Bioactive Sphingolipids in Response to Chemotherapy: A Scope on Leukemias

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Abstract: Sphingolipids are major constituents of the cells with emerging roles in the regulation of cellular processes. Deregulation of sphingolipid metabolism is reflected as various pathophysiological conditions including metabolic disorders and several forms of cancer. Ceramides, ceramide-1-phosphate (C1P), glucosyl ceramide (GluCer), sphingosine and sphingosine-1-phosphate (S1P) are among the bioactive sphingolipid species that have important roles in the regulation of cell death, survival and chemotherapeutic resistance. Some of those species are known to accumulate in the cells upon chemotherapy while some others are known to exhibit an opposite pattern. Even though the length of fatty acid chain has a deterministic effect, in general, upregulation of ceramides and sphingosine is known to induce apoptosis. However, S1P, C1P and GluCer are proliferative for cells and they are involved in the development of chemoresistance. Therefore, sphingolipid metabolism appears as a good target for the development of novel therapeutics or supportive interventions to increase the effectiveness of the chemotherapeutic drugs currently in hand. Some approaches involve manipulation of the synthesis pathways yielding the increased production of apoptotic sphingolipids while the proliferative ones are suppressed. Some others are trying to take advantage of cytotoxic sphingolipids like short chain ceramide analogs by directly delivering them to the malignant cells as a distinct chemotherapeutic intervention. Numerous studies in the literature show the feasibility of those approaches especially in acute and chronic leukemias. This review compiles the current knowledge about sphingolipids and their roles in chemotherapeutic response with the particular attention to leukemias.

Keywords: Ceramide, ceramide-1-phosphate, chemotherapeutic response, dihydroceramide, glucosylceramide, leukemia, sphingosine, sphingosine-1-phosphate.

INTRODUCTION

Sphingolipids form a major class of lipids found within the eukaryotic membranes and an unusual class of bacterial genus called *Sphingobacteria*. General structure of sphingolipids contains a long-chain sphingosine base and an amide-linked fatty acid chain. These molecules have amphiphatic structures having polar and nonpolar parts like phospholipids. Various polar head groups and differing lengths of fatty acid chain produce a wide range of sphingolipids and give different characteristics to them. Following their discovery in late 1800s, versatile nature of sphingolipids appeared enigmatic to scientific community and for a long time, they were thought to be important solely for structural purposes in cells. Last decades have shown that they are not simple cellular building blocks; instead they are involved in several other processes including but not limited to cell cycle control, apoptosis and differentiation [1-3]. Sphingolipids are involved in the regulation of essential cellular processes by participating to signal transduction pathways as secondary messengers.

Studies pointing out the roles of sphingolipids in pathophysiology of various diseases including cancer and metabolic disorders have increased the attention to lipid research lately and therapeutic interventions are started to incorporate approaches targeting sphingolipid synthesis pathways. By means of those efforts, numerous sphingolipid species with promising therapeutic importance are identified by manipulating their cellular levels. Ceramide (Cer), ceramide-1-phosphate (C1P), glucosylceramide (GluCer), dihydroceramide (dhCer), sphingosine (Sph), and sphingosine-1-phosphate (S1P) are among those sphingolipid species that have important regulative roles in cells (for their chemical structures please see Fig. **1**). Some of those species play opposing roles while some others are working in concert for the similar outcome; and cellular fate is determined by their interactions and ratios in the cell [4-6].

This review attempts to provide an overview of what is known for particular roles of such sphingolipids, and their implications in cancer area. Roles of sphingolipids will be examined in the perspective of chemotherapeutic response with the emphasis on targeting these mechanisms for the treatment of leukemias. Towards the end, possibility of targeting sphingolipid metabolism as an alternative or a supportive therapeutic intervention will be discussed.

METABOLISM OF SPHINGOLIPIDS

There are many inter-conversions among different sphingolipid species but one of them, ceramide, is particularly important as being located in the middle of this complex metabolic pathway. Therefore, ceramide is essential for the synthesis of other sphingolipids and back-reactions catalyzed by different enzymes contribute to the ceramide pool by converting other types of sphingolipids, indicating that this is not a one way process, instead, it has a dynamic nature [7-9]. Ceramide is formed by the addition of a fatty acid chain with varying number of carbon atoms to sphingosine base. There are multiple mechanisms responsible for ceramide production in cells. Ceramide can be produced from serine and palmitoyl CoA in endoplasmic reticulum (ER) via *de novo* synthesis (Fig. **2**) [10, 11]. This pathway involves several chemical reactions and separate enzymes catalyzing each step. First condensation step is catalyzed by serine palmitoyltransferase producing 3-ketosphinganine (3-ketodihydrosphingosine) [12]. In subsequent steps, 3-ketosphinganine is converted to sphinganine (dihydrosphingosine) that would in turn serve as a substrate for dihydroceramide synthase (known as Lass or CerS) for acylation to dhCer [13, 14]. This step is reversible and the back-reaction is catalyzed by ceramidases (CDase). Desaturation of dhCer produces ceramide in the last step of *de novo* synthesis pathway (Fig. **1**) [15, 16]. Alternatively, ceramides can be synthesized from sphingomyelin by the catalysis of sphingomyelinase (SMase) [17, 18]. According to pH optima, SMases are named as acid SMase, neutral SMase, and alkaline SMase. Not surprisingly, these classes of enzymes have preference for subcellular localizations due to their optimal pH ranges. Moreover, generation of ceramides by different SMases is thought to attribute different functions to the ceramide products, which might have distinct and profound effects in signaling [19]. Reverse reaction is also possible by the catalysis of sphingomyelin

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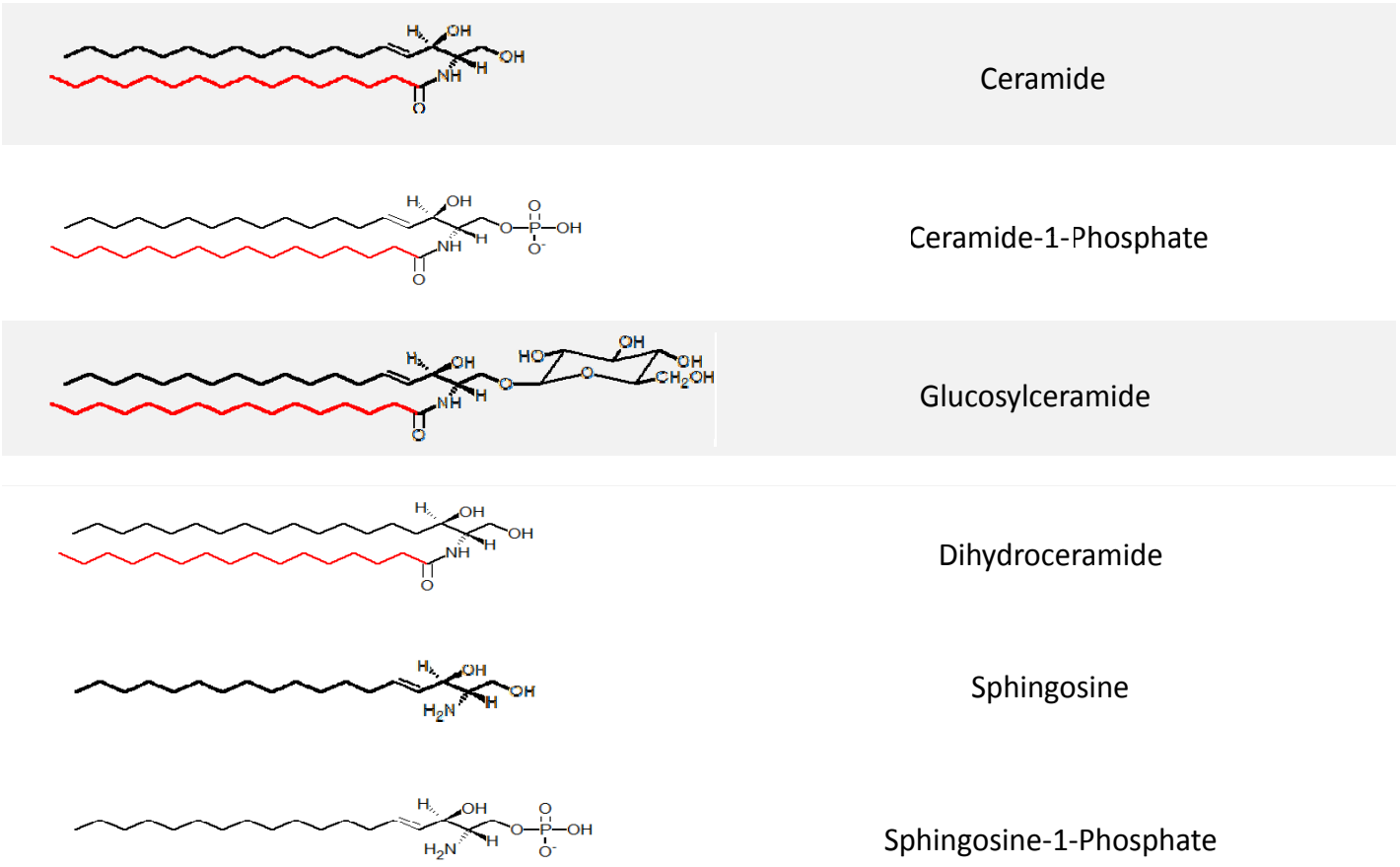

Fig. (1). Chemical structures of various bioactive sphingolipids. Ceramide and its derivatives shown in this figure contain 16 carbon atoms in the fatty acid chain and hence named as C16-ceramides.

synthase producing sphingomyelin from ceramide with a process involving utilization of phosphatidylcholine and liberation of diacylglycerol meanwhile [20]. This reaction specifically takes place in Golgi and ceramides that would be used for SM production are transported to this compartment by ceramide transfer protein, CERT [21]. As the last mechanism, degradation of complex sphingolipids can also result in the production of ceramides by a process called "salvage pathway" [22]. This pathway involves intermediates such as GluCer and galactosylceramide (GalCer) as the direct products of degradation; but they are hydrolyzed to ceramides by specific enzymes subsequently (Fig. **2**) [23, 24].

Sphingosine is synthesized from ceramides by CDases by the removal of amide-linked fatty acid chain. Similar to SMases, there are different types of CDases classified according to their optimal pH for enzymatic catalysis as acid CDases, neutral CDases and alkaline CDases [25-27]. In this mechanism, ceramides can also be derived from sphingosine by ceramide synthase (encoded by a family of genes called *Lass* genes - longevity assurance homologue-1 of yeast Lag1) in the reverse reaction. Upon its synthesis, sphingosine can be modified to sphingosine-1-phosphate (S1P) by sphingosine kinase (SphK) [28]. Sphingosine phosphatase can catalyze the reverse reaction by removing the phosphate group. S1P can be further metabolized by S1P lyase yielding ethanolamine phosphate and hexadecanal. This is an irreversible step but products can be converted into palmitate for re-utilization in the sphingolipid metabolism (Fig. **2**) [29].

Glucosylceramide (GluCer) is synthesized from ceramides in Golgi by GluCer synthase (GCS) and it serves as a substrate for the synthesis of complex glycosphingolipids in the same subcellular compartment. Unlike CERT-mediated transport that is seen during SM production, ceramides are carried to Golgi by vesicles budding off directly from ER without the direct involvement of a protein transporter [30]. However, GluCer associates with some proteins for transport through Golgi in the subsequent reactions leading to the synthesis of complex glycosphingolipids [31, 32]. GluCer can be converted back to ceramide by glucosylceramidase [33]. However, ceramide is not limited to the biochemical conversions listed above. It can also undergo phosphorylation by the catalysis of ceramide kinase [34] and this modification changes its characteristics and downstream targets profoundly as it will be discussed in the following section.

CELLULAR ROLES OF BIOACTIVE SPHINGOLIPIDS

Accumulating body of evidence for decades suggests that bioactive sphingolipids are involved in the regulation of many cellular processes. Deregulation of sphingolipid metabolism is implicated in several forms of metabolic disease and malignancies [35-37]. Moreover, different forms of sphingolipids exert distinct regulatory functions. Therefore, a delicate balance among lipid players needs to be maintained for homeostasis under varying environmental conditions [38-41].

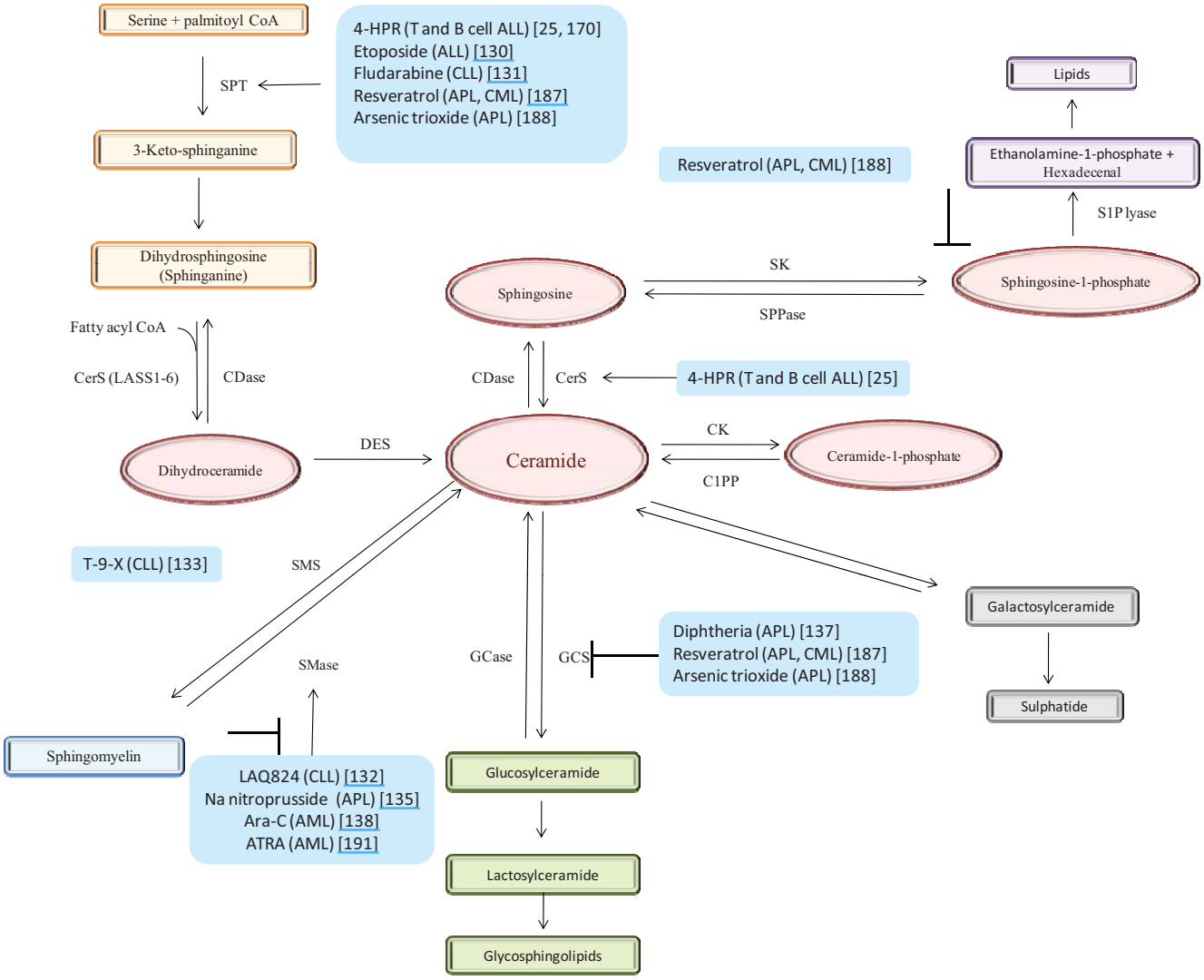

Fig. (2). Biochemical inter-conversions of sphingolipid species and compounds inhibiting or inducing the specific enzymes in this pathway. CerS (LASS), ceramide synthase; CDase, ceramidase; DES, dihydroceramide desaturase; SMS, sphingomyelin synthase; SMase, sphingomyelinase; GCS, glucosyl ceramide synthase; GCase, glucosyl ceramidase; CK, ceramide kinase; S1P, sphingosine-1-phosphate; SPPase, S1P phosphatase; SK, sphingosine kinase; C1PP, ceramide-1-phosphate phosphatase. T-9-X, tricyclodecan-9-yl-xanthogenate; LAQ824, histone deacetylase inhibitor; 4-HPR, N-(4-hydroxyphenyl) retinamide; ATRA, all-trans-retinoic acid; Ara-C, cytosine arabinoside; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CLL, chronic lymphoblastic leukemia; CML, chronic myeloid leukemia; APL, acute promyelocytic leukemia.

a). Ceramide Derivatives: Ceramide, C1P, GluCer, and dhCer

Ceramide is maybe the most intensely studied sphingolipid among the others. Therefore there is a wide array of data about the roles of ceramide in different physiological and pathophysiological conditions. One of the well known functions of ceramides is involvement in type-I programmed cell death, or apoptosis (this is not valid for every single type of ceramide; in fact fatty acid chain length has a deterministic effect on the behavior of the ceramide as more will be discussed about this below). In numerous different model systems, ceramides are shown to induce apoptosis with some overlapping and non-overlapping mechanisms. For instance, *de novo* ceramide generation was found to be important for triggering apoptosis specifically in melanoma cells as evidenced by the lack of apoptosis when serine palmitoyltransferase activity is blocked [42]. However in prostate cancer cancer cell lines, ceramide-mediated apoptosis appeared to be driven by Akt dephosphorylation through protein phosphatase 2A activity [43]. Similarly, in another study, ceramide analogs were shown to induce apoptosis by affecting mitochondria along with the concomitant dephosphorylation of Akt [44]. Supporting this mechanism, ceramide channels formed on the mitochondrial membranes were documented to be important for the release of pro-apoptotic factors to cytosol [45]. This finding was recapitulated by various other studies involving different model systems. Interestingly, a recent report has shown that mitochondrially-targeted ceramide analog LCL29 is more potent for retarding cell growth in MCF7 breast cancer cell line through autophagy and apoptosis compared to uncharged ceramides suggesting that targeting ceramides to mitochondria might enhance the efficacy of the compound for therapeutic purposes [46]. In another report, upregulation of ceramide correlated with the activation of proapoptotic genes; and apoptosis is inhibited by blocking the ceramide pathway in breast cancer cells indicating the importance of ceramides in the actual apoptotic induction [47]. Cytochrome C release is mediated directly by ceramides as shown in the isolated mitochondria [48]. Ceramide-mediated apoptosis is not only important for cancer cell death but also thought to be present in various healthy cells includ-

ing macrophages and developing embryos which suggests its importance for maintaining homeostasis in healthy cells as well [49, 50]. Furthermore, apoptotic induction by ceramides is a known mechanism for stress-induced cell death as shown by numerous studies [51-54]. As an example, conversion of sphingomyelin to ceramide was documented during apoptosis upon heat-stress [55]. A similar pathway is responsible for insulinoma cell death induced by ER-stress [56, 57]. However, there are some other studies indicating that ceramides might be dispensable for cell death under certain stress conditions [54, 58] preventing us to generalize ceramide-mediated apoptosis as "the mechanism" for stress-induced cell death. Moreover, ceramides having long fatty acid chains behave somewhat differently in the regulation of cellular processes. While ceramides are generally known to exert growth suppressive and apoptotic functions, C16-ceramide was documented to be anti-apoptotic and provide protection against cell death upon ER stress [59]. Similar observations were done in other models attributing anti-apoptotic roles to long chain ceramides [60, 61].

C1P has opposing regulatory roles compared to ceramides. Unlike majority of ceramides, C1P is known to induce DNA replication, suppress apoptosis and favor survival of cells [62, 63]. Protein kinase C-α was shown to be essential for the proliferative effects of C1P [64]. In a study involving macrophages, suppression of apoptosis by C1P was shown to be taking place via inhibition of *de novo* ceramide synthesis pathway [65]. Nitric oxide (NO) is also thought to have roles in C1P-mediated survival as evidenced by the abrogation of pro-survival effects in the presence of inhibitors for NO synthase [66]. In macrophages, C1P activates PI3 kinase, JNK and ERK1/2 pathways to mediate pro-survival effects [67]. On the other hand, C1P analogs were documented to exert anti-inflammatory functions through suppression of TNF-α and induction of IL-10 production in macrophages [68]. This role was confirmed by various other groups as well, attributing the importance to C1P in the regulation of inflammatory response [69-71]. Besides this, C1P is also thought to have roles in the regulation of intracellular Ca^{2+} levels [72-74].

GluCer is another type of proliferative sphingolipids and it has roles in the development of chemotherapeutic resistance [75-77]. Multidrug transporter, P-glycoprotein (P-gp), was shown to potentiate ceramide glycosylation, and addition of GCS inhibitors sensitized cells to chemotherapy [78]. Chemoresistance conferred by GluCer is mediated by the inhibition of NADPH oxidase; hence augmenting NADPH oxidase activity provides sensitivity to chemotherapy [75]. GluCer was also shown to directly upregulate MDR1 expression via cSrc and β-catenin pathways producing chemoresistant cancer subtypes [79]. In leukemic cell lines, GCS was found to contribute drug resistance by upregulating anti-apoptotic Bcl-2 [80]. Upregulation of GCS correlates with poor prognosis and aggressive nature of the neoplasm as shown by studies on different cancer models [81, 82]. GluCer is not only important for conferring chemotherapeutic resistance to cancer cells, but it is also important for the pathophysiology of several disorders including Gaucher disease, polycystic kidney disease and asthma [83-85]. Membrane trafficking, natural killer lymphocyte polarization and neuronal activation can be given as examples of other cellular processes involving GluCer [86-88].

Dihydroceramide (dhCer) has several functions in cells some of which looks a bit controversial. By some groups dhCer was shown not to be important for inducing apoptosis and cell cycle arrest [89, 90]. Opposite to those observations, in some other studies, a low but considerable level of apoptotic induction is linked to dhCer [91, 92]. dhCer was shown to increase in response to 4-HPR chemotherapy in ovarian cancers concomitantly with the increased levels of apoptosis by a process reversed by sphingosine kinase activity [25, 93]. In another study, inhibition of dhCer synthesis correlated with reduced viability in T-cell leukemia line [94]. Ceramide-to-dhCer ratio was shown to be a determinant in the apoptotic decision in another report, possibly by modulating ceramide channels through conversion of ceramide to dhCer [95]. Loss of dhCer was correlated with rapid cell growth and increased apoptosis interestingly, and this phenotype was rescued with the activation of dhCer synthesis in some neurodegerative disorders [96]. Autophagy, self-destruction of the cellular components, is another process in which dhCer is thought to have regulatory roles [97, 98]. In some studies it was documented that not the increased intracellular dhCer level; but the conversion of dhCer into other sphingolipids per se is responsible for some of the listed phenotypes above [99].

b). Sphingosine Derivatives: Sphingosine and S1P

Sphingosine is a growth suppressive and apoptotic sphingolipid, like ceramide. This feature of sphingosine is documented in several cell types including both malignant and healthy cells [100]. Leukemic cells including multidrug resistant subtypes [101-103], various forms of carcinoma [104], and soft tissue sarcoma cells [105] were shown to undergo apoptosis upon exposure to sphingosine or in response to increased intracellular levels of sphingosine. Apoptotic induction mechanism of sphingosine was shown to be dependent on cytochrome c release from mitochondria in neurons and astrocytes [106]. In some other studies, sphingosine was found to exert its functions through the inhibition of protein kinase C [107]. This particular sphingolipid also has regulative functions for phagocytosis as shown in alveolar macrophages [108]. In this study, conversion of ceramides into sphingosine impaired clearance of apoptotic bodies by macrophages in a dose-dependent manner.

Sphingosine-1-phosphate (S1P) plays a totally different role in the regulation of cellular fate. It antagonizes ceramide and sphingosine and favors cell survival. When endothelial cells are beamed with ionizing radiation, S1P was shown to activate AKT pathway to protect the cells from apoptosis [109]. Loss of sphingosine kinase activity was shown to increase the sensitivity to the DNA damaging chemotherapy with the concomitant increase in reactive oxygen species [110]. Blocking S1P production was shown to have possible implications in the suppression of angiogenesis which is an instrumental process for tumor growth [111, 112]. Other than its implications in cancer, S1P signaling was shown to be important in various other processes as well. For instance, recruitment of inflammatory macrophages in atherosclerosis appears to be mediated by S1P-driven chemotaxis [113]. It is also known to be important for the migration of other cell types including myofibroblasts [114], osteoclast precursors [115] and hepatic stellate cells [116]. S1P signaling is also thought to be important in multiple sclerosis provided the drugs modulating S1P receptor activity could be used as good therapeutic options for the relapsing disease [117, 118]. Furthermore, S1P appears to be a promising target for the treatment of rheumatoid arthritis (as reviewed in [119]) and asthma [120]. Interestingly, in a study of murine collagen-induced arthritis model, two isoenzymes responsible for the production of S1P (sphingosine kinase 1 and sphingosine kinase 2) were shown to have distinct cellular functions [121].This surprising finding was also confirmed by other groups in different models [122-124]. In one of those studies, SphK1 and SphK2 were shown to have opposing functions for the regulation of ceramide biosynthesis [124]. Unlike SphK1, SphK2 was found to be inhibitory for cell growth through calcium-mediated apoptosis. Authors of this study argue that cellular location of the S1P product is deterministic to its function as evidenced by anti-apoptotic to pro-apoptotic conversion of SphK1 upon targeting to ER.

SPHINGOLIPIDS IN THE PERSPECTIVE OF CHEMOTHERAPEUTIC RESPONSE

As seen above, bioactive sphingolipids play important roles in the regulation of several cellular processes. Programmed cell death and the opposing activation of pro-survival mechanisms are among

those processes which are regulated by sphingolipids, at least in part. Therefore, not surprisingly, sphingolipids are implicated in the chemotherapeutic response of various anti-cancer agents on various types of malignancies. By numerous studies it was shown that accumulation of apoptotic sphingolipids such as ceramide and sphingosine is responsible for the cytotoxicity of the chemotherapeutic agent in question. For instance, accumulation of ceramide was found to damage mitochondria and induce apoptosis in a caspase-independent manner upon curcumin treatment in prostate cancer cells [125]. Another study supported this observation by showing C6 ceramides contribute to sensitization to curcumin in melanoma cells [126]. Generation of ceramides was shown to reduce the viability of hormone-resistant prostate cancer cells upon treatment with the cannabinoid R+ methanandamide as evidenced by the reversal of the cytotoxicity by the addition of ceramide synthase inhibitor Fumosin B1 [127]. In gastrointestinal tumors, combination of targeted therapeutics *vorinostat* and *sorafenib* upregulated CD95 through ceramide signaling which in turn resulted in the increase of reactive oxygen species for killing the cancerous cells [128]. *De novo* pathway is suspected to be the important mechanism in this type of cytotoxicity as evidenced by the increased levels of dhCer which is the intermediate product of this pathway. A similar observation was done in ovarian cancer cells treated with synthetic retinoids [129]. In this study, mass spectrometry analysis identified more than 30 species of sphingolipids, dhCer species being in particular, increased upon drug treatment,. Etoposide induced apoptosis through increasing intracellular ceramides by activating serine palmitoyltransferase enzyme in Molt-4 human ALL cells [130]. Fludarabine induced apoptosis in WSU and JVM-2 CLL cells through inducing *de novo* ceramide generation and increasing sphingomyelinase enzyme activity [131]. Histone deacetylase inhibitor LAQ824 triggered apoptosis in U937 cells through ceramide generation by activating acid sphingomyelinase [132]. Tricyclodecan-9-yl-xanthogenate induced apoptosis in U937 cells in a time and dose-dependent manner by inhibiting SMS activity and increasing intracellular levels of ceramides [133]. Photodynamic treatment resulted in a time-dependent ceramide accumulation in U937 cells [134]. Sodium nitroprusside elevated ceramide levels through increasing the activity of neutral sphingomyelinase enzyme activity in acute promyelocytic leukemia cells [135]. Ionizing radiation resulted in hydrolysis of SM and generation of ceramide through the activation of neutral sphingomyelinase in TF-1-33 AML cells [136]. Diphtheria toxin conjugated to granulocyte-macrophage colony-stimulating factor (DT(388)-GM-CSF) elevated ceramide levels significantly and decreased the viability in vincristine resistant HL60 cells. Similar results were obtained for parental sensitive counterparts [137]. Treatment of HL60 cells with cytosine arabinoside increased ceramide levels in a time- and dose-dependent manner. Researchers detected these increases as early as 5 min after cytosine arabinoside exposure. On the other hand, cytosine arabinoside also activated neutral sphingomyelinase [138]. Forced expression of SK1 in LAMA84 CML cells inhibited imatinib-induced apoptosis while inhibition of SK1 with F-12509a or application of SK1 siRNA induced apoptosis in both parental sensitive and imatinib-resistant LAMA84 cells [139].

In majority of the studies presented above and shown in Fig. (**2**), elevation of the apoptotic sphingolipids was found to be coincident with the chemotherapy, suggesting that these sphingolipids may be playing effector roles in those mechanisms. In agreement with this point of view, there are numerous other reports in which exogenous sphingolipids (short chain ceramides in particular) were provided to the cells and cancer eradication was obtained as well [140-142]. In fact, providing synthetic sphingolipids might have better outcomes for therapeutic purposes since availability and rapid metabolism of natural sphingolipids limit their utilization. FTY720 is a chemically synthesizable analog for sphingosine [143] which was shown to be effective for the treatment of CLL and CML [144, 145]. Mechanism of action is dependent on protein phosphatase 2A (PP2A) activation resulting in shutting down of the signaling pathways driving leukomogenesis [146-148]. While FTY720 is phosphorylated by sphingosine kinases (where SphK2 has higher potential for this process) [149, 150]; VPC23019 is a synthetic compound analogous to S1P which directly antagonizes S1P(1) and S1P(2) receptors by competing with S1P [151]. However novel compounds are still being synthesized to enhance the solubility and *in vivo* efficacy of such antagonists [152]. These studies show that particular sphingolipid species can be used for obtaining better clearance of cancerous cells as distinct chemotherapeutic options. Addressing the same issue, many other papers have shown that manipulating sphingolipid metabolism might have important applications for obtaining better responses to conventional chemotherapy and for overcoming chemotherapeutic resistance developed by many tumors in the course of cancer progression. The main logic behind those studies can be summarized as suppressing the enzymes responsible for the production of growth-promoting sphingolipids [153, 154] and/or activating enzymes responsible for the production of apoptotic ceramides and sphingosine [155, 156]. Since efficacy of such an approach has been proven by several studies, it is currently a hot topic for developing new therapies to the variety of cancer types (as reviewed in [35, 157]). Short chain ceramides with apoptotic induction capabilities [158]; GluCer and S1P with undesired contribution to drug resistance and cancer cell survival [78, 79, 159] come forward as good targets for manipulation in this manner. Sphingolipids might play effector roles as seen in the numerous studies above; but they are also utilized for better drug delivery options. There are some studies indicating that sphingolipid-conjugated nanoparticles are more effective in delivering the chemotherapeutic agent to cancer cells specifically [160, 161]. This approach was tested in various xenograft and syngeneic tumor models in mice and proven to be effective for better responses to chemotherapy [162, 163]. Taken together, gathered data attribute an important role to sphingolipid metabolism and its individual components for the development of effective therapeutics and for overcoming drug resistance which is a major challenge in clinic for obtaining the desired response to the chemotherapeutic options in hand.

TARGETING SPHINGOLIPID METABOLISM FOR THE TREATMENT OF LEUKEMIAS

Leukemia research is one of broadest areas in which targeted therapies are most advanced lately. The more we understand the characteristics of malignant transformation in blood-forming tissues, the more we develop therapeutic options that work specifically on cancer cells and give the least damage to healthy cells in the body as possible. However, sadly enough, not all types of blood cancers are provided with such therapeutic options. Besides that, in the course of treatment, some leukemias develop resistance even to targeted drugs and disease relapses with more aggressive phenotypes being refractory to multiple other chemotherapeutic drugs. These undesired outcomes make researchers look for new therapies overcoming the caveats of the current options. Sphingolipid metabolism appears to be a good target for that purpose. This section of the article is concentrated on possible applications of this approach for the treatment of leukemias.

Acute lymphoblastic leukemia (ALL) occurs mainly at childhood with unknown direct causative factors. However some genetic changes altering the expressions of hematopoietic transcription factors are suspected to be responsible for the malignant transformation [164] (for more detailed information about characteristics of ALL, please see reviews [165] and [166]). A recent report showed that membrane-bound sialidase, an enzyme responsible for the degradation of gangliosides, was downregulated in ALL; and when sialidase was overexpressed, apoptotic induction was obtained with the concomitant increase in ceramide levels and decrease in lactosylceramide [167]. By another report, ganglioside GD3 was found to be upregulated [168] and O-acetylated in ALL cells rendering it

unable to induce apoptosis suggesting that it is a survival trick adopted by leukemic cells [169]. As mentioned briefly in the previous sections, retinoid 4-HPR causes elevation of ceramide levels and induces apoptosis. This induction was shown to be malignancy-specific for ALL cells as evidenced by increased cytotoxicity in leukemic cells whereas non-transformed cells were unaffected [170]. Taken together, those data indicate interventions increasing cellular ceramide levels might be a promising approach for effective ALL therapies. Gangliosides such as GD3 might have important implications for the development of immunotherapy to ALL on the other hand. In this approach, highly upregulated gangliosides on the cell surface could be used as markers of malignant cells. Another study showed that significant amounts of antibody-dependent cellular cytotoxicity could be obtained in serum when ALL cells are treated with monoclonal antibodies recognizing GD3 [171]. In addition to this, CD1d-bearing ALL subsets are known to present α-galactosylceramide to CD1d-restricted T-cells [172]. Even though this event was shown as a poor prognostic marker, it may lead to new immunotherapeutic interventions. For instance, α-galactosylceramide-pulsed antigen presenting cells and *in vitro* expanded natural killer T cells (NKT cells) were shown to be effective for immunotherapeutic clearance of solid tumors when delivered to tumor microenvironment [173-175]. While not all α-galactosylceramide analogs are effective in the same manner [176, 177], KRN7000 in particular, was shown to stimulate invariant natural killer T cells (iNKT cells) and evoke an immune response against viruses and tumors [178-180]. Because of its potential, there has been phase-I and phase-II trials assessing the efficacy of KRN7000 in various solid tumors and myelomas (with the identifiers of NCT00003985 and NCT00698776 respectively) [181-185].

Acute myeloid leukemia (AML) originates from myeloid lineage unlike ALL. Radiation and chemotherapy are among the causative factors identified so far [186]. Ceramide appears to be important in the chemotherapeutic cytotoxicity for AML cells as well [187, 188]. Studies with AML cells showed that ceramides might be important for heat-shock induced apoptosis [189]. Immunologic clearance of leukemic cells by TRAIL-induced apoptosis was observed after pulsing dendritic cells with α-galactosylceramide but not prior to pulsing, suggesting that other sphingolipids might have roles for other effective therapeutic approaches [190]. Interestingly, ceramides were shown to be potent to induce differentiation in AML cells which somewhat show the characteristics of immature stem cells [131, 191]. Some gangliosides, neolacto-series and GM3 in particular, have similar roles for the differentiation of leukemic cells [192, 193]. P-glycoprotein, P-gp, (protein product of *multi drug resistance 1* gene, *MDR1*) is responsible for the drug resistance developed by different types of blood cancers including AML. P-gp was shown to induce conversion of ceramide to GluCer allowing cancer cell survival in addition to its direct drug-efflux functions [194, 195]. Overexpression of sphingosine kinase which is responsible for the production of proliferative S1P was shown to cause *MDR1*-associated chemotherapeutic resistance in AML cell lines [196]. This observation was supported by other papers indicating that S1P upregulation is an important oncogenic achievement in the cancer progression producing refractory subtypes [197]. In the light of the data attributing essential roles to sphingolipids in the leukomogenesis and drug resistance, targeting sphingosine kinase pathway might be a promising approach to overcome chemoresistance for AML in particular.

Chronic lymphocytic leukemia (CLL) is the most common form of leukemia and it is characterized by elevated levels of immature white blood cells in bloodstream similarly to other leukemias, but CD5-positive B cells being in particular for this case. Various studies have shown that CLL is manifested by the lack of apoptosis as evidenced by accumulation of G_0 quiescent cells [198]. Therefore re-activation of apoptotic pathway might be an effective approach to eradicate CLL. One aggressive form of this leukemia was shown to be efficiently eradicated and complete remission was obtained in rats through the delivery of liposomal short chain ceramides that target survivin pathway [199]. Besides this, lipid rafts and particular gangliosides are thought to have implications for obtaining better clearance of leukemic cells. GM1 and GM3 were found to be elevated in B-cell neoplasms as evidenced by various biochemical assessments [200, 201]. Even though studies making this discovery couldn't provide a satisfying explanation about their specific roles in malignancy, those sphingolipid species may have importance as diagnostic and therapeutic markers. Alemtuzumab, an antibody recognizing CD52 membrane proteins for immunotherapy, was shown to induce caspase-dependent cell death through lipid rafts which are rich in GM1 ganglioside [202]. Similar to ALL cases mentioned above, CD1d marker is also expressed on CLL cells and functions as α-galactosylceramide presenting protein which may lead to development of novel immunotherapies to CLL through a similar approach that targets CD1d [203].

Chronic myeloid leukemia (CML) is maybe the most well-characterized leukemia type among others. A reciprocal translocation between chromosomes 9 and 22 is the driving force of malignant transformation in majority of the CML cases. That translocation results in the production of a fusion protein called BCR-ABL which shows constitutive tyrosine kinase activity in the cells [204]. BCR-ABL phosphorylates key proteins which in turn initiate proliferation and cell division. CML is the first leukemia type to which targeted chemotherapies are developed. There are drugs specifically binding to the fusion protein and blocking its kinase activity yielding reduction of leukemic cells in bloodstream to almost undetectable levels [205, 206]. Similar to what is seen above; ceramides are thought to be important mediators of apoptosis in CML cells, and the delicate balance in sphingolipid rheostat was shown to be important for chemoresistance [207]. BCR-ABL might be competing with ceramides for providing survival abilities to leukemic cells as shown by a study indicating Abl kinase as a negative regulator of Fas-mediated cell death which is a convergent pathway for ceramide signaling [208, 209]. In addition to the importance of ceramides, some gangliosides (GM3 in particular here as well) were shown to be potent for inducing megakaryocytic differentiation of CML blasts [210, 211]. Moreover, desialylation of some glycoproteins by Neu2, a cytosolic sialidase, might block BCR-ABL/Src signaling [212]. Another report might be providing supportive evidence for such convergent mechanisms by showing that BCR-ABL regulates membrane GM1 levels and expression of ligands for natural killer cell receptors [213]. In this study, treatment of BCR-ABL positive cells with imatinib, a specific tyrosine kinase inhibitor, modulated levels of those molecules indicating that BCR-ABL is the actual factor responsible for this regulation. Some surface glycosphingolipids such as fucosylated gangliosides were shown as possible targets for immunotherapeutic interventions for CML since they may participate into the antigen presentation processes [214, 215].

CONCLUSION AND FUTURE PERSPECTIVES

Regulation of cell death and survival is a complex process involving numerous pathways and dozens of proteins. Accumulated scientific knowledge so far attributed new roles to sphingolipids in this regulation besides their structural roles in membranes. Involvement of sphingolipids in these mechanisms appears to be not less important than previously known proteins and other small signaling molecules. In fact, deregulation of sphingolipid metabolism is now known as the causative factors for several diseases including but not limited to metabolic disorders and cancer. Moreover, with each study completed, scientists are discovering that sphingolipids are important mediators for mechanisms of action of several drugs, adding more importance to sphingolipids for the development of better therapeutic options, especially in the cancer area. Numerous reports have shown that altered ceramide metabolism upon chemo-

therapy is a major contributor to the cytotoxicity [216-218]. These experiments showed that inhibition of enzymes responsible for the production of apoptotic sphingolipid species (or in some cases overexpression of opposing enzymes) abrogates the cytotoxicity of the drug, and conversely overexpression of the same enzymes correlated with increased chemotherapeutic sensitivity suggesting that certain sphingolipid species are important for cytotoxicity of the drug [219-221]. Some other studies have also shown that therapeutic efficacy of cancer drugs can be significantly enhanced in combination with growth-suppressive sphingolipids such as ceramides and sphingosine [222-224]. Therefore, manipulation of sphingolipid metabolism comes forward as a good intervention approach for novel therapies [225]. Importance of some sphingolipid species in chemotherapeutic response and upregulation of proliferative types in the case of drug resistance make researchers think that chemotherapeutic outcome can be improved significantly through altering sphingolipid metabolism. This approach provides an exciting hot topic for the development of better therapeutics in the future (as reviewed in [226] and [35]). Acute and chronic leukemias are among the cancer types upon which we built majority of our knowledge about the involvement of sphingolipids in cancer therapeutics. Hence, leukemias might be good starting points for incorporation of sphingolipids for new treatments. Specificity of some sphingolipid species for malignant cells makes this approach feasible and exciting enough to pursue. However, for applying sphingolipid based therapeutics to several other types of leukemias and cancers, we may need to develop ways to deliver cytotoxic species specifically to the malignant cells. By this way, this approach might be generalized for other types of cancers as well to obtain enhanced eradication of the malignant cells. In the light of the accumulating literature in this area, it is suffice to say that sphingolipid metabolism holds a significant importance for the future therapeutics of various cancers and shows promise for better results in clinic that would bring us closer to the ultimate aim: changing the nature of cancer from being deadly in many cases, to being curable.

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