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Review

Sphingolipid players in the leukemia arena

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Abstract

Sphingolipids function as bioactive mediators of different cellular processes, mostly proliferation, survival, differentiation and apoptosis, besides being structural components of cellular membranes. Involvement of sphingolipid metabolism in cancerogenesis was demonstrated in solid tumors as well as in hematological malignancies. Herein, we describe the main biological and clinical aspects of leukemias and summarize data regarding sphingolipids as mediators of apoptosis triggered in response to anti-leukemic agents and synthetic analogs as inducers of cell death as well. We also report the contribution of molecules that modulate sphingolipid metabolism to development of encouraging strategies for leukemia treatment. Finally we address how deregulation of sphingolipid metabolism is associated to occurrence of therapy resistance both in vitro and in vivo. Sphingolipids can be considered promising therapeutic tools alone or in combination with other compounds, as well as valid targets in the attempt to eradicate leukemia and overcome drug resistance.

Keywords: Leukemia; Sphingolipids; Ceramide; Apoptosis; Chemotherapeutics; Resistance

Contents

Abbreviations: 4-HPR, fenretinide; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; AMMoL, acute myelomonocytic leukemia; APL, acute promyelocytic leukemia; Ara-c, cytosine arabinoside; aSMase, acid sphingomyelinase; ATRA, all–trans retinoic acid; B-CLL, chronic B-cell lymphoid leukemia; Cer, Ceramide; CML, chronic myelogenous leukemia; D609, tricyclodecan-9-yl-xanthogenate; d-e-MAPP, (1S,2R)-d-erythro-2-(N-myristoylamino)-1-phenyl-1-propanol; DMS, *N,N*-Dimethylsphingosine; DNR, daunorubicin; doxo, doxorubicin; DT, diphtheria toxin; EBV, Epstein–Barr Virus; Epo, erythropoietin; GCS, GlucosylCeramideSynthase; GlcCer, GlucosylCeramide; GM-CSF, granulocyte-macrophage colony-stimulating factor; GSH, glutathione; HDACIs, histone deacetylases inhibitors; HSC, hematopoietic stem cells; IM, Imatinib Mesylate; IR, ionizing radiation; LSC, leukemic stem cells; nSMase, neutral sphingomyelinase; Pgp, P-glycoprotein; Ph, Philadelphia Chromosome; PKC, protein kinases C; PPPP, dl-threo-phenyl-2-hexadecanoylamino-3-pyrrolidino-1-propanol; ROS, reactive oxygen species; S1P, sphingosine-1-phosphate; SAHA, suberoylanilide hydroximic acid; SK1, sphingosine kinase 1; SM, sphingomyelin; SMS, sphingomyelin synthase; SPLs, sphingolipids; SPT, serine-palmitoyl transferase; T-ALL, acute T-lymphoblastic leukemia; VCR, vincristine

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1. Introduction

In the last decades, many studies have demonstrated that sphingolipids (SPLs) are biologically active molecules that play pivotal roles in different cellular processes. Ceramide (Cer) triggers differentiation and apoptosis, whereas its metabolite sphingosine-1-phosphate (S1P) exerts an anti-apoptotic effect and enhances cell growth and survival [1,2] (Figs. 1 and 2). The balance between pro-apoptotic and pro-survival stimuli determines cell fate, according to the model of a sphingolipid rheostat [3]. Using in vitro models of solid and hematological tumors, it is possible to establish a link between SPLs and cancer formation and progression [4–8]. The involvement of Cer metabolism in cellular responses to various stimuli, like serum deprivation, cytokines (e.g. TNF-α), ionizing radiation (IR), heat shock and chemotherapeutic agents was reported [9]. This review focuses on the implications of the dual role of SPLs, as pro-apoptotic as well as anti-apoptotic effectors, on the treatment of leukemias. SPLs mediate chemotherapeutics-induced apoptosis but synthetic analogs display the capacity to trigger cell death as well. However, when SPL metabolism is altered, ability of leukemic cells to undergo apoptosis is impaired, leading to enhanced growth and survival and reduced sensitivity to treatment. Strategies that cause accumulation of endogenous Cer either by increasing its production via de novo synthesis and sphingomyelin (SM) hydrolysis, or by decreasing its metabolism are emerging, thus opening new therapeutic perspectives in the treatment of leukemias. Modulation of SPL metabolism through the use of SPL analogs alone or in combination with other agents as well as inhibition of key enzymes represent a promising therapeutic option to effectively block the growth of leukemic cells and redirect them towards apoptosis, contributing also to overcome drug resistance. Thus, SPLs can be considered valid tools and targets at the same time for the treatment of leukemias.

2. The multiple facets of leukemias

Organization of hematopoietic system follows a hierarchical pattern: from the pool of pluripotent stem cells (hematopoietic stem cells, HSC), characterized by self-renewal and multi-lineage differentiation potential, committed lymphoid and myeloid progenitors derive. The hallmark of stem cells is self-renewal, that is the ability to generate daughter cells with the same stem cell characteristics. Through this capacity, the continuous repopulation of the hematopoietic system is ensured. Under normal physiological conditions, HSC divide by asymmetric duplication, producing an identical stem cell and

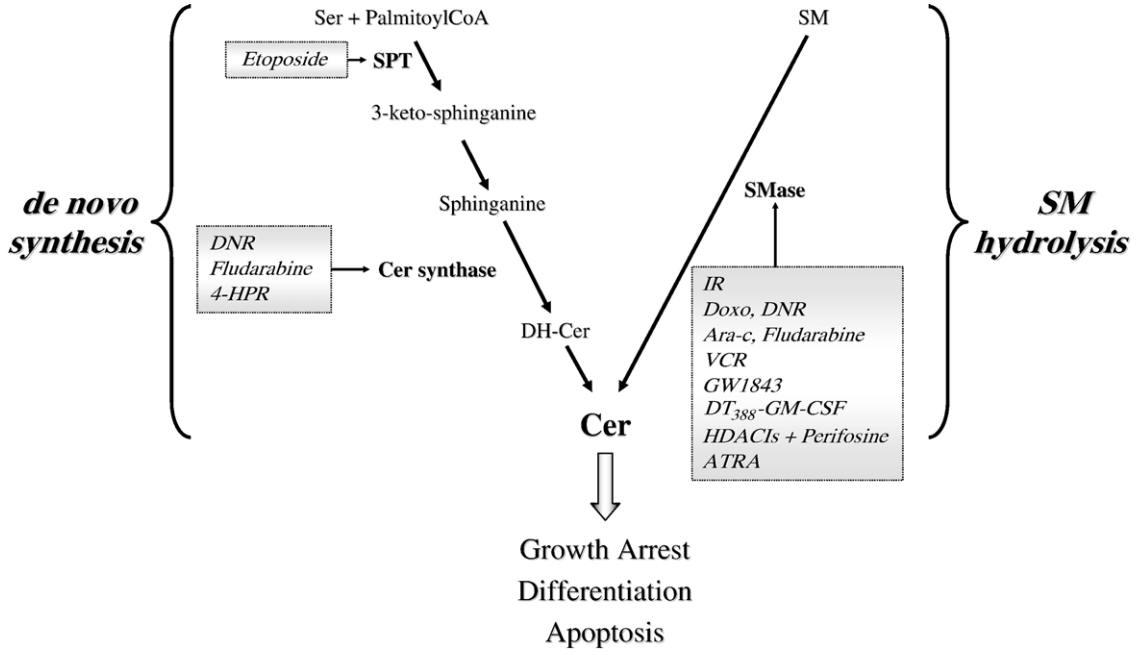

Fig. 1. Anti-leukemic agents that cause Cer increase by targeting different enzymes of SPL metabolism. Cer production occurs through de novo synthesis or SM hydrolysis. Different compounds have been found to augment endogenous Cer accumulation by activating SPT, Cer synthase and nSMase and/or aSMase, thus triggering apoptotic cell death.

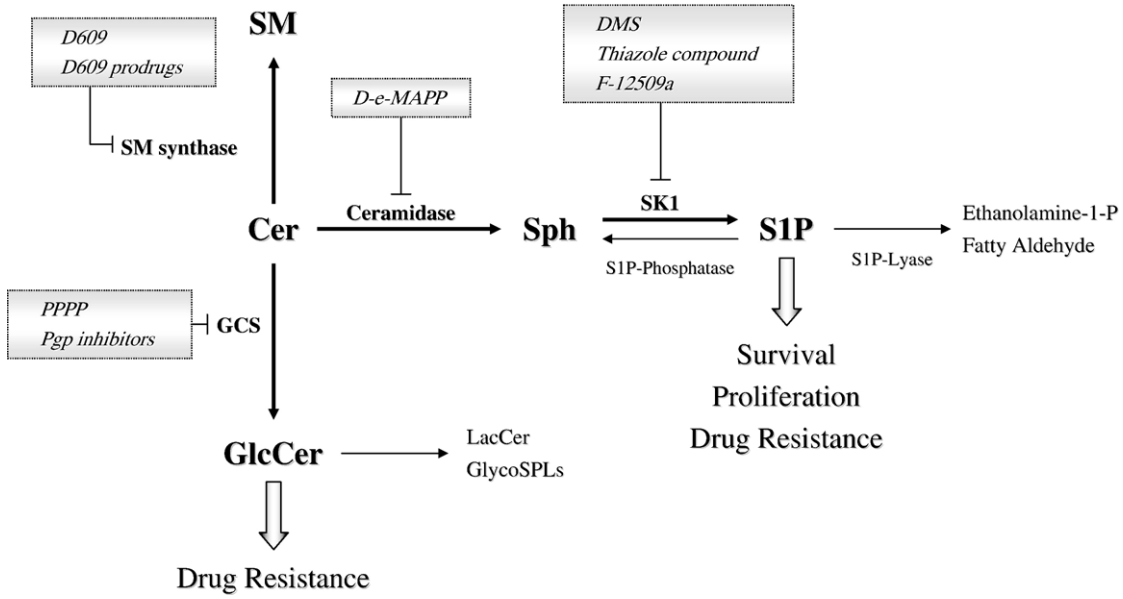

Fig. 2. Inhibition of crucial enzymes controlling different steps of SPL metabolism leads to endogenous Cer accumulation and consequent cell death of leukemia cells.

a committed progenitor that will further undergo differentiation and proliferation. However, in particular situations (e.g. bone marrow transplantation), HSC may divide symmetrically, generating preferentially daughter stem cells, in order to restore and maintain the pool [10]. Therefore, hematopoiesis depends on the balance between self-renewal and differentiation.

Acquired genetic aberrations altering mechanisms controlling self-renewal, cell survival and differentiation can lead to abnormal expansion and defective maturation of hematopoietic progenitors that distinguish leukemias [11,12].

Leukemias are a heterogeneous group of hematological malignancies that are classified as acute or chronic based upon the natural history of the disease. Furthermore, according to the cell types primarily involved, leukemias are classified as lymphoid or myeloid. In acute leukemias block of differentiation occurs at very early stages (stem cell level) and disease rapidly progresses, resulting in death of patients not effectively treated within a short period of time. In the chronic varieties of leukemias, aberrant cell maturation occurs with consequent production of partially functional elements, which over time become numerically predominant on normal differentiated ones. Chronic leukemias are generally characterized by a slower clinical course and a more prolonged natural history.

Recently, the World Health Organization proposed a new classification of myeloid and lymphoid neoplasms based on revised criteria previously established by other systems [13–18].

As far as pathogenesis is concerned, many subtypes of acute leukemias are characterized by genetic mutations that activate signal transduction pathways, conferring a proliferative and/or survival advantage to hematopoietic progenitors, as well as by mutations that alter transcriptional pathways involved in differentiation of hematopoietic progenitors. These latter pathways are critical for generation of leukemic stem cells (LSC) that are ultimately responsible for the maintenance of the malignant phenotype [19].

In the last decade, increasing evidences supported the model of myeloid leukemia as a stem cell disease (reviewed in [20–26]). According to this model, leukemia is composed by a heterogeneous cell population made of a small fraction of LSC and a bulk of blasts originating from partial differentiation of precursors and biologically distinct from LSC, as shown by the inability of blasts to self-renew and by the presence of quiescent cells in the LSC population [20,27,28]. The exact origin of LSC is still unknown.

The longer lifespan of stem cells increases the probability to acquire further mutations necessary for leukemic transformation. Lesions enhancing self-renewal over differentiation but also proliferation and survival over apoptosis may lead to formation of LSC. Among molecules involved in the control of these cellular functions are the Notch and Hox families and Bmi-1 for self-renewal; transcription factors like CBF and RARα for differentiation; receptor tyrosine kinases like Flt-3 and c-kit and the Ras pathway for proliferation; NF-κB, the PI3K pathways and Bcl-2 for cell survival [21–24,26].

3. Present and future in the treatment of leukemias

In most patients with acute leukemia, intensive induction chemotherapy is required to achieve hematological remission generally with combination of cytotoxic drugs (e.g. Ara-C and anthracyclines). When successful, the induction phase is followed by consolidation and maintenance regimens of variable duration. Under some circumstances, treatment strategies may include autologous or allogeneic transplantation of HSC. In chronic leukemias abnormal cellular proliferation is usually antagonized with chemotherapy. In acute as well as in chronic leukemias the normal hematopoietic compartment might be stimulated by the administration of growth factors

like the granulocyte-colony stimulating factor and erythropoietin (Epo). In some cases, when known pathogenetic molecular abnormalities are involved, treatment may include targeted drugs, i.e. agents that have been developed to specifically inhibit the pathogenetic event at molecular level, with the endpoint to achieve either differentiation or apoptosis of leukemic cells.

Best examples of targeted therapeutics currently available are all-trans retinoic acid (ATRA) for treatment of acute promyelocytic leukemia (APL) and Imatinib Mesylate (IM) for treatment of chronic myelogenous leukemia (CML). APL is characterized by the chromosomal translocation t(15;17)(q22;q12) associated with the formation of the PML–RARα fusion gene. Through the aberrant recruitment of the nuclear co-repressor complex, the fusion protein leads to the block of myeloid cell differentiation at the promyelocytic stage. ATRA, a ligand for RARα, has proved to be an effective therapy for APL in relation to its ability to bind to the fusion protein, causing dissociation of the nuclear co-repressor complex. Promyelocytes are then able to undergo normal differentiation finally resulting in apoptotic cell death [19].

CML represents the form of clonal myeloproliferative disease which is best characterized at the molecular level. The natural history of the disease is characterized by a chronic phase of variable duration (3 to 5 years) followed by progression into an acute blastic phase, generally passing through a brief accelerated phase. Eighty-five percent of patients are diagnosed in the chronic phase. This disorder is associated with the acquired karyotypic aberration known as Philadelphia Chromosome (Ph), which originates from the reciprocal translocation t(9;22)(q34;q11). The result is generation of the BCR/ABL fusion gene that encodes for a chimeric protein with deregulated tyrosine kinase activity, which leads to increased proliferation and survival of transformed cells [29]. IM is a small molecule that competitively inhibits the interaction of ATP with Bcr/Abl tyrosine kinase, blocking its ability to phosphorylate and activate downstream pathways. The use of IM dramatically increased the rate of remission of patients in chronic phase (up to 97%) and demonstrated efficacy also in accelerated and blastic phases [30–32].

Another class of targeted therapeutic agents is represented by the small molecules acting as inhibitors of the Flt-3 tyrosine kinase receptor. Mutations of FLT-3 either as internal tandem duplications within the juxtamembrane domain of the receptor or as point mutations within the activation loop of the kinase domain, represent the most frequent known genetic abnormality in acute myeloid leukemia (AML) blast cells (30–35% of patients). Both types of mutation result in constitutive receptor activation and consequent proliferative advantage for leukemic cells [33,34]. Pharmacologic inhibition of Flt-3 blocks its tyrosine kinase activity and results in a significant antileukemic effect. In recent clinical trials, this approach has shown evidence of activity in refractory or relapsed AML patients [35–37].

It has been clearly documented that impaired cell capacity to undergo apoptosis represents one of the central mechanisms involved in leukemogenesis, due to alterations of crucial components of the apoptotic cascade like the Bcl-2 family members [38,39]. Thus, targeting specific molecules involved in these alterations represents an alternative approach to restore apoptotic pathways and redirect cells towards physiologic death. For example small antagonists and antisense oligonucleotides against Bcl-2, FLIP and XIAP, respectively, part of the mitochondrial, death receptor and common pathways, proved effective in a variety of hematological malignancies [40].

Differentiation rather than malignant cell toxicity is the goal of therapeutic epigenetics, that targets DNA methylation and histone modifications to restore expression and function of genes abnormally silenced (e.g. $p15^{INK4B}$). Demethylating agents like 5-aza-2′-deoxycytidine and decitabine and histone deacetylases inhibitors (HDACIs) like valproic acid proved effective in treatment of hematological malignancies in recent clinical trials [41].

Finally, emerging data indicate SPLs as a new class of molecules involved in the apoptotic cascade in different in vitro models, providing favorable targets for anti-cancer therapy.

4. To tackle drug resistance

The occurrence of multi-drug resistance, namely resistance to multiple, structurally unrelated chemotherapeutic agents remains a major issue in the treatment of leukemias. Different mechanisms are implicated in this phenomenon, which may be developed by cancer cells to escape the selective drug pressure. Among these, expression of transmembrane drug-efflux pumps encoded by the ATP-Binding Cassette transporter superfamily like the P-glycoprotein (Pgp), has been investigated in leukemia [42]. Moreover, attempts to overcome drug resistance and promote apoptosis of leukemic cells unvealed an association between Pgp and SPLs, whose altered metabolism has been shown to contribute to resistant phenotype of leukemic cells.

Drug resistance represents an important issue also with the more recently developed targeted therapies. A good example is resistance to IM, which has been shown to be likely multifactorial (reviewed in [43–45]): in the majority of relapsed patients, gene point mutations are detected (predominantly in the kinase domain) and interfere with the binding of IM to Bcr/Abl. However, gene amplification and protein overexpression, activation of alternative pathways, extracellular sequestration of the drug by α1 acid glycoprotein in the plasma and expression of drug efflux-pumps were also reported to contribute to resistant phenotype.

Another element that may contribute to treatment failure and relapse of disease is the biology of LSC. In myeloid leukemias LSC are emerging as the crucial target to eradicate leukemia, therefore elucidation of the mechanisms involved in malignant transformation into leukemic stem cells may have therapeutic consequences. For example, LSC have been shown to be resistant to the anthracycline daunorubicin (DNR) [46] and to IM, the latter exerting a cytostatic rather than cytotoxic effect on this cell population [47]. In addition, since unlimited self-renewal is a prerequisite for tumor growth, inhibition of this property may have marked effects on cancer progression, and could lead to blockade of LSC besides the blast populations,

thus increasing the incidence of remission and reducing that of relapse.

Importantly, identification of molecules differentially expressed between HSC and LSC would contribute to design therapeutic regimens that specifically target cancer cells while sparing normal HSC [26,33,48].

Clearly, development of alternative strategies implying the use of combination therapies for leukemia treatment are warranted and a better understanding of the biology of LSC would provide a useful tool for this purpose.

5. Cell lines are a model to investigate the role of sphingolipids in leukemogenesis

Metabolism of SPLs and their role in cellular growth, differentiation, apoptosis and resistance to therapy were largely investigated by means of in vitro models of myeloid and lymphoid leukemias (cell lines are summarized in Table 1). Leukemic cell lines are obtained by selection of the most undifferentiated tumor cells through in vitro culture of bone marrow or peripheral blood (in the case of acute leukemias with high percentages of circulating blasts) samples. However, while these models well represent the undifferentiated blast population typical of acute leukemias, in the context of chronic leukemias they represent only the tumor cell population arisen after progression into acute phase. In fact chronic leukemias are characterized by the presence of cells that still retain the ability to partially differentiate and undergo cell death (since the pathways involved in these processes are not fully compromised and impaired yet) and thus they would last shortly when cultured in vitro.

Table 1
List of cell lines used to study the role of SPLs in leukemias

Cell lines	Morphological and molecular characteristics	References
KG1a	AML Pgp^+, TNF-α-resistant	[115,116,121]
HL-60	APL PML-$RAR\alpha^-$, c-myc amplicons	[2,52,54,62,63,65,66,69,70,72,73,75,76,80,82,85,87–89,92,98,99,103,107,121,122]
JVM-2	B-CLL (transformed with EBV) t(11;14)(q13;q32) associated with Cyclin D1 activation	[58]
TF-1	Erythroleukemia (AML FAB M6) Pgp^+	[51,117,121]
U937	Histiocytic lymphoma, expressing markers and properties of monocytes (AML FAB M5; hystomonocytoid morphology) Pgp^-, TNF-α-sensitive	[2,53,54,56,57,65–67,70,72,73,76,79,81,82,85,87,90,97,106,111,112,115,121]
JFP1	Ph^+ CML in blast crisis derived from a patient refractory to different combination of chemotherapy regimens	[107]
K562	Ph^+ CML in blast crisis	[107]
ALL-697	pre-B ALL	[59]
CCRF-CEM	T-ALL	[49,60,84,91]
HPB-ALL	T-ALL	[87]
Jurkat	T-ALL	[2,49,55,66,71,81,105,112,120]
Molt-4	T-ALL; p53 is not expressed	[50,59,61,64,74,83,94,96,123]
HS1	Non-tumorigenic pro-erythroblasts from Sp-1-transgenic mice arrested in differentiation, Epo-dependent growth and survival	[104]
HS2	Tumorigenic pro-erythroblasts from Sp-1-transgenic mice malignant, Epo-independent growth and survival	[104]
NB4	APL PML-$RAR\alpha^+$	[68]

6. Sphingolipids are mediators of apoptosis induced by chemotherapeutics

An increasing body of evidence supports the hypothesis that exposure of leukemic cells to IR and many chemotherapeutics results in the onset of apoptosis via alterations of SPL metabolism (Fig. 1). In this regard, accumulation of Cer produced by SM hydrolysis, as well as de novo synthesis, was shown to play a major role as mediator of leukemic cell death. Apoptosis triggered in response to IR was associated to increase of endogenous Cer in lymphoid cell lines [49,50] and in erythromyeloblastic cells, these last undergoing apoptosis only if the SM/Cer pathway was intact, and this was associated to activation of neutral sphingomyelinase (nSMase) [51].

Induction chemotherapy may include anthracyclines like doxorubicin (doxo) and DNR and nucleoside analogs like cytosine-arabinoside (Ara-c) and fludarabine. Anthracyclines are capable to induce apoptosis not only by intercalating to DNA and targeting topoisomerase II, but also by stimulating Cer production. Doxo-induced apoptosis was shown to be mediated by Cer increase in acute T-lymphoblastic leukemia (T-ALL), where Cer activated the CD95 (APO-1/Fas) death pathway [49] and in HL-60 cells via inhibition of sphingosine kinase 1 (SK1) [52]. Cytotoxic effect of DNR was mediated by Cer accumulation in myeloid leukemia cell lines through activation of Cer synthase and nSMase [53,54] but was independent of Cer accumulation in Jurkat E6.1 cells [55]. In addition, Ara-c and fludarabine were reported to exert their pro-apoptotic effect through increased level of Cer, respectively, in myeloid cells via nSMase activation [54,56,57] and in lymphoid leukemia cell lines through SM hydrolysis as well as de novo Cer synthesis [58].

Another chemotherapeutic agent that was shown to induce Cer-mediated apoptosis of lymphoblastic cells is the vinca alcaloid vincristine (VCR) [59,60], that exerts cytotoxicity by interfering with tubulin, thus blocking mitosis.

Cer generation was involved also in apoptosis triggered by etoposide, another topoisomerase II inhibitor, through activation of serine-palmitoyl transferase (SPT) in Molt-4 cells [61] and via SK1 inhibition in HL-60 cells [52]. However, in HL-60 cells the early increase of endogenous Cer associated to apoptosis resulting from exposure to the synthetic retinoid fenretinide (4-HPR) was due to stimulation of de novo synthesis [62,63].

Activation of both acid and neutral SMase that led to Cer accumulation in a caspase-independent fashion, was instead

implicated in apoptosis induced in Molt-4 cells by the folate analog thymidylate synthase inhibitor GW1843 [64].

A strategy to effectively target the leukemic blast population in AML, which express the granulocyte-macrophage colony-stimulating factor (GM-CSF) receptor, is designed on the use of the fusion toxin DT_{388}-GM-CSF, constructed by conjugating the catalytic and translocation domains of diphtheria toxin (DT) with the ligand of GM-CSF receptor. When tested on different myeloid leukemia cell lines, DT_{388}-GM-CSF triggered apoptosis associated to strong increase of Cer level via SM hydrolysis [65].

HDACIs have been recently shown to induce apoptosis by increasing Cer production. Growth inhibition and apoptosis occurred in leukemic cells following exposure to combination of different HDACIs (sodium butyrate, suberoylanilide hydroximic acid SAHA or trichostatin) and perifosine, in a synergistic manner. Molecular mechanism involved inactivation of ERK1/2 and Akt that caused activation of aSMase and consequent Cer increase [66]. Moreover, the novel HDACI LAQ-824 induced either G_0/G_1 arrest and differentiation or G_2/M arrest and apoptosis in U937 cells according to the dose, leading to Cer generation through SM hydrolysis [67].

Finally, ATRA was shown to induce myeloid differentiation of NB4 cells (PML-RARα^+) via up-regulation of aSMase and subsequent increase of endogenous Cer [68].

Altogether, the studies reviewed here demonstrate a crucial role for Cer metabolism in mediating anti-leukemic effects of many agents. Importantly, the observation that stimuli including ionizing radiations and bioactive compounds, having different molecular targets, can all act by modulating the SPLs metabolism, highlights the high versatility of SPLs pathway. Moreover, it suggests the use of combination regimens to enhance the increase of endogenous Cer level with the endpoint of triggering apoptotic cascade in leukemic cells.

The discrepancies observed may be due at least in part to different in vitro models and experimental conditions used.

7. Sphingolipid analogs are pro-apoptotic agents in leukemias

Commonly, to investigate the role of SPLs in cellular processes and particularly Cer-mediated apoptotic cell death, analogs of SPLs have been used. Many of these molecules have been reported to exert anti-tumor activity in different leukemic cell lines, confirming that they behave like endogenous SPLs and suggesting their use as pro-apoptotic therapeutics in leukemia treatment. Although some compounds have a natural origin (e.g. bacterial SPLs extracted from the Sphingobacterium genus, capable of triggering apoptosis in HL-60 cells [69]), most are chemically synthesized. The reason is that mammalian SPLs are difficult to dissolve in water and are poorly permeable in cell membrane. Thus, short-chain, cell-permeable analogs have been employed (e.g. C_2-, C_6-, C_8-Cer) and correlation between structure and biological activity of Cers has been demonstrated [70].

Treatment of leukemia cells with short chain Cer analogs resulted in growth arrest, differentiation, apoptosis induction, restored sensitivity to IR [51] and chemotherapeutics and potentiated the activity of anti-leukemic agents (e.g. paclitaxel [71]). Synthetic C_2-Cer, chiral C_2-Cer, C_6-Cer and C_8-Cer were shown to induce cell cycle arrest in G_0/G_1 phase, DNA breakage and cell death in myeloid [70,72,73] and lymphoid [74] leukemia cell lines. Moreover, sphingoid bases like trans-4-sphinganine (Sph) and 4,5-dihydrosphingosine (Sphinganine) induced PMA-driven differentiation of HL-60 into macrophages and apoptosis in undifferentiated HL-60 and U937 cells [75,76].

Moreover, the use of analogs helped to define involvement of different molecules in SPLs-mediated signaling. Reactive oxygen species (ROS) production (e.g. superoxide anion radical H_2O_2) takes place at the mitochondrial electron transport chain and is part of the normal cellular metabolism but may also be induced by various stimuli. Data are available about the involvement of ROS in Cer-induced cell death in leukemia [77,78]. For example, in U937 cells exposure to C_6-Cer triggered apoptosis and DNA fragmentation mediated by significant H_2O_2 production which, in turn, activated the transcription factors NF-κB and AP-1 [79]. In HL-60 cells C_2-Cer-mediated apoptosis led to increased oxidative damage by caspase-3 dependent inactivation of the ROS-scavenger catalase, as shown by in vitro proteolytic cleavage of the enzyme [80]. Moreover, C_2- and C_6-Cer triggered different responses in U937 and Jurkat cells, respectively growth arrest accompanied by transient loss of glutathione (GSH) and apoptosis associated with complete depletion of the ROS-scavenger GSH. Since total cellular GSH concentration in U937 was 3-fold higher than in Jurkat cells and inhibition of mitochondrial respiration restored apoptosis in U937, authors suggested that the balance between mitochondrial peroxide production and the intrinsic anti-oxidant capacity of cells determine the response to Cer [81].

The Bcl-2 family comprises proteins with both pro- and anti-apoptotic activity. In particular, involvement of Bcl-2 in Cer-mediated cell death in leukemic models has been investigated. In this context, down regulation of Bcl-2 was observed in association to C_2 Cer-induced apoptosis in myeloid leukemia cells [82], while in ALL-697 Bcl-2 acted downstream of Cer but did not affect Rb dephosphorylation caused by Cer-induced cell cycle arrest in Molt-4 cells, suggesting that these Cer-effectors function independently [59]. Experiments on lymphoblastic leukemia showed that Bcl-2 over expression antagonized apoptosis induced by Cer analogs: however, in Molt-4 cells Bcl-2 prevented prICE activation and thus PARP cleavage [83], while in CCRF-CEM the anti-apoptotic protein had no effect on CrmA-inhibitable proteases like ICE or ICE-related proteases [84]. In contrast, cell-permeant Cers were able to induce apoptosis in different myeloid leukemia cells through nSMase activation, SM hydrolysis and endogenous Cer generation independently of Bcl-2 [85].

Protein kinases C (PKC) are a family of serine/threonine kinases involved in different cellular processes [86]. There are different isoenzymes classified according to the stimuli that can activate them, like Ca^{2+}, diacylglycerol and phorbol esters. However, the precise role of PKC in the apoptotic cascade is controversial and is probably due to the presence of different combination of isoforms in different cell systems. A role for these kinases in SPL-mediated apoptosis emerged. Following

exposure to sphingoid bases, an apoptotic response was observed in myeloid leukemia cells, enhanced by inhibition of total PKC activity [76]. Sawai H. and coworkers demonstrated that Cer-induced cell death in HL-60, U937 and HPB-ALL is associated with translocation of PKC-δ and -ε from the membrane to the cytosol, whose inhibition had a cytoprotective effect [87], with overexpression of c-jun through PKC activation in HL-60 [88] and with activation of the transcription factor AP-1, as shown by prevention of growth inhibition and DNA fragmentation in HL-60 after inhibition of AP-1 and c-jun [89].

Activation of the transcription factors AP-1 and NF-κB was induced also by ROS generation in U937 cells following treatment with C_6-Cer [79]. In the same cell line, Cer analogs induced apoptosis through strong stimulation of p46-JNK1/p54-JNK2 activity, increased c-Jun mRNA and protein expression and weak inhibition of p42-ERK1/p44-ERK2 activity. In contrast, Sph analogs led to apoptosis via moderate stimulation of p46-JNK1/p54-JNK2 activity and no change in c-Jun expression and immediate and complete inhibition of p42-ERK1/p44-ERK2 activity [90].

Finally, in some cases SPLs were chemically modified and the biological effects of new compounds were tested on leukemia cells. Growth inhibition or apoptosis was induced in leukemia cells by several synthetic SPL analogs including: (i) conformationally restrained analogs of Cer where the polar portion of the molecule was replaced by a thiouracil uracyl ring [91], (ii) non-natural stereoisomers of C_2-Cer [92], (iii) cis- and acetylene type derivatives of Cer [93], (thus suggesting that the trans configuration is not a prerequisite for Cer-induced apoptosis), (iv) analogs having a para-substituted phenyl ring in the sphingoid moiety or an allylic fluoride [94], (v) N-acylphenylaminoalcohol Cer analogs [95], (vi) N-acetylthio-sphingosine and 4-dodecanoylamino-decan-5-ol [96], (vii) C_8-ceramine, a derivative where the Cer carbonyl group is replaced by a methylene group [97], (viii) N-lactylsphingosine and N-lactyldihydrosphingosine [98], (ix) aminophenyl Cer analogs [99], (x) phenetylisothiocyanate derivatives of sphingosine and sphinganine [100].

All the efforts in the design of novel Cer analogs with potent apoptotic properties helped in clarifying the function–structure relationship of the natural Cer effects, but additionally led to the discovery of innovative drugs with potential antileukemic efficacy.

8. Sphingolipids are targets for novel therapy of leukemias

Intracellular levels of Cer decrease following activation of specific, compartmentalized enzymes that convert it into different metabolites (Fig. 2). Discovery and availability of inhibitors for enzymes of SPL metabolism helped to clarify their role in cellular processes and to develop alternative therapeutic strategies to augment Cer-mediated apoptosis in cancer cells [5,7,101,102].

Data regarding the effect of ceramidases inhibition in leukemic cells are poor. The Cer analog (1S,2R)-d-erythro-2-(N-myristoylamino)-1-phenyl-1-propanol (d–e-MAPP) but not its enantiomer l-e-MAPP (that is metabolized inside cells) inhibited alkaline ceramidase, inducing G_0/G_1 growth arrest of HL-60 cells and increasing Cer level [103].

One of the most studied enzyme of SPL metabolism in leukemic models is SK1, which has been implicated in leukemia cells transformation, growth and survival [104,105]. The most used SK1 inhibitor is *N,N*-Dimethylsphingosine (DMS), whose specificity was reported to be restricted to SK1 in a model of myeloid leukemia [106]. Anti-leukemic action of DMS was demonstrated on cell lines as well as blasts from patients with acute and chronic myeloid leukemia in blast crisis [75,107]. Intriguingly, the cytotoxic effect of DMS was more evident in drug-resistant than in drug-sensitive cell lines, suggesting a new therapeutic option to overcome drug-resistance [108]. DMS also enhanced the pro-apoptotic effect of TNF-α and Fas monoclonal antibody in lymphoid and myeloid leukemia suggesting its use in combination with other agents [2]. Recently, other non-competitive inhibitors of SK1 were developed, that showed in vitro and in vivo anti-cancer activity against a panel of solid tumors [108]. Preliminary data from our lab (Ricci C., et al, unpublished data) showed that one of these compounds [2-(p-Hydroxyanilino)-4-(p-chlorophenyl) thiazole] exerts a time- and dose-dependent cytotoxic effect on different myeloid leukemia cell lines. Moreover, inhibition of SK1 by F-12509a (a sesquiterpene quinone isolated from a culture broth of a discomycete [109,110] led to apoptosis of both parental and chemoresistant sublines of HL-60 [52]. In particular, in drug-resistant cells S1P level decreased while Cer level increased, and this effect was reverted by addition of S1P. Thus, inhibition of SK1 associated to alterations of the balance between Cer and S1P, namely Cer increase and S1P decrease, may overcome drug resistance in AML.

Inhibitors were also developed for GlucosylCeramide Synthase (GCS), whose association to drug resistance has been largely documented (see below).

Another possible strategy to restore high intracellular level of Cer is based on inhibition of Sphingomyelin Synthase (SMS). In particular, tricyclodecan-9-yl-xanthogenate (D609), a tumor cytotoxic xanthate, was shown to induce apoptosis in U937 through dose-dependent inhibition of SM synthase and subsequent increase of intracellular Cer level and decrease of SM content [111]. However, despite anti-cancer activity in vitro, D609 displayed poor in vivo efficacy, probably due to oxidative instability. Thus S-(alkoxyacyl) D609 prodrugs were generated: in particular, the prodrug methyleneoxybutyryl D609 exhibited higher chemical stability and higher cytotoxicity versus parental D609 in U937 and Jurkat cells, where a significant increase of different species of Cer was observed [112].

Overall, inhibition of key enzymes of SPL metabolism leading to accumulation of intracellular Cer, may be used as an alternative strategy to induce cell death of leukemia cells, with positive consequences on treatment design.

9. Sphingolipids are involved in resistance of leukemia cells to therapy

Deficiency in the SM/Cer pathway, disruption of lipid composition of cellular membranes by Pgp and increased

activity of key enzymes of SPLs metabolism, in particular GCS, SMS and SK1, proved evidence for new mechanisms of multi-drug resistance in leukemia (Fig. 2).

Van Helvoort and coworkers proposed that Pgp, besides its drug-efflux pump activity, may function as a lipid translocase of broad specificity, by translocating phospholipids across cell membranes [113,114]. Moreover, it was suggested that Pgp may alter membrane composition of lipids like SM, thus interfering with the apoptotic cascade induced by stimuli that activate SMase to produce Cer. In most cells, SM is concentrated in the outer leaflet of plasma membrane and following exposure to stimuli like TNF-α, it is hydrolyzed within the inner leaflet by activation of SMase. In this regard, it was demonstrated that the Pgp^+ cell line KG1a, that is inherently resistant to TNF-α because does not produce Cer via SM hydrolysis in response to the cytokine, carried a pool of SM in the inner leaflet of the membrane that was 7-fold lower in size compared to the Pgp^-, TNF-α sensitive U937 cell line [115]. Exposure of KG1a cells to the Pgp inhibitor PSC833 restored apoptosis through increase of Cer by activation of nSMase. PSC833 alone led to increase of membrane-bound nSMase activity and of SM content on the inner leaflet of plasma membrane, without affecting total SM content. Thus, by modifying SM distribution across plasma membrane, inhibition of Pgp rendered the substrate SM available for TNF-α-activated nSMase. This converted SM into Cer and triggered apoptotic cascade [116]. The existence of a drug-efflux pump-independent activity of Pgp was confirmed by the observation that inhibition of Pgp augmented cell death induced by growth factors and serum deprivation in Pgp^+ as well as in Pgp^- AML samples. Moreover, inhibition of Pgp led to SM accumulation in association to apoptotic cell death, indicating that Pgp alters SM distribution by decreasing the SM pool available for Cer generation [117]. In addition, a functional link between Pgp and GCS has been demonstrated, suggesting at least the partial involvement of Pgp in resistance of AML cells to drugs. Pgp, that localizes also on the Golgi membrane where GCS is located, can transport GlucosylCeramide (GlcCer) into the luminal side of the Golgi, thus increasing GCS activity [118–121].

It is now evident that modulation of the activity of different enzymes of Cer metabolism may revert resistance of leukemic cells to chemotherapeutic agents. For example, co-exposure of lymphoblastic cells to VCR and to the GCS inhibitor dl-threo-phenyl-2-hexadecanoylamino-3-pyrrolidino-1-propanol (PPPP) resulted in cell cycle arrest, cytotoxicity and higher Cer levels than upon VCR alone. In addition, chemosensitizing activity of PPPP was more pronounced in VCR-resistant sublines: taken together these results suggested that inhibition of GCS may be used to overcome drug-resistance [60]. Lower basal levels of Cer due to activation of GCS and SMS were found in a drug-resistant subline of HL-60 compared to parental cells, the first being resistant to doxo-induced apoptosis until exposed to C_2-Cer. Moreover, in samples from chemoresistant and chemo-sensitive AML patients, lower Cer levels and higher activity of GCS and SMS were detected, indicating the role of deregulated GCS and SMS activity in development of chemoresistance in vitro as well as in vivo [122]. Finally, overexpression of SK1 has been recently implicated in multi-drug resistance of myeloid leukemia cells to anti-neoplastic agents. Inhibition of the kinase caused apoptosis of both chemosensitive and chemoresistant cells, increasing Cer level concomitantly to S1P decrease [52].

10. Perspectives

Involvement of SPL metabolism in the processes of leukemogenesis and disease progression emerged. Therefore, by exploiting SPL pro-apoptotic effect as well as by blocking their anti-apoptotic, pro-survival capacities, it would be possible to contribute to leukemia eradication and overcome resistance to treatment, which still represents a major hurdle in the field of hematological malignancies. SPLs represent powerful tools to induce cell death of leukemia cells, suggesting the employment of SPL analogs as single agents or in combination regimens to potentiate the anti-leukemic action of chemotherapeutic drugs that increase Cer level. In addition, by targeting specific enzymes crucial for SPL metabolism, it would be possible to kill neoplastic cells as well as sensitizing them to anti-leukemic therapeutics. A new function of the Pgp protein as a lipid translocase has been identified and links it to deregulation of SM distribution across the plasma membrane, GlcCer transport inside the Golgi apparatus and modulation of GCS activity, which contribute to resistant phenotype. Moreover, increased activity of GCS, SMS and SK1 leads to resistance of leukemic cells to drug-induced apoptosis, by impairing the level of Cer and its metabolites. In the present review we have outlined the potential role of SPL biology in leukemia. The extensive number of publications testifies the interest in this exciting and innovative field. Hopefully, the design of novel SPL-based drugs, as well as of chemotherapeutic agents that specifically target SPL metabolism by inducing Cer accumulation, inhibiting its clearance and S1P generation, may help worldwide researchers to overcome some of the hurdles, still present in the therapy of most resistant leukemias.

References

[1] O. Cuvillier, G. Pirianov, B. Kleuser, P.G. Vanek, O.A. Coso, S. Gutkind, S. Spiegel, Suppression of ceramide-mediated programmed cell death by sphingosine-1-phosphate, Nature 381 (1996) 800–803.
[2] O. Cuvillier, T. Levade, Sphingosine 1-phosphate antagonizes apoptosis of human leukemia cells by inhibiting release of cytochrome *c* and Smac/DIABLO from mitochondria, Blood 98 (2001) 2828–2836.
[3] S. Spiegel, Sphingosine 1-phosphate: a prototype of a new class of second messengers, J. Leukoc. Biol. 65 (1999) 341–344.
[4] N.S. Radin, Killing cancer cells by poly-drug elevation of ceramide levels: a hypothesis whose time has come? Eur. J. Biochem. 268 (2001) 193–204.
[5] S. Spiegel, R. Kolesnick, Sphingosine 1-phosphate as a therapeutic agent, Leukemia 16 (2002) 1596–1602.
[6] N.S. Radin, Killing tumours by ceramide-induced apoptosis: a critique of available drugs, Biochem. J. 371 (2003) 243–256.
[7] B. Ogretmen, Y.A. Hannun, Biologically active sphingolipids in cancer pathogenesis and treatment, Nat. Rev., Cancer 4 (2004) 604–616.
[8] H. Sawai, N. Domae, T. Okazaki, Current status and perspectives in ceramide-targeting molecular medicine, Curr. Pharm. Des. 11 (2005) 2479–2487.

[9] T. Okazaki, T. Kondo, T. Kitano, M. Tashima, Diversity and complexity of ceramide signalling in apoptosis, Cell Signalling 10 (1998) 685–692.
[10] A.D. Ho, Kinetics and symmetry of divisions of hematopoietic stem cells, Exp. Hematol. 33 (2005) 1–8.
[11] T. Reya, S.J. Morrison, M.F. Clarke, I.L. Weissman, Stem cells, cancer, and cancer stem cells, Nature 414 (2001) 105–111.
[12] E. Passegue, C.H. Jamieson, L.E. Ailles, I.L. Weissman, Normal and leukemic hematopoiesis: are leukemias a stem cell disorder or a reacquisition of stem cell characteristics? Proc. Natl. Acad. Sci. U. S. A. 1 (2003) 11842–11849.
[13] N.L. Harris, E.S. Jaffe, H. Stein, P.M. Banks, J.K. Chan, M.L. Cleary, G. Delsol, C. De Wolf-Peeters, B. Falini, K.C. Gatter, et al., A revised European-American classification of lymphoid neoplasms: a proposal from the International Lymphoma Study Group, Blood 84 (1994) 1361–1392.
[14] J.M. Bennett, D. Catovsky, M.T. Daniel, G. Flandrin, D.A. Galton, H.R. Gralnick, C. Sultan, Proposals for the classification of the acute leukaemias. French–American–British (FAB) co-operative group, Br. J. Haematol. 33 (1976) 451–458.
[15] J.M. Bennett, D. Catovsky, M.T. Daniel, G. Flandrin, D.A. Galton, H.R. Gralnick, C. Sultan, Proposals for the classification of the myelodysplastic syndromes, Br. J. Haematol. 51 (1982) 189–199.
[16] N.I. Berlin, Diagnosis and classification of the polycythemias, Semin. Hematol. 12 (1975) 339–351.
[17] S. Murphy, P. Peterson, H. Iland, J. Laszlo, Experience of the Polycythemia Vera Study Group with essential thrombocythemia: a final report on diagnostic criteria, survival, and leukemic transition by treatment, Semin. Hematol. 34 (1997) 29–39.
[18] J.W. Vardiman, N.L. Harris, R.D. Brunning, The World Health Organization (WHO) classification of the myeloid neoplasms, Blood 100 (2002) 2292–2302.
[19] S. Frohling, C. Scholl, D.G. Gillil, R.L. Levine, Genetics of myeloid malignancies: pathogenetic and clinical implications, J. Clin. Oncol. 23 (2005) 6285–6295.
[20] D. Bonnet, J.E. Dick, Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell, Nat. Med. 3 (1997) 730–737.
[21] J.K. Warner, J.C. Wang, K.J. Hope, L. Jin, J.E. Dick, Concepts of human leukemic development, Oncogene 23 (2004) 7164–7177.
[22] C.T. Jordan, M.L. Guzman, Mechanisms controlling pathogenesis and survival of leukemic stem cells, Oncogene 23 (2004) 7178–7187.
[23] D. Bonnet, Normal and leukaemic stem cells, Br. J. Haematol. 130 (2005) 469–479.
[24] B.J. Huntly, D.G. Gilliland, Leukaemia stem cells and the evolution of cancer-stem-cell research, Nat. Rev., Cancer 5 (2005) 311–321.
[25] L.J. Elrick, H.G. Jorgensen, J.C. Mountford, T.L. Holyoake, Punish the parent not the progeny, Blood 105 (2005) 1862–1866.
[26] M.A. Moore, Converging pathways in leukemogenesis and stem cell self-renewal, Exp. Hematol. 33 (2005) 719–737.
[27] T. Holyoake, X. Jiang, C. Eaves, A. Eaves, Isolation of a highly quiescent subpopulation of primitive leukemic cells in chronic myeloid leukemia, Blood 94 (1999) 2056–2064.
[28] K.J. Hope, L. Jin, J.E. Dick, Acute myeloid leukemia originates from a hierarchy of leukemic stem cell classes that differ in self-renewal capacity, Nat. Immunol. 5 (2004) 738–743.
[29] S. Faderl, M. Talpaz, Z. Estrov, S. O'Brien, R. Kurzrock, H.M. Kantarjian, The biology of chronic myeloid leukemia, N. Engl. J. Med. 341 (1999) 164–172.
[30] H. Kantarjian, C. Sawyers, A. Hochhaus, F. Guilhot, C. Schiffer, C. Gambacorti-Passerini, D. Niederwieser, D. Resta, R. Capdeville, U. Zoellner, M. Talpaz, B. Druker, J. Goldman, S.G. O'Brien, N. Russell, T. Fischer, O. Ottmann, P. Cony-Makhoul, T. Facon, R. Stone, C. Miller, M. Tallman, R. Brown, M. Schuster, T. Loughran, A. Gratwohl, F. Mandelli, G. Saglio, M. Lazzarino, D. Russo, M. Baccarani, E. Morra, Hematologic and cytogenetic responses to imatinib mesylate in chronic myelogenous leukemia, N. Engl. J. Med. 346 (2002) 645–652.
[31] M. Talpaz, R.T. Silver, B.J. Druker, J.M. Goldman, C. Gambacorti-Passerini, F. Guilhot, C.A. Schiffer, T. Fischer, M.W. Deininger, A.L. Lennard, A. Hochhaus, O.G. Ottmann, A. Gratwohl, M. Baccarani, R. Stone, S. Tura, F.X. Mahon, S. Fernandes-Reese, I. Gathmann, R. Capdeville, H.M. Kantarjian, C.L. Sawyers, Imatinib induces durable hematologic and cytogenetic responses in patients with accelerated phase chronic myeloid leukemia: results of a phase 2 study, Blood 99 (2002) 1928–1937.
[32] H.M. Kantarjian, J. Cortes, S. O'Brien, F.J. Giles, M. Albitar, M.B. Rios, J. Shan, S. Faderl, G. Garcia-Manero, D.A. Thomas, D. Resta, M. Talpaz, Imatinib mesylate (STI571) therapy for Philadelphia chromosome-positive chronic myelogenous leukemia in blast phase, Blood 99 (2002) 3547–3553.
[33] D.G. Gilliland, C.T. Jordan, C.A. Felix, The molecular basis of leukemia, Hematology (Am. Soc. Hematol. Educ. Program) (2004) 80–97.
[34] D.G. Gillil, J.D. Griffin, The roles of FLT3 in hematopoiesis and leukemia, Blood 100 (2002) 1532–1542.
[35] W. Fiedler, R. Mesters, H. Tinnefeld, S. Loges, P. Staib, U. Duhrsen, M. Flasshove, O.G. Ottmann, W. Jung, F. Cavalli, R. Kuse, J. Thomalla, H. Serve, A.M. O'Farrell, M. Jacobs, N.M. Brega, P. Scigalla, D.K. Hossfeld, W.E. Berdel, A phase 2 clinical study of SU5416 in patients with refractory acute myeloid leukemia, Blood 102 (2003) 2763–2767.
[36] B.D. Smith, M. Levis, M. Beran, F. Giles, H. Kantarjian, K. Berg, K.M. Murphy, T. Dauses, J. Allebach, D. Small, Single-agent CEP-701, a novel FLT3 inhibitor, shows biologic and clinical activity in patients with relapsed or refractory acute myeloid leukemia, Blood 103 (2004) 3669–3676.
[37] R.M. Stone, D.J. DeAngelo, V. Klimek, I. Galinsky, E. Estey, S.D. Nimer, W. Grandin, D. Lebwohl, Y. Wang, P. Cohen, E.A. Fox, D. Neuberg, J. Clark, D.G. Gillil, J.D. Griffin, Patients with acute myeloid leukemia and an activating mutation in FLT3 respond to a small-molecule FLT3 tyrosine kinase inhibitor, PKC412, Blood 105 (2005) 54–60.
[38] S. Kitada, I.M. Pedersen, A.D. Schimmer, J.C. Reed, Dysregulation of apoptosis genes in hematopoietic malignancies, Oncogene 21 (2002) 3459–3474.
[39] S. Kitada, J.C. Reed, MCL-1 promoter insertions dial-up aggressiveness of chronic leukemia, J. Natl. Cancer Inst. 96 (2004) 642–643.
[40] J.C. Reed, M. Pellecchia, Apoptosis-based therapies for hematologic malignancies, Blood 106 (2005) 408–418.
[41] L.R. Silverman, E.P. Demakos, B.L. Peterson, A.B. Kornblith, J.C. Holland, R. Odchimar-Reissig, R.M. Stone, D. Nelson, B.L. Powell, C. M. DeCastro, J. Ellerton, R.A. Larson, C.A. Schiffer, J.F. Holland, Randomized controlled trial of azacitidine in patients with the myelodysplastic syndrome: a study of the cancer and leukemia group B, J. Clin. Oncol. 20 (2002) 2429–2440.
[42] S.L. McKenna, R.A. Padua, Multidrug resistance in leukaemia, Br. J. Haematol. 96 (1997) 659–674.
[43] S.R. Hingorani, D.A. Tuveson, Targeting oncogene dependence and resistance, Cancer Cell 3 (2003) 414–417.
[44] H. Daub, K. Specht, A. Ullrich, Strategies to overcome resistance to targeted protein kinase inhibitors, Nat. Rev., Drug Discov. 3 (2004) 1001–1010.
[45] M. Deininger, E. Buchdunger, B.J. Druker, The development of imatinib as a therapeutic agent for chronic myeloid leukemia, Blood 105 (2005) 2640–2653.
[46] R.T. Costello, F. Mallet, B. Gaugler, D. Sainty, C. Arnoulet, J.A. Gastaut, D. Olive, Human acute myeloid leukemia CD34+/CD38− progenitor cells have decreased sensitivity to chemotherapy and Fas-induced apoptosis, reduced immunogenicity, and impaired dendritic cell transformation capacities, Cancer Res. 60 (2000) 4403–4411.
[47] S.M. Graham, H.G. Jorgensen, E. Allan, C. Pearson, M.J. Alcorn, L. Richmond, T.L. Holyoake, Primitive, quiescent, Philadelphia-positive stem cells from patients with chronic myeloid leukemia are insensitive to STI571 in vitro, Blood 99 (2002) 319–325.
[48] M.L. Guzman, S.J. Neering, D. Upchurch, B. Grimes, D.S. Howard, D.A. Rizzieri, S.M. Luger, C.T. Jordan, Nuclear factor-kappaB is constitutively activated in primitive human acute myelogenous leukemia cells, Blood 98 (2001) 2301–2307.

[49] I. Herr, D. Wilhelm, T. Bohler, P. Angel, K.M. Debatin, Activation of CD95 (APO-1/Fas) signaling by ceramide mediates cancer therapy-induced apoptosis, EMBO J. 16 (1997) 6200–6208.
[50] W. El-Assaad, L. Kozhaya, S. Araysi, S. Panjarian, F.F. Bitar, E. Baz, M.E. El-Sabban, G.S. Dbaibo, Ceramide and glutathione define two independently regulated pathways of cell death initiated by p53 in Molt-4 leukaemia cells, Biochem. J. 376 (2003) 725–732.
[51] A.P. Bruno, G. Laurent, D. Averbeck, C. Demur, J. Bonnet, A. Bettaieb, T. Levade, J.P. Jaffrezou, Lack of ceramide generation in TF-1 human myeloid leukemic cells resistant to ionizing radiation, Cell Death Differ. 5 (1998) 172–182.
[52] E. Bonhoure, D. Pchejetski, N. Aouali, H. Morjani, T. Levade, T. Kohama, O. Cuvillier, Overcoming MDR-associated chemoresistance in HL-60 acute myeloid leukemia cells by targeting shingosine kinase-1, Leukemia 20 (2006) 95–102.
[53] R. Bose, M. Verheij, A. Haimovitz-Friedman, K. Scotto, Z. Fuks, R. Kolesnick, Ceramide synthase mediates daunorubicin-induced apoptosis: an alternative mechanism for generating death signals, Cell 82 (1995) 405–414.
[54] J.P. Jaffrezou, T. Levade, A. Bettaieb, N. Andrieu, C. Bezombes, N. Maestre, S. Vermeersch, A. Rousse, G. Laurent, Daunorubicin-induced apoptosis: triggering of ceramide generation through sphingomyelin hydrolysis, EMBO J. 15 (1996) 2417–2424.
[55] K.J. Turnbull, B.L. Brown, P.R. Dobson, Caspase-3-like activity is necessary but not sufficient for daunorubicin-induced apoptosis in Jurkat human lymphoblastic leukemia cells, Leukemia 13 (1999) 1056–1061.
[56] C. Bezombes, I. Plo, V. Mansat-De Mas, A. Quillet-Mary, A. Negre-Salvayre, G. Laurent, J.P. Jaffrezou, Oxidative stress-induced activation of Lyn recruits sphingomyelinase and is requisite for its stimulation by Ara-C, FASEB J. 15 (2001) 1583–1585.
[57] C. Bezombes, A. de Thonel, A. Apostolou, T. Louat, J.P. Jaffrezou, G. Laurent, A. Quillet-Mary, Overexpression of protein kinase Czeta confers protection against antileukemic drugs by inhibiting the redox-dependent sphingomyelinase activation, Mol. Pharmacol. 62 (2002) 1446–1455.
[58] S.S. Biswal, K. Datta, G.K. Acquaah-Mensah, J.P. Kehrer, Changes in ceramide and sphingomyelin following fludarabine treatment of human chronic B-cell leukemia cells, Toxicology 154 (2000) 45–53.
[59] J. Zhang, N. Alter, J.C. Reed, C. Borner, L.M. Obeid, Y.A. Hannun, Bcl-2 interrupts the ceramide-mediated pathway of cell death, Proc. Natl. Acad. Sci. U. S. A. 93 (1996) 5325–5328.
[60] R.S. Olshefski, S. Ladisch, Glucosylceramide synthase inhibition enhances vincristine-induced cytotoxicity, Int. J. Cancer 93 (2001) 131–138.
[61] D.K. Perry, J. Carton, A.K. Shah, F. Meredith, D.J. Uhlinger, Y.A. Hannun, Serine palmitoyltransferase regulates de novo ceramide generation during etoposide-induced apoptosis, J. Biol. Chem. 275 (2000) 9078–9084.
[62] A. Dipietrantonio, T.C. Hsieh, J.M. Wu, Differential effects of retinoic acid (RA) and N-(4-hydroxyphenyl) retinamide (4-HPR) on cell growth, induction of differentiation, and changes in p34cdc2, Bcl-2, and actin expression in the human promyelocytic HL-60 leukemic cells, Biochem. Biophys. Res. Commun. 224 (1996) 837–842.
[63] A.M. DiPietrantonio, T.C. Hsieh, S.C. Olson, J.M. Wu, Regulation of G1/S transition and induction of apoptosis in HL-60 leukemia cells by fenretinide (4HPR), Int. J. Cancer 78 (1998) 53–61.
[64] R.M. Laethem, Y.A. Hannun, S. Jayadev, C.J. Sexton, J.C. Strum, R. Sundseth, G.K. Smith, Increases in neutral, Mg2+-dependent and acidic, Mg2+-independent sphingomyelinase activities precede commitment to apoptosis and are not a consequence of caspase 3-like activity in Molt-4 cells in response to thymidylate synthase inhibition by GW1843, Blood 91 (1998) 4350–4360.
[65] A. Senchenkov, T.Y. Han, H. Wang, A.E. Frankel, T.J. Kottke, S.H. Kaufmann, M.C. Cabot, Enhanced ceramide generation and induction of apoptosis in human leukemia cells exposed to DT(388)-granulocyte-macrophage colony-stimulating factor (GM-CSF), a truncated diphtheria toxin fused to human GM-CSF, Blood 98 (2001) 1927–1934.
[66] M. Rahmani, E. Reese, Y. Dai, C. Bauer, S.G. Payne, P. Dent, S. Spiegel, S. Grant, Coadministration of histone deacetylase inhibitors and perifosine synergistically induces apoptosis in human leukemia cells through Akt and ERK1/2 inactivation and the generation of ceramide and reactive oxygen species, Cancer Res. 65 (2005) 2422–2432.
[67] R.R. Rosato, S.C. Maggio, J.A. Almenara, S.G. Payne, P. Atadja, S. Spiegel, P. Dent, S. Grant, The histone deacetylase inhibitor LAQ-824 induces human leukemia cell death through a process involving XIAP down-regulation, oxidative injury, and the acid sphingomyelinase-dependent generation of ceramide, Mol. Pharmacol. 69 (2006) 216–225.
[68] T. Murate, M. Suzuki, M. Hattori, A. Takagi, T. Kojima, T. Tanizawa, H. Asano, T. Hotta, H. Saito, S. Yoshida, K. Tamiya-Koizumi, Up-regulation of Acid Sphingomyelinase during retinoic acid-induced myeloid differentiation of NB4, a human acute promyelocytic leukemia cell line, J. Biol. Chem. 277 (2002) 9936–9943.
[69] M. Minamino, I. Sakaguchi, T. Naka, N. Ikeda, Y. Kato, I. Tomiyasu, I. Yano, K. Kobayashi, Bacterial ceramides and sphingophospholipids induce apoptosis of human leukaemic cells, Microbiology 149 (2003) 2071–2081.
[70] L.M. Obeid, C.M. Linardic, L.A. Karolak, Y.A. Hannun, Programmed cell death induced by ceramide, Science 259 (1993) 1769–1771.
[71] D. Myrick, D. Blackinton, J. Klostergaard, N. Kouttab, A. Maizel, H. Wanebo, S. Mehta, Paclitaxel-induced apoptosis in Jurkat, a leukemic T cell line, is enhanced by ceramide, Leuk. Res. 23 (1999) 569–578.
[72] W.D. Jarvis, R.N. Kolesnick, F.A. Fornari, R.S. Traylor, D.A. Gewirtz, S. Grant, Induction of apoptotic DNA damage and cell death by activation of the sphingomyelin pathway, Proc. Natl. Acad. Sci. U. S. A. 91 (1994) 73–77.
[73] M.Y. Baek, H.S. Yoo, K. Nakaya, D.C. Moon, Y.M. Lee, Sphingolipid metabolic changes during chiral C2-ceramides induced apoptosis in human leukemia cells, Arch. Pharm. Res. 24 (2001) 144–149.
[74] S. Jayadev, B. Liu, A.E. Bielawska, J.Y. Lee, F. Nazaire, M. Pushkareva, L.M. Obeid, Y.A. Hannun, Role for ceramide in cell cycle arrest, J. Biol. Chem. 270 (1995) 2047–2052.
[75] H. Ohta, E.A. Sweeney, A. Masamune, Y. Yatomi, S. Hakomori, Y. Igarashi, Induction of apoptosis by sphingosine in human leukemic HL-60 cells: a possible endogenous modulator of apoptotic DNA fragmentation occurring during phorbol ester-induced differentiation, Cancer Res. 55 (1995) 691–697.
[76] W.D. Jarvis, F.A. Fornari, R.S. Traylor, H.A. Martin, L.B. Kramer, R.K. Erukulla, R. Bittman, S. Grant, Induction of apoptosis and potentiation of ceramide-mediated cytotoxicity by sphingoid bases in human myeloid leukemia cells, J. Biol. Chem. 271 (1996) 8275–8284.
[77] B. Liu, N. Andrieu-Abadie, T. Levade, P. Zhang, L.M. Obeid, Y.A. Hannun, Glutathione regulation of neutral sphingomyelinase in tumor necrosis factor-alpha-induced cell death, J. Biol. Chem. 273 (1998) 11313–11320.
[78] N. Andrieu-Abadie, V. Gouaze, R. Salvayre, T. Levade, Ceramide in apoptosis signaling: relationship with oxidative stress, Free Radic. Biol. Med. 31 (2001) 717–728.
[79] A. Quillet-Mary, J.P. Jaffrezou, V. Mansat, C. Bordier, J. Naval, G. Laurent, Implication of mitochondrial hydrogen peroxide generation in ceramide-induced apoptosis, J. Biol. Chem. 272 (1997) 21388–21395.
[80] K. Iwai, T. Kondo, M. Watanabe, T. Yabu, T. Kitano, Y. Taguchi, H. Umehara, A. Takahashi, T. Uchiyama, T. Okazaki, Ceramide increases oxidative damage due to inhibition of catalase by caspase-3-dependent proteolysis in HL-60 cell apoptosis, J. Biol. Chem. 278 (2003) 9813–9822.
[81] D.C. Phillips, K. Allen, H.R. Griffiths, Synthetic ceramides induce growth arrest or apoptosis by altering cellular redox status, Arch. Biochem. Biophys. 407 (2002) 15–24.
[82] M. Chen, J. Quintans, Z. Fuks, C. Thompson, D.W. Kufe, R.R. Weichselbaum, Suppression of Bcl-2 messenger RNA production may mediate apoptosis after ionizing radiation, tumor necrosis factor alpha, and ceramide, Cancer Res. 55 (1995) 991–994.
[83] M.J. Smyth, D.K. Perry, J. Zhang, G.G. Poirier, Y.A. Hannun, L.M. Obeid, prICE: a downstream target for ceramide-induced apoptosis and

for the inhibitory action of Bcl-2, Biochem. J. 316 (Pt 1) (1996) 25–28.
[84] S. Geley, B.L. Hartmann, R. Kofler, Ceramides induce a form of apoptosis in human acute lymphoblastic leukemia cells that is inhibited by Bcl-2, but not by CrmA, FEBS Lett. 400 (1997) 15–18.
[85] J.P. Jaffrezou, N. Maestre, V. de Mas-Mansat, C. Bezombes, T. Levade, G. Laurent, Positive feedback control of neutral sphingomyelinase activity by ceramide, FASEB J. 12 (1998) 999–1006.
[86] Y. Nishizuka, Intracellular signaling by hydrolysis of phospholipids and activation of protein kinase C, Science 258 (1992) 607–614.
[87] H. Sawai, T. Okazaki, Y. Takeda, M. Tashima, H. Sawada, M. Okuma, S. Kishi, H. Umehara, N. Domae, Ceramide-induced translocation of protein kinase C-delta and -epsilon to the cytosol. Implications in apoptosis, J. Biol. Chem. 272 (1997) 2452–2458.
[88] H. Sawai, T. Okazaki, N. Domae, Sphingosine-induced c-jun expression: differences between sphingosine- and C2-ceramide-mediated signaling pathways, FEBS Lett. 524 (2002) 103–106.
[89] H. Sawai, T. Okazaki, H. Yamamoto, H. Okano, Y. Takeda, M. Tashima, H. Sawada, M. Okuma, H. Ishikura, H. Umehara, et al., Requirement of AP-1 for ceramide-induced apoptosis in human leukemia HL-60 cells, J. Biol. Chem. 270 (1995) 27326–27331.
[90] W.D. Jarvis, F.A. Fornari Jr., K.L. Auer, A.J. Freemerman, E. Szabo, M.J. Birrer, C.R. Johnson, S.E. Barbour, P. Dent, S. Grant, Coordinate regulation of stress- and mitogen-activated protein kinases in the apoptotic actions of ceramide and sphingosine, Mol. Pharmacol. 52 (1997) 935–947.
[91] M. Macchia, S. Barontini, S. Bertini, V. Di Bussolo, S. Fogli, E. Giovannetti, E. Grossi, F. Minutolo, R. Danesi, Design, synthesis, and characterization of the antitumor activity of novel ceramide analogues, J. Med. Chem. 44 (2001) 3994–4000.
[92] K. Shikata, H. Niiro, H. Azuma, K. Ogino, T. Tachibana, Apoptotic activities of C2-ceramide and C2-dihydroceramide homologues against HL-60 cells, Bioorg. Med. Chem. 11 (2003) 2723–2728.
[93] E. Kishida, M. Kasahara, Y. Takagi, M. Matsumura, T. Hayashi, S. Kobayashi, Y. Masuzawa, Evaluation of a trans configuration for the apoptosis-inducing activity of ceramide, J. Lipid Mediators Cell Signal. 16 (1997) 127–137.
[94] S. De Jonghe, I. Van Overmeire, J. Gunst, A. De Bruyn, C. Hendrix, S. Van Calenbergh, R. Busson, D. De Keukeleire, J. Philippe, P. Herdewijn, Synthesis and apoptogenic activity of fluorinated ceramide and dihydroceramide analogues, Bioorg. Med. Chem. Lett. 9 (1999) 3159–3164.
[95] A. Bielawska, C.M. Linardic, Y.A. Hannun, Ceramide-mediated biology. Determination of structural and stereospecific requirements through the use of N-acyl-phenylaminoalcohol analogs, J. Biol. Chem. 267 (1992) 18493–18497.
[96] T. Wieder, C.C. Geilen, T. Kolter, F. Sadeghlar, K. Sandhoff, R. Brossmer, P. Ihrig, D. Perry, C.E. Orfanos, Y.A. Hannun, Bcl-2 antagonizes apoptotic cell death induced by two new ceramide analogues, FEBS Lett. 411 (1997) 260–264.
[97] N. Karasavvas, R.K. Erukulla, R. Bittman, R. Lockshin, Z. Zakeri, Stereospecific induction of apoptosis in U937 cells by N-octanoyl-sphingosine stereoisomers and N-octyl-sphingosine. The ceramide amide group is not required for apoptosis, Eur. J. Biochem. 236 (1996) 729–737.
[98] H. Azuma, R. Takao, K. Shikata, H. Niiro, T. Tachibana, K. Ogino, Synthesis and potent antileukemic activities of N-lactylsphingosine and N-lactyldihydrosphingosine, J. Med. Chem. 46 (2003) 3445–3447.
[99] A. Dagan, C. Wang, E. Fibach, S. Gatt, Synthetic, non-natural sphingolipid analogs inhibit the biosynthesis of cellular sphingolipids, elevate ceramide and induce apoptotic cell death, Biochim. Biophys. Acta 1633 (2003) 161–169.
[100] C.R. Johnson, J. Chun, R. Bittman, W.D. Jarvis, Intrinsic cytotoxicity and chemomodulatory actions of novel phenethylisothiocyanate sphingoid base derivatives in HL-60 human promyelocytic leukemia cells, J. Pharmacol. Exp. Ther. 309 (2004) 452–461.
[101] O. Cuvillier, T. Levade, Enzymes of sphingosine metabolism as potential pharmacological targets for therapeutic intervention in cancer, Pharmacol. Res. 47 (2003) 439–445.
[102] J.W. Kok, H. Sietsma, Sphingolipid metabolism enzymes as targets for anticancer therapy, Curr. Drug Targets 5 (2004) 375–382.
[103] A. Bielawska, M.S. Greenberg, D. Perry, S. Jayadev, J.A. Shayman, C. McKay, Y.A. Hannun, (1S,2R)-D-erythro-2-(N-myristoylamino)-1-phenyl-1-propanol as an inhibitor of ceramidase, J. Biol. Chem. 271 (1996) 12646–12654.
[104] E. Le Scolan, D. Pchejetski, Y. Banno, N. Denis, P. Mayeux, W. Vainchenker, T. Levade, F. Moreau-Gachelin, Overexpression of sphingosine kinase 1 is an oncogenic event in erythroleukemic progression, Blood 106 (2005) 1808–1816.
[105] A. Olivera, T. Kohama, L. Edsall, V. Nava, O. Cuvillier, S. Poulton, S. Spiegel, Sphingosine kinase expression increases intracellular sphingosine-1-phosphate and promotes cell growth and survival, J. Cell Biol. 147 (1999) 545–558.
[106] L.C. Edsall, J.R. Van Brocklyn, O. Cuvillier, B. Kleuser, S. Spiegel, *N,N*-dimethylsphingosine is a potent competitive inhibitor of sphingosine kinase but not of protein kinase C: modulation of cellular levels of sphingosine 1-phosphate and ceramide, Biochemistry 37 (1998) 12892–12898.
[107] D.B. Jendiroba, J. Klostergaard, A. Keyhani, L. Pagliaro, E.J. Freireich, Effective cytotoxicity against human leukemias and chemotherapy-resistant leukemia cell lines by N-N-dimethylsphingosine, Leuk. Res. 26 (2002) 301–310.
[108] K.J. French, R.S. Schrecengost, B.D. Lee, Y. Zhuang, S.N. Smith, J.L. Eberly, J.K. Yun, C.D. Smith, Discovery and evaluation of inhibitors of human sphingosine kinase, Cancer Res. 63 (2003) 5962–5969.
[109] K. Kono, M. Tanaka, T. Ogita, T. Hosoya, T. Kohama, F-12509A, a new sphingosine kinase inhibitor, produced by a discomycete, J. Antibiot. (Tokyo) 53 (2000) 459–466.
[110] K. Kono, M. Sugiura, T. Kohama, Inhibition of recombinant sphingosine kinases by novel inhibitors of microbial origin, F-12509A and B-5354c, J. Antibiot. (Tokyo) 55 (2002) 99–103.
[111] A. Meng, C. Luberto, P. Meier, A. Bai, X. Yang, Y.A. Hannun, D. Zhou, Sphingomyelin synthase as a potential target for D609-induced apoptosis in U937 human monocytic leukemia cells, Exp. Cell Res. 292 (2004) 385–392.
[112] A. Bai, G.P. Meier, Y. Wang, C. Luberto, Y.A. Hannun, D. Zhou, Prodrug modification increases potassium tricyclo[5.2.1.0(2,6)]-decan-8-yl dithiocarbonate (D609) chemical stability and cytotoxicity against U937 leukemia cells, J. Pharmacol. Exp. Ther. 309 (2004) 1051–1059.
[113] A. van Helvoort, A.J. Smith, H. Sprong, I. Fritzsche, A.H. Schinkel, P. Borst, G. van Meer, MDR1 P-glycoprotein is a lipid translocase of broad specificity, while MDR3 P-glycoprotein specifically translocates phosphatidylcholine, Cell 87 (1996) 507–517.
[114] A. vanHelvoort, M.L. Giudici, M. Thielemans, G. van Meer, Transport of sphingomyelin to the cell surface is inhibited by brefeldin A and in mitosis, where C6-NBD-sphingomyelin is translocated across the plasma membrane by a multidrug transporter activity, J. Cell Sci. 110 (Pt. 1) (1997) 75–83.
[115] A. Bettaieb, M. Record, M.G. Come, A.C. Bras, H. Chap, G. Laurent, J.P. Jaffrezou, Opposite effects of tumor necrosis factor alpha on the sphingomyelin-ceramide pathway in two myeloid leukemia cell lines: role of transverse sphingomyelin distribution in the plasma membrane, Blood 88 (1996) 1465–1472.
[116] C. Bezombes, N. Maestre, G. Laurent, T. Levade, A. Bettaieb, J.P. Jaffrezou, Restoration of TNF-alpha-induced ceramide generation and apoptosis in resistant human leukemia KG1a cells by the P-glycoprotein blocker PSC833, FASEB J. 12 (1998) 101–109.
[117] M. Pallis, N. Russell, P-glycoprotein plays a drug-efflux-independent role in augmenting cell survival in acute myeloblastic leukemia and is associated with modulation of a sphingomyelin-ceramide apoptotic pathway, Blood 95 (2000) 2897–2904.
[118] P. Lala, S. Ito, C.A. Lingwood, Retroviral transfection of Madin–Darby canine kidney cells with human MDR1 results in a major increase in globotriaosylceramide and 10(5)- to 10(6)-fold increased cell sensitivity

to verocytotoxin. Role of p-glycoprotein in glycolipid synthesis, J. Biol. Chem. 275 (2000) 6246–6251.
[119] J.A. Shabbits, L.D. Mayer, P-glycoprotein modulates ceramide-mediated sensitivity of human breast cancer cells to tubulin-binding anticancer drugs, Mol. Cancer Ther. 1 (2002) 205–213.
[120] M.F. De Rosa, D. Sillence, C. Ackerley, C. Lingwood, Role of multiple drug resistance protein 1 in neutral but not acidic glycosphingolipid biosynthesis, J. Biol. Chem. 279 (2004) 7867–7876.
[121] J. Turzanski, M. Grundy, S. Shang, N. Russell, M. Pallis, P-glycoprotein is implicated in the inhibition of ceramide-induced apoptosis in TF-1 acute myeloid leukemia cells by modulation of the glucosylceramide synthase pathway, Exp. Hematol. 33 (2005) 62–72.
[122] M. Itoh, T. Kitano, M. Watanabe, T. Kondo, T. Yabu, Y. Taguchi, K. Iwai, M. Tashima, T. Uchiyama, T. Okazaki, Possible role of ceramide as an indicator of chemoresistance: decrease of the ceramide content via activation of glucosylceramide synthase and sphingomyelin synthase in chemoresistant leukemia, Clin. Cancer Res. 9 (2003) 415–423.
[123] B. Liu, Y.A. Hannun, Inhibition of the neutral magnesium-dependent sphingomyelinase by glutathione, J. Biol. Chem. 272 (1997) 16281–16287.