased doxorubicin resistance (for review see [143]; see also Cabot and Gouaze-Andersson [226]).

Abnormally increased GlcCer synthesis may not be the sole mechanism implicating glycolipids in the MDR phenotype. For instance, Kok and coworkers have reported that multidrug resistant ovarian cancer cells exhibit a defect in the coupling of GlcCer and lactosylceramide biosynthesis [144], and that up-regulation of lactosylceramide synthase is also associated with resistance [145]. In addition, at least in some instances, GCS expression does not influence cancer cell resistance to anticancer drugs or regimens [146–148]. Whether the ability of GCS to modulate the sensitivity to anticancer therapy is cell type and/or drug-specific remains to be established.

Whatever the cause for a shift towards an increased ceramide glucosylation, inhibition of the ceramide to GlcCer conversion would be expected to prevent tumor development. As a matter of fact, a number of observations have supported this tenet whereby incubation of malignant cells with pharmacological inhibitors of GCS resulted in chemo and/or radiosensitization, and in delayed tumor formation (see Section 4). Of note, these findings do not allow to distinguish between two potential modes of action, that is, the suppression of glycosphingolipids such as gangliosides which appear as crucial determinants in tumor development and invasion (see Section 2.1), or the increased intracellular content of the tumor-suppressor ceramide.

Our group has been investigating the role of ceramide and ceramide metabolism notably in daunorubicin (DNR) and aracytine (Ara-C)-induced apoptosis of leukemic cells. Using clinically relevant DNR and Ara-C concentrations (1 μM and 40 μM, respectively), we have shown that these chemotherapeutic drugs induced rapid (<6 h) interphasic apoptosis, and stimulated an early (5–10 min) SM cycle (hydrolysis and resynthesis) and subsequent ceramide generation in both U937 and HL-60 leukemic cells through the stimulation of a neutral SMase [149,150]. It latter became apparent that ceramide metabolites had a direct influence on cell response [56]. However, the role of GCS in these non-MDR cells had largely been overlooked. Surprisingly, we observed that while 1-phenyl-2-decanoylamino-3-morpholino-1-propanol (PDMP) derivatives sensitized the MDR variants of U937 and HL-60 cells to both DNR and Ara-C, these GCS inhibitors completely blocked drug-induced apoptosis in the parental cell lines. Moreover, blocking GCS did not lead to an increase in drug-induced ceramide generation but to an increased GalCer content, which was not observed in the MDR models [70]. To determine the potential role of GalCer in ‘protecting’ these leukemic cell lines, we preincubated the cells with GalCer before drug treatment, and observed significant decrease in apoptosis induction. Moreover, treatment of Krabbe and Gaucher lymphoblasts, which are inherently defective in GalCer and GlcCer lysosomal breakdown, respectively, with both drugs led to an intriguing result. Indeed, compared to normal lymphoblasts, cells originating from Krabbe patients were more resistant to both DNR and Ara-C induced apoptosis, whereas Gaucher cells presented significantly greater sensitivity [70].

The contradictory observations concerning GCS inhibition and cell response to cytotoxic effectors may lie in the complexity of ceramide metabolism, which is likely cell and/or tissue-specific. For example, in drug-sensitive Jurkat cells which overexpress GCS, the ceramide generated by ligation of the death receptor CD95, or etoposide, or ionizing radiation, was not glycosylated [146]. In contrast, de novo synthesized ceramide as well as an exogenously supplied cell-permeable ceramide analogue were efficiently glycosylated. The authors concluded that GCS, located at the Golgi, is topologically segregated from ceramide produced in the plasma membrane [146]. Therefore, the ability of GCS overexpression to protect cells from possible detrimental effects of ceramide accumulation is not a clear-cut observation.

To make matters more complex, ceramide has long been considered a pro-apoptotic mediator, and inhibition of GlcCer synthesis leading to increased ceramide content has been considered to be the mechanism by which PDMP sensitizes MDR cells [151]. However, there is some controversy in the matter (see below). There are also several studies showing that apoptosis induction by ceramide requires its conversion to GD3 (for reviews see [68,152]). Since the first step in this conversion is the glucosylation of ceramide, one would expect that cells enriched in GlcCer would present higher sensitivity to apoptosis. Indeed, Tepper and coworkers did notice a modest sensitization of GCS-overexpressing Jurkat cells to CD95 ligation [146].

A close look at the literature suggests that blocking GlcCer synthesis does not necessarily lead to increased apoptosis [153,154]. Such discrepancies may be explained by the lack of specificity of PDMP compounds towards GCS. Another possible explanation would be differences in the basal levels of GlcCer and GalCer. MDR cells indeed present elevated GalCer as well as GlcCer levels [144,155]. It is therefore tempting to propose that the relative balance (or imbalance) of basal GalCer and GlcCer levels could represent a predisposition for sensitivity or resistance to rapid interphasic SM-derived ceramide-mediated apoptosis.

4. Manipulation of sphingolipid metabolism and sphingolipid analogues as anticancer tools

4.1. Genetic manipulation of sphingolipid metabolism

To clarify the functions of SLs in cancer cell death, a series of studies using genetic or epigenetic approaches either to over-express or knockdown enzymes of SL metabolism have been undertaken. In addition, the sensitivity of cells derived from
mouse or patients affected with inborn disorders of SL metabolism has been evaluated in response to different stress stimuli. However, from the studies summarized in Table 1 that illustrate the importance of SL metabolism in the modulation of cancer cell death, it appears that some conflicting observations have been published, which still make difficult to delineate a clear-cut function for some enzymes in the regulation of cell death. Besides possible bias due to the genetic alteration per se, the impact of manipulation of SL metabolism is likely to be dependent on cell type, stress stimulus and/or type of cell death.

4.2. Sphingolipid analogues

During the last decade, a number of compounds sharing structural homology with simple SLs have been isolated from natural sources or chemically synthesized. These SL analogues have proved to be valuable tools for assessing the functions of SLs in the regulation of cancer cell death.

4.2.1. Ceramide analogues

It has long been known that short-chain ceramides can (at least in part) trigger cell death. More recently, a series of ceramide analogues have been synthesized and shown to be cytotoxic for leukemia or solid tumor cells both under cell culture conditions and in animals (Table 2). Of interest, some of these synthetic derivatives proved to be active also on drug-resistant cancer cells (e.g., [66,156,157]) or hormone-insensitive tumor cells [158], whereas they were less cytotoxic on non-cancer cells (e.g., [157,159]). Recently, systemic delivery of liposomal preparations of C6-ceramide proved to exhibit growth inhibitory effects on breast carcinoma tumors transplanted to mice [160].

4.2.2. Sphingosine analogues

Two derivatives of sphingosine, N,N-dimethylsphingosine and dihydro sphingosine, are potent inducers of cell death in a variety of malignant cell types (see [67] and references therein). Some novel sphingosine analogues have recently been shown to be even more cytotoxic for leukemia cells than the previous derivatives [161].

FTY720 is another synthetic sphingosine derivative, with analogy with myriocin. Several studies have highlighted the FTY720 immunosuppressive properties that rely mainly on the capacity of its phosphorylated form to desensitize S1P receptor 1 on lymphocyte cell surface [162]. This phenomenon is accompanied by the sequestration of lymphocytes into lymph nodes. Recent publications have demonstrated the putative anti-tumor effect of FTY720. In vitro, FTY720 induces cell death of leukemia [163–166], glioma [167], breast cancer [168], prostate cancer [169–171], bladder cancer [172], hepatocellular carcinoma [173,174], and multiple myeloma cells [175]. FTY720 was shown to activate caspase and promote apoptosis in cancer cells [164,169,172,173,175], and to trigger mitochondrial events such as cytochrome c release [175]. FTY720-induced cell death is likely independent of S1P receptor cross-linking because micromolar concentrations are needed for triggering cytotoxicity. Moreover, an analogue of FTY720 that cannot bind S1P receptors is equally capable of inducing cell death within the same concentration range [176]. In vivo, FTY720 inhibits tumor growth without notable side effects in mouse models [168,171–174,177]. This phenomenon is likely a consequence of FTY720-induced cancer cell death as well as an inhibition of angiogenesis and metastasis [171,174], making FTY720 and/or its derivatives promising candidate(s) for anti-cancer therapy.

4.2.3. Glycosphingolipid analogues/inhibitors

As mentioned in Section 3.3, GlcCer appears to be an important ceramide metabolite implicated in MDR. GlcCer was initially proposed to stimulate cell proliferation [178]; also, inhibitors of GCS could present antitumor effects [153,179]. These observations suggested that GCS plays a role in cell survival. Indeed, cells overexpressing GCS were found to be quite resistant towards anthracyclines [180], while transfection of an antisense oligonucleotide directed against GCS sensitized cells [181]. In MDR cells treated by chemotherapeutic agents, ceramide is rapidly converted to GlcCer which makes GCS an attractive target in MDR reversal [182]. A number of studies using GCS inhibitors, which are structural analogues of GlcCer, notably PDMP, 1-phenyl-2-palmitoylamine-3-morpholinol-1-propanol (PPMP), and 1-phenyl-2-hexadecanoylamino-3-pyrrolidino-1-propanol (P4) enhance chemotherapeutic drug efficacy in MDR models [183–189]. Moreover, most agents which reverse MDR (e.g., cyclosporine A, tamoxifen, verapamil) are potent GCS inhibitors, and some others (e.g., PSC833) led to increased ceramide levels through the activation of ceramide synthase [190,191]. Attenuation of GlcCer synthesis (and, consequently, of ganglioside biosynthesis) has also led to significant reduction in tumor or metastasis development in experimental settings using tumor xenografts or injection of cancer cells in animals [19,187,192–194] (see Table 1).

As discussed earlier (Section 2.1), NKT lymphocytes are a T cell subpopulation mediating cytotoxicity against various tumor cells, either directly by using FasL, TNF or TRAIL, or indirectly by enhancing antitumor immunity. NKT lymphocytes are activated via antigen presentation by CD1d that is capable to bind hydrophobic molecules such as glycolipids. Only recently, iGb3 has been identified as a physiological CD1d ligand in mice and humans. In addition, in the past few years, α-galactosylceramide has been shown to be a potent stimulator of NKT cells. This molecule is absent from mammals and was originally purified from Agelas mauritianus. KRN7000 is a chemically synthesized α-galactosylceramide and may be useful for the treatment of cancer, directly by the way of NKT activation and indirectly by stimulating dendritic cell functions and IFNγ/IL-12 production [195]. Several phase I trials in patients with solid tumors have been recently performed, demonstrating good drug tolerance [196].

5. Concluding remarks

In conclusion, we now know that SLs play an essential part in the regulation of cancer cell death and tumor development, and it will most certainly be of great importance to delineate their exact role in cell signaling (survival, death, proliferation). This review
incites for a reinterpretation of the actions of SLs in the light of recent advances in the basic sciences which should allow, for example, for cellular pharmacology of anti-neoplastic agents to continue gathering momentum in the perspective of overcoming drug resistance by defining new strategies capable of sensitizing resistant tumor cells and/or protecting normal physiological cells.

Acknowledgments

The authors’ studies were supported by INSERM, Université Paul Sabatier, Association pour la Recherche sur le Cancer (grant 3417 to BS), Ligue Nationale Contre le Cancer, and Comités Départementaux du Gers, de l’Aveyron, du Lot, de la Haute-Garonne et du Tam-tam-Garonne de la Ligue Contre le Cancer (to JPJ and TL).

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B. Ségui et al. / Biochimica et Biophysica Acta 1758 (2006) 2104–2120


