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Minireview

Role of Sphingolipids and Metabolizing Enzymes in Hematological Malignancies

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Sphingolipids such as ceramide, sphingosine-1-phosphate and sphingomyelin have been emerging as bioactive lipids since ceramide was reported to play a role in human leukemia HL-60 cell differentiation and death. Recently, it is well-known that ceramide acts as an inducer of cell death, that sphingomyelin works as a regulator for microdomain function of the cell membrane, and that sphingosine-1-phosphate plays a role in cell survival/proliferation. The lipids are metabolized by the specific enzymes, and each metabolite could be again returned to the original form by the reverse action of the different enzyme or after a long journey of many metabolizing/synthesizing pathways. In addition, the metabolites may serve as reciprocal biomodulators like the rheostat between ceramide and sphingosine-1-phosphate. Therefore, the change of lipid amount in the cells, the subcellular localization and the downstream signal in a specific subcellular organelle should be clarified to understand the pathobiological significance of sphingolipids when extracellular stimulation induces a diverse of cell functions such as cell death, proliferation and migration. In this review, we focus on how sphingolipids and their metabolizing enzymes cooperatively exert their function in proliferation, migration, autophagy and death of hematopoetic cells, and discuss the way developing a novel therapeutic device through the regulation of sphingolipids for effectively inhibiting cell proliferation and inducing cell death in hematological malignancies such as leukemia, malignant lymphoma and multiple myeloma.

INTRODUCTION

Sphingolipids have been emerged as a bioactive lipid to regulate a myriad of cell functions including cell death, proliferation/survival, autophagy, migration, secretion and immunity (Adada et al., 2014; Maceyka and Spiegel, 2014; Truman et al., 2014). Among sphingolipids, ceramide was firstly reported to induce cell differentiation and death in human leukemia HL-60 cells (Obeid et al., 1993; Okazaki et al., 1989). To clarify the mechanism to induce cell death, the subcellular compartmentalization of active ceramide, the putative diverse function among ceramide molecular species and its regulation by metabolic enzymes have been investigated in several kinds of cancers (Gault et al., 2010; Park et al., 2014). Recently, sphingosine-1-phosphate (S1P) and sphingomyelin (SM) are also recognized as biological regulators in many cell functions. Ceramide induces apoptosis, autophagy and senescence (Truman et al., 2014) while S1P acts as an extracellular mediator for cell survival and migration, an inhibitor of autophagy and a regulator of innate and adaptive immunity (Degagne and Saba, 2014). SM has been thought as a reservoir of ceramide because ceramide is generated through hydrolysis of SM by sphingomyelinases (SMases). Additionally, it becomes clear that SM *per se* modulates the trans-membrane signals through microdomains and the intracellular vesicular trafficking (Taniguchi and Okazaki, 2014). Therefore, at present the metabolic regulation of bioactive sphingolipids takes an attention as a key for understanding cell death, proliferation and migration in the pathological malignant condition. It is critical to clarify the role for sphingolipids in the regulation of the signal balance between cell proliferation/survival and death, in order to develop a novel therapy for hematological malignant disorders such as leukemia, malignant lymphoma and multiple myeloma.

In this review, we focus on the recent progress of the research for investigating the biological implications of sphingolipids in the regulation of hematological malignant cells and show a bird's-eye view image of sphingolipid action for developing a novel therapeutic way. We also discuss the intracellular localization of sphingolipids and their metabolizing enzymes and the reciprocal role of sphingolipids in cell proliferation/survival and death in hematopoietic cells.

METABOLISM OF SPHINGOLIPIDS (CERAMIDE, S1P, AND SM)

Sphingolipid anabolism and catabolism are important for cellular homeostasis and a number of enzymes are involved in the metabolism. Intensive studies revealed individual responsible genes, biochemical characteristics, subcellular localization and regulation (Fig.1 and Tables 1-4).

Individual enzyme is compartmentalized in specific subcellu-

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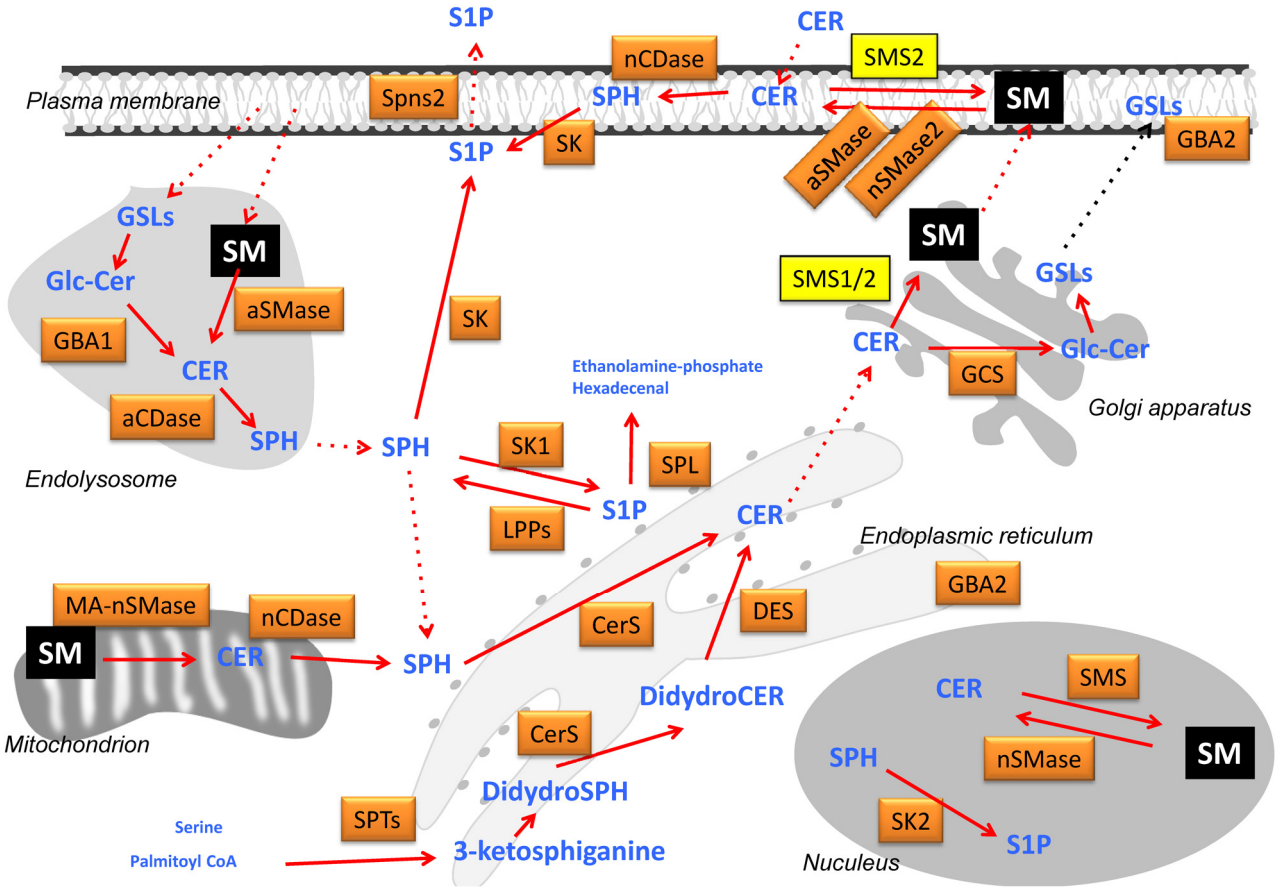

Fig. 1. Sphingolipid metabolism and subcellular compartmentalization. A diverse sphingolipids are synthesized and degraded through complex metabolizing pathways in each intracellular compartment. For example, SM (colored by yellow) is synthesized in Golgi apparatus by SMS1 and SMS2, and in plasma membrane by SMS2, and resides in endosomes, lysosomes, mitochondria and nucleus. Each SM is suggested to play a role as a regulator of micro-organelles and a source of other bioactive sphingolipids.

lar organelle. In endoplasmic reticulum, *de novo* synthesis of sphingolipids begins with the condensation of palmitoyl-CoA and serine by the catalytic action of serine palmitoyl acyltransferase (SPT) (Hanada, 2003; Hanada et al., 2000), generating 3-ketosphinganine. This intermediate is converted to dihydrosphingosine that serves as a substrate for ceramide synthase (CerS) to form dihydroceramide. Ceramide desaturase (DES1) (Rodriguez-Cuenca et al., 2015) catalyzes the synthesis of ceramide from dihydroceramide, which is the last step for the *de novo* synthesis of ceramide. Those steps occur in endoplasmic reticulum, and ceramide serves as a building block for most of sphingolipid species. Transport of ceramide by ceramide transfer protein (CERT) (Hanada et al., 2003; Yamaji and Hanada, 2015) and/or other transporting protein (s) to the Golgi are required for the synthesis of ceramide-1-phosphate, SM, galactosylceramide, and glucosylceramide. The last glycolipids are further metabolized to complex sphingolipids.

Most of sphingolipid catabolizing enzymes are localized in endolysosomes, resulting in the formation of lysosomal ceramide (Futerman and Hannun, 2004; Futerman and Riezman, 2005). For instance, lysosomal acid-β-glucosidase (GBA1) (Dinur et al., 1986; Grabowski, 1993) cleaves glucosylceramide to form ceramide. The lysosomal ceramide is further catabolized to sphingosine by ceramidase (CDase) (Park and Schuchman, 2006). This sphingolipid backbone sphingosine is exploited to generate ceramide through the catalytic action of ceramide synthase at endoplasmic reticulum. This is termed the "salvage pathway" of sphingolipid synthesis (Kitatani et al., 2008; Tettamanti et al., 2003). Exogenous short chain ceramide such as C_6-ceramide is recycled into long chain ceramide via that pathway (Kitatani et al., 2008). Alternatively, sphingosine is phosphorylated by sphingosine kinase (SK) (Pitson, 2011), forming S1P that is also degraded or dephosphorylated by S1P lyase (SPL) (Saba and de la Garza-Rodea, 2013) or S1P phosphatase (SPP) (Pyne et al., 2009), respectively.

Importantly, ceramide transported to Golgi by CERT is converted to SM by the catalytic action of SM synthase (SMS), and then SM is distributed to plasma membranes and functions as a component of lipid microdomains. On the other hand, SMase hydrolyses SM to form ceramide in various subcellular organelles. This metabolic cycle between ceramide and SM is known as a "SM-ceramide cycle." Ceramide serves a central lipid in sphingolipid metabolism and is produced *via* three pathways including *de novo* synthesis, salvage pathway, and SM-ceramide cycle.

Table 1. SMS family

Name	Developer	SMS1	SMS2	SMSr
Gene symbol (Locus)	Human	*SGMS1* (Chr.10 q11.2)	*SGMS2* (Chr.4 q25)	*SAMD8* (Chr.10 q22.2)
	Mouse	*Sgms1* (Chr.19 C1)	*Sgms2* (Chr.3 G3)	*Samd8* (Chr.14 B)
Amino Acids	Human	413	365	414
	Mouse	419	365	432
Localization		Golgi	Golgi, Plasma membrane (PM)	ER
Expression (high)		Ubiquitous (islet, testis)	Ubiquitous (liver, kidney, intestine)	Ubiquitous (brain)
Enzymatic effect	Substrate ↓ Product	Cer + PC ↓ SM + DAG Cer + PE ↓ CPE	Cer + PC ↓ SM + DAG Cer + PE ↓ CPE	Cer + PE ↓ CPE

Cer, Ceramide; CPE, Ceramide phosphoethanolamine; DAG, Diacylglycerol; PC, Phosphatidylcholine; PE, Phosphatidylethanolamine

Table 2. SMase family

		Acid		Neutral				Alkaline
Protein		L-aSMase (lysosome)	S-aSMase (secreted)	nSMase1	nSMase2	nSMase3	MA-nSMase	alk-SMase
Gene symbol (Locus)	Human	*SMPD1* (Chr.11 p15.4-15.1)		*SMPD2* (Chr.6 q21)	*SMPD3* (Chr.16 q22.1)	*SMPD4* (Chr.2 q21.1)	—	*ENPP7* (Chr.17 q25.3)
	Mouse	*Smpd1* (Chr.7 E3)		*Smpd2* (Chr.10 B2)	*Smpd3* (Chr.8 D2)	*Smpd4* (Chr.16 B1)	*Smpd5* (Chr.15 D3)	*ENPP7* (Chr.11 E2)
Amino acids	Human	629		423	655	866	—	458
	Mouse	627		498	655	823	483	446
Localization		Lysosome /Endosome PM	Extracellular	ER	Golgi PM	ER Golgi PM	Mitochondria	—
Expression (High)		Ubiquitous		Ubiquitous (kidney)	Ubiquitous (brain, spleen)	Ubiquitous (muscle, heart)	Ubiquitous (testis, pancreas, brain)	Intestinal mucosa
Enzyme Substrate/product		SM/Cer		SM/Cer (in *vitro*)	SM/Cer	SM/Cer	SM/Cer	SM/Cer
Cation		no need	Zn^{2+}	Mg^{2+}	Mg^{2+}	Mg^{2+}	Mg^{2+}·Mn^{2+}	Inhibition by Zn^{2+}
Optimal pH		pH 5.0		pH 7.5	pH 7.5	pH 7.5	pH 7.5	pH 9.0

Cer, Ceramide

SMS

SMS is an enzyme that catalyzes the transfer of the phosphocholine head group of phosphatidylcholine to ceramide, generating SM (Table 1). SMS family consists of three isoforms including SMS1, SMS2, and SMSr (Tafesse et al., 2006; Taniguchi and Okazaki, 2014; Yamaoka et al., 2004). The former two enzymes, but not last one, catalyze sphingomyelin synthesis, whereas all three have the activities in synthesis of the sphingomyelin analogue ceramide phosphoethanolamine even though its ability in SMS1 and SMS2 is so faint (Ding et al., 2015). SMS1 is a Golgi-resident enzyme and SMS2 is localized at plasma membrane and Golgi (Huitema et al., 2004; Tafesse et al., 2006; Taniguchi and Okazaki, 2014). SM synthesized at the Golgi is delivered to plasma membrane through the vehicle trafficking. The possible existence of SMS was shown by the enzymatic activity in the nucleus (Watanabe et al., 2004), but the precise implication of SMS and SM in the nucleus is still unclear. Plasma membrane-resident SMS2 is probably in-

Table 3. CerS family

		CerS1	CerS2	CerS3	CerS4	CerS5	CerS6
Other names		LASS1 UOG1	LASS2 TRH3	LASS3 T3I	LASS4 TRH1	LASS5 TRH4	LASS6
Gene symbol (Locus)	Human	*CERS1* (Chr.19 p12)	*CERS2* (Chr.1 q21.3)	*CERS3* (Chr.15 q26.3)	*CERS4* (Chr.19 P13.2)	*CERS5* (Chr.12 q13.12)	*CERS6* (Chr.2 q24.3)
	Mouse	*Cers1* (Chr.8 E3.3)	*Cers2* (Chr.3 F2)	*Cers3* (Chr.7 C)	*Cers4* (Chr.8 A1.2)	*Cers5* (Chr.15 F1)	*Cers6* (Chr.2 C2)
Amino acids	Human	239, 337, 350	380	383, 394	394	334, 392	384, 392
	Mouse	350	380	419	393	414	384
Localization		ER					
Expression (High)		Brain, testis skeletal muscle	Kidney, liver	Testis, skin	Ubiquitous:low (skin, heart, liver)	Ubiquitous: low	Ubiquitous: low
Enzyme Substrate/product				Sph/Cer (salvage pathway)	dHSph/dHCer (*de novo* synthesis)		
Acyl-chain length specificity		C18	C20-C26	C22-C26	C18, C20	C14, C16	C14, C16
K_m towards dHSph (μM)		2.5 ± 1.7	4.8 ± 0.4	1.7 ± 0.4	1.8 ± 0.4	1.8 ± 0.4	2.0 ± 0.6

Cer, Ceramide; dHCer, Dihydroceramide; dHSph, Dihydroshingosine; Sph, sphingosine

Table 4. CDase family

		Acid	Neutral	Alkaline		
Protein		aCDase	nCDase	Alk-CDase1	Alk-CDase2	Alk-CDase3
Gene symbol (Locus)	Human	*ASAH1* (Chr.8 p22)	*ASAH2* (Chr.10 q11.21)	*ACER1* (Chr.19 p13.3)	*ACER2* (Chr.9 p22.1)	*ACER3* (Chr.11 q13.5)
	Mouse	*Asah1* (Chr.8 A4)	*Asah2* (Chr.19 C3)	*Acer1* (Chr.17 D)	*Acer2* (Chr.4 C4)	*Acer3* (Chr.7 E2)
Amino acids	Human	389, 411	745^{short},780^{long}	264	275	172, 230, 267
	Mouse	394	756	273	219, 229, 275	267
Localization		Lysosome	PM^{long} $Mitochondria^{short}$	ER	Golgi	ER Golgi
Expression (High)		Kidney, lung, placenta brain, lskeltal muscle (heart)	Ubiquitous (kidney, skeletal muscle, heart)	Ubiquitous (skin)	Pancreas, heart (placenta)	Ubiquitous (placenta)
Enzyme Substrate/product		Cer/Sph	Cer/Sph	Cer/Sph	Cer/Sph	Cer/Sph
Cation	Activated	—	None	Ca^{2+}	Ca^{2+}	Ca^{2+}
	Inhibited	—	None	Zn^{2+}, Cu^{2+}, Mn^{2+}	None	Zn^{2+}
Optimal pH		pH 4.5	pH 7.5-8.5	pH 8.5	pH 9.0	pH 9.5

Cer, Ceramide; Sph, sphingosine

volved in turnover of the SM-ceramide cycle where SMase generates ceramide (Tani and Kuge, 2009). Whereas SMSr localizes in endoplasmic reticulum and mainly produces ceramide phosphoethanolamine (Vacaru et al., 2009). Moreover, cellular ceramide phosphoethanolamine amount is little and its function remains unknown. SMSr seems to work for maintaining ceramide balance rather than producing ceramide phosphoethanolamine.

SMase

SM is the most abundant in sphingolipids and constitutes microdomains at the membrane (Miyaji et al., 2005). SMase hydrolyses SM to form phosphocholine and ceramide and has been classified into three groups including acid SMase (aSMase) (Jenkins et al., 2009), alkaline SMase (Alk-SMase) (Duan, 2006) and neutral SMase (nSMase) (Airola and Hannun,

2013; Clarke et al., 2006), according to the biochemical characteristics (Table 2). Presently, five human SMases encoded by 5 distinct genes have been cloned: aSMase (*SMPD1*), nSMase1 (*SMPD2*), nSMase2 (*SMPD3*), nSMase3 (*SMPD4*), and Alk-SMase (*ENPP7*). MA-nSMase (*smpd5*) is found in mouse but not in human resides in mitochondria and its optimal pH is 7.5.

aSMase

aSMase (Jenkins et al., 2009) is characterized by acidic pH optima for enzymatic activity and contributes sphingolipid catabolism. Its cDNA rises to a polypeptide of 629 amino acids. Enzymatic dysfunction of aSMase results in Niemann-Pick disease type A and B (Schulze and Sandhoff, 2011). aSMase exists as two enzymatic isoforms such a zinc-independent lysosomal aSMase and a zinc-dependent secretary aSMase. Lysosomal aSMase arises from mannose-6-phosphorylation of N-glycans required for targeting to the endolysosomal compartments. This enzyme is predominantly localized in the endolysosomal compartments, although it has been shown to translocate to the plasma membranes in responses to stimuli (Grassme et al., 2003; Zeidan et al., 2008). Secretary aSMase is trafficked through the Golgi pathway and then extracellularly released.

nSMase1

nSMase1 was cloned by homology search of bacterial SMase as a Mg^{2+}-dependent nSMase (Tomiuk et al., 1998) and has 423 amino acids. nSMase1 exhibits SMase activity *in vitro*, but could not regulate amounts of ceramide or SM in nSMase1-overexpressing cells or knockout mice (Sawai et al., 1999; Zumbansen and Stoffel, 2002). Instead of SMase activity, therefore, nSMase1 may act as phospholipase C that hydrolyzes lyso-phosphatidylcholine or lyso-platelet activation factor.

nSMase2

nSMase2 (Airola and Hannun, 2013; Clarke et al., 2006) is also a Mg^{2+}-dependent nSMase and human nSMase2 cDNA rises to a polypeptide of 655 amino acids. This enzyme containing two hydrophobic segments near the N-terminal region is localized to the plasma membrane and Golgi apparatus. In cell-based studies, nSMase2 is well-investigated to produce ceramide in response to inflammatory cytokine or apoptosis-inducing agents (Shamseddine et al., 2015).

nSMase3

nSMase3 has been found from homology search of nSMase1 and nSMase2 (Krut et al., 2006; Moylan et al., 2014). Human nSMase3 encodes 866 amino acids and exhibits ubiquitous expression in organs. Especially, nSMase3 strongly expresses in skeletal muscle and heart. Its enzyme is localized in endoplasmic reticulum, Golgi apparatus, and plasma membrane.

MA-nSMase

MA-nSMase has recently identified from homology search of zebrafish mitochondrial SMase in mouse, and its cDNA is translated to polypeptides of 483 amino acids (Wu et al., 2010). MA-nSMase has mitochondrial localization signal in N-terminus and requires cations such as Mg^{2+} and Mn^{2+} for its full catalytic activity. However, human orthologue of MA-nSMase is not found yet.

Alk-SMase

Alk-SMase has 458 amino acids in human and expresses in intestine. Its enzyme has been implicated in digestion of dietary SM in intestinal mucosa to produce ceramide that is further catabolized to sphingoid base (Nilsson and Duan, 2006). Moreover, Alk-SMase was shown to contribute to intestinal absorption of cholesterol (Zhang et al., 2014).

De novo ceramide synthesis

CerS (Mizutani et al., 2009; Park et al., 2014) catalyzes N-acylation of sphinganine or sphingosine at the C2-position to form dihydroceramide or ceramide, respectively. CerS activity has been firstly identified in microsome fractions (Morell and Radin, 1970). The enzyme was known to utilize a variety of fatty acyl-CoA. So far, six genes encoding mammalian CerS have been identified, named as CerS1-6, and all of which are endoplasmic reticulum-resident proteins (Table 3) (Mizutani et al., 2009; Park et al., 2014). Those CerS family enzymes display distinct substrate specificity toward carbon chain length of acyl-CoAs, although the hydroxylation and saturation status in the acyl-CoAs is unlikely to influence their specificity.

CDase

CDase catalyzes hydrolysis of ceramide to generate sphingosine (Mao and Obeid, 2008) (Table 4). According to the primary structure and the optimal pH in the enzymatic activity, CDase has been classified into three groups: acid CDase (aCDase) (Park and Schuchman, 2006), neutral CDase (nCDase) (Ito et al., 2014), and alkaline CDase (Alk-CDase) (Mao and Obeid, 2008). aCDase and nCDase are encoded by *ASAH1* and *ASAH2*, respectively. At present, 3 distinct human Alk-CDase (Mao and Obeid, 2008) have been cloned: Alk-CDase1 (*ACER1*), Alk-CDase2 (*ACER2*), and Alk-CDase3 (*ACER3*).

aCDase

aCDase is a lysosome-resident enzyme with a pH optimum of 4.5. This enzyme activity was first identified over five decade ago and is deficient in the inherited sphingolipid lysosomal storage disorder, Farber disease (Park and Schuchman, 2006). The full length human cDNA was isolated from skin fibroblasts and pituitary in 1996 (Koch et al., 1996). This aCDase is synthesized as a polypeptide of 389 amino acids (53 kDa), which is processed into a mature heterodimeric enzyme composed of α subunit (13 kDa) and highly-glycosylated β subunit (40 kDa).

nCDase

Human nCDase activity has a pH optimum ranging from 7.5-8.5. Two isoforms of human nCDase have been cloned, a long form composed of 780 amino acids and a short form composed of 745 amino acids (Ito et al., 2014). The long form is predominantly localized at the plasma membrane (Hwang et al., 2005), whereas the short form appeared to be localized to mitochondria (El Bawab et al., 2000)

Alk-CDase1

Human Alk-CDase1 (Mao and Obeid, 2008) is a 264-amino acid protein with a predicted molecular weight of 31 kDa. The optimum pH for enzyme activation is 8.5. It contains several putative transmembrane domains and is localized to the endoplasmic reticulum.

Alk-CDase2

Human Alk-CDase2 (Mao and Obeid, 2008) is a 275-amino acid protein with a predicted molecular weight of 31.3 kDa. The optimum pH for enzyme activation is 9.0. Its protein sequence is highly homologous to Alk-CDase1 and has several transmembrane domains. This enzyme is localized to t Golgi apparatus.

Alk-CDase3

Human Alk-CDase3 (Mao and Obeid, 2008) is a 267-amino acid protein with a predicted molecular weight of 31.6 kDa. The optimum pH for enzyme activation is 9.5. Its protein sequence is also homologous to Alk-CDase1 and Alk-CDase2 and has several transmembrane domains. This enzyme is localized to the endoplasmic reticulum and Golgi apparatus.

SK

SK catalyzes phosphorylation of sphingosine to form S1P (Pitson, 2011; Pyne and Pyne, 2010). This lipid kinase exists as two isoforms, SK1 and SK2. SK1 and SK2 are encoded by different genes, respectively, *SPHKL1* and *SPHK2*. SK1 has three splicing variants that differ only in their N-terminal, termed SK1a, SK1b, and SK1c. SK2 also has two isoforms that appear to arise from alternative start codon usage, termed SK2a and SK2b.

SK1

SK1 (Pitson, 2011; Pyne and Pyne, 2010) normally resides in the cytoplasm. A diverse of range of growth factors, cytokines and other stimuli have been shown to increase SK1 activity and induce its translocation to plasma membrane. The translocated SK1 phosphorylates sphingosine formed by nCDase catalytic action, generating S1P. Importantly, phosphorylation of SK1 at the Ser225 residue is essential for the translocation.

SK2

SK2 (Pitson, 2011; Pyne and Pyne, 2010) has nuclear localization signal in the N-terminal portion, and its subcellular localization is mainly the nucleus and cytoplasm. During cellular responses, it has been shown to be relocalized to the endoplasmic reticulum where S1P phosphatase residues. SK2 shares protein sequence similarity with SK1, although the region containing the Ser225 phosphorylation site responsible for SK1 activation is not conserved in SK2.

SPPs and SPL

SPPs

S1P is generated from sphingosine by a catalytic action of SKs. Sphingosine is regenerated from S1P by the catalytic action of SPP1 and SPP2 which belong to a family of lipid phosphatases (LPPs) such as LPP1, LPP2, LPP3, the *Drosophila* homologues Wunen and Wunen2 (Pyne et al., 2009). SPP1 and SPP2 consist of 430 and 399 amino acids, respectively. The LPPs have broad substrate specificity *in vitro* and are integral membrane proteins with six transmembrane domains, while SPPs have eight transmembrane domains and show a substrate specificity that is limited to S1P, dihydroS1P and phytoS1P. SPPs are magnesium-independent and localized to the endoplasmic reticulum. The main tissue distribution of SPP1 is in kidney and placenta whereas SPP2 predominates in the heart and kidney.

SPL

SPL (Serra and Saba, 2010) is an enzyme that cleaves S1P to form hexadecenal and ethanolamine-phosphate. This enzymatic reaction is irreversible, thereby suggesting that SPL plays an important regulator in determining S1P levels. Human SPL is a 568-amino acid protein with a predicted molecular weight of 63.5 kDa. This protein is an integral membrane protein localized to the endoplasmic reticulum, and the active site of SPL faces the cytosolic compartment. Uniquely, SPL protein was also found in the mitochondrial-associated membrane where is associated with the mitochondria and the endoplasmic reticulum.

Glucosylceramidase

Glucosylceramidase is a β-glucosidase that catalyzes hydrolysis of glucosylceramide to form ceramide and glucose. Three types have been identified, GBA1 (Dinur et al., 1986), GBA2 (Boot et al., 2007; Yildiz et al., 2006), and GBA3 (Hayashi et al., 2007). Lysosomal GBA1 is acid β-glucosidase known as a responsible gene for Gaucher disease. This enzyme is involved in sphingolipid catabolism and also contributes to the re-synthesis of ceramide through the salvage pathway. GBA2 is an endoplasmic reticulum-resident protein. GBA3, identified as a Klotho-related protein (Hayashi et al., 2007), is a cytosolic protein and its activity is insensitive to conduritol B known as an inhibitor for GBA1 and GBA2. Those GBA2 and GBA3 have neutral optimal pH for enzyme activities.

Glucosylceramide synthase (GCS)

Glucosylceramide synthase (Bleicher and Cabot, 2002; Ishibashi et al., 2013) catalyzes the transfer of glucose residue from UDP-glucose to ceramide, generating glucosylceramide that serves as a backbone of 300-400 glycosphingolipids. In 1996, Ichikawa et al. discovered human gene encoding GCS (Ichikawa and Hirabayashi, 1998). This enzyme is localized at Golgi apparatus where ceramide is transported by CERT from endoplasmic reticulum. Interestingly, GCS is unlikely to utilize CERT-transported ceramide for glucosylceramide synthesis, whereas SMS synthesizes SM from ceramide transported by CERT (Hanada et al., 2003; Yamaji and Hanada, 2014).

ROLE OF SPHINGOLIPIDS AND METABOLIZING ENZYMES IN HEMATOLOGICAL MALIGNANCIES

Leukemia

Sphingolipids

Ceramide

A diverse kind of anti-leukemic agents was reported to increase ceramide level in the malignant cells when the cells underwent apoptotic cell death (Carpinteiro et al., 2008; Mullen and Obeid, 2012; Saddoughi and Ogretmen, 2013). Arsenic trioxide using for the treatment of acute promyelocytic leukemia increased ceramide through the activation of *de novo* ceramide synthesis and the inhibition of GCS (Dbaibo et al., 2007). Instead of unsaturated ceramide, a C4-C5 saturated ceramide dihydroceramide was believed not to show cytotoxic effect in leukemia cells. A synthetic retinoid treatment was reported to induce cell death with increase of dihydroceramide in leukemia cells, but the inhibition of *de novo* synthesis by myriocin did not prevent the loss of cell viability even though dihyroceramide decreased (Apraiz et al., 2012). On the other hand, dihydroceramides including $C_{22:0}$- and $C_{24:0}$-dihydroceramides were suggested to contribute to synthetic retinoid-induced cytotoxicity in T cell ALL CCRF-CEM cells (Holliday et al., 2013; Rodriguez-Cuenca et al., 2015). The investigation for function and regulation of dihydroceramides in the process of cell death has just begun.

A growing body of evidence suggests the involvement of ceramide in death ligand- and stress-induced apoptosis. Fas engagement by Fas ligand (FasL) induces apoptosis with ceramide increases in cancer cells. In human leukemia Jurkat cells, inhibition of SMS is proposed to facilitate Fas-induced ceramide increase resulting in caspase-9 activation and cell death. Interestingly, caspases such as caspase-2, -7, -8 and -9 were shown to cleave and inactivate SMS1, suggesting post-translational regulation of SMS (Lafont et al., 2010).

When the patients in acute myeloid leukemia (AML) and chronic myeloid leukemia (CML) became resistant to chemotherapy, the activities of SMS and GCS in the chemo-resistant leukemia blasts increased higher as compared to chemo-sensitive ones, leading to a decrease of ceramide-dependent cell death (Itoh et al., 2003). Therefore, inhibition of SMS and/or GCS is possible therapeutics for chemotherapy-resistant hematological malignancy. Although knockout mice of GCS gene were embryonic lethal, those of SMS1 or SMS2 gene were not lethal. Considering those, specific inhibition of SMS 1 or SMS2 may be clinically valuable not to induce indiscriminate damage in indispensable tissues.

The balance between pro-apoptotic ceramide and pro-survival S1P may be important in drug-induced cell death in K562 CML cells (Baran et al., 2007). Recently in order to increase the cure rate of leukemia, there are many basic investigations to overcome the resistance to anti-cancer reagents by modulating the balance between pro-apoptotic and pro-survival sphingolipids. When acute promyelocytic leukemia-derived HL-60 cells and their multidrug-resistant counterpart (HL-60/VCR cells) were treated with curcumin, firstly nSMase2 was activated and then SMS was inhibited to make biphasic increase of ceramide generation, resulting in the overcome of the chemo-resistance in HL-60/VCR cells (Shakor et al., 2014). In addition, a second generation of BCR-ABL tyrosine kinase inhibitor nilotinib was shown to up-regulate CerS and down-regulate SK1 in chemo-resistant Meg-01 CML cells (Camgoz et al., 2011). When monocytic leukemia U937 cells were treated with a combination of a Bcl-2 family inhibitors ABT-263 (2 μM) and a GCS inhibitor PDMP (45 μM), the synergistic effect on the induction of cell death occurred (Casson et al., 2013). CML K562/IMA cells resistant to imatinib showed the increase of cell death by the simultaneous treatment with PDMP (Baran et al., 2011). Since the concentration of PDMP used in the experiments was too high to keep its specificity for the inhibition of GCS, this combination seems to be difficult to apply to the animal experiment without investigating unknown adverse effects. Importantly, those showed clearly that the increase of ceramide enhanced cell death even in the chemo-resistant leukemia cells.

Short chain ceramide and ceramide liposomes

D-erythro-N-acetylsphingosine (C_2-ceramide) was shown to inhibit the synthesis of a physiological SM due to the production of short chain SM like C_2-SM and to increase physiological ceramide through acylation of sphingosine by CerS, resulting in the induction of a significant level of apoptosis in HL-60 cells (Allan, 2000; Baek et al., 2001). Short chain ceramides such as C_2-ceramide and *D-erythro-N*-hexanoylsphingosine (C_6-ceramide) seem to increase long chain ceramide through recycling/salvage pathway using sphingosine backbone recycling system in KG1 leukemia cells (Chapman et al., 2010; Kitatani et al., 2008). High concentration of C_6-ceramide liposomes (25 μM for 24 h) induced apoptosis of natural killer leukemia cells *in vitro*, and systemic intravenous treatment with C_6-ceramide nanoliposomes induced complete remission in the syngeneic Fischer F344 rat model of aggressive natural killer-large granular leukemia (Liu et al., 2010). Although the accumulation of C_6-NBD-ceramide liposomes in the cells was suggested, the precise manner of the metabolism of nanoliposomal ceramide remains to be elucidated.

Recently the way to regulate glycolysis in cancer cells has been stressed as a target of a novel therapy for the cancer because tumor cells switch the machinery of ATP generation from TCA cycle to glycolysis as described in “Warburg effect”. Since glyceraldehyde-3-phosphate dehydrogenase is a key glycolytic enzyme, it has been emerged as a target for cancer therapy (Ganapathy-Kanniappan et al., 2013). In CLL JVM3 cells, treatment with C_6-ceramide nanoliposomes was shown to promote caspase 3/7-independent necrosis by down-regulating glyceraldehyde-3-phosphate dehydrogenase and its-dependent ATP production (Ryland et al., 2013). Moreover, treatment of natural killer cell leukemia with C_6-ceramide liposomes also enhanced the induction of cell death by the increase of intracellular ceramide in the presence of a GCS inhibitor 1-phenyl-2-palmitoylamino-3-morpholino-1-propanol (Watters et al., 2013). It is thought that ceramides derived from incorporated liposomes are partially metabolized to glucosylceramide and the metabolic inhibition by 1-phenyl-2-palmitoylamino-3-morpholino- 1-propanol promotes the accumulation of ceramide.

Short chain SM and SM liposomes

SM is recognized as an important component to maintain the structure of membrane microdomain while it has been thought as a source of bioactive lipids such as ceramide and S1P. Internalization of the complex of transferrin and transferrin receptor through clathrin-coated pits were up-regulated followed by increases in cell viability, when SM-deficient T lymphoid WR19L/Fas-SMS(-) cells were treated with exogenous C_6-SM (Shakor et al., 2011). Those suggest the modulation of cell proliferation through SM-regulated microdomains.

The effect of SM liposomes consisting of SM has been examined on the regulation of drug pump MDR1 in human lymphoblastic leukemia CEM cells. Unexpectedly SM liposomes did not affect MDR1 activity and chemo-sensitivity in drug-resistance of CEM/ADR5000 cells (Zembruski et al., 2013). Interestingly, SM contents increased in non-raft fractions, but not in raft fraction where MDR1 was distributed. It has just started investigating the role for SM lioposomes as a novel therapeutics for leukemia.

S1P

Ceramide-S1P rheostat was reported to be critical in the resistant mechanism to imatinib-induced apoptosis in K562 CML cells (Baran et al., 2007). In HL-60 cells, ceramide has been shown to promote autophagy-associated cell death by ceramide-activated protein phosphatases-dependent inactivation of mTOR, whereas S1P treatment suppressed ceramide-induced cell death by transactivation of mTOR via S1P receptor (S1PR) 3. Taken together, ceramide-S1P rheostat is suggested to play an important role in determining cell fates.

Food components

Withanolide D, a pure herbal compound isolated from *Withania somnifera,* was reported to induce cell death in K562 and MOLT4 cells through JNK and p38 MAPK by an increase of ceramide, which was generated via nSMase (Mondal et al., 2010). Treatment with stichoposide C from *Thelenota anax* induced apoptosis with activation of aSMase and nSMase in HL-60 cells (Yun et al., 2012). Resveratrol treatment increased expression of CerS and showed synergistic cytotoxicity with short chain ceramide, GCS inhibitor and SK1 inhibitor in K562 cells (Kartal et al., 2011). Uptake of natural compounds in hematologic cancer cells appears to promote the generation of ceramide that mediates cell death.

Sphingolipids-metabolizing enzymes

SK

The inhibitor for both SK1 and SK2 named SKi was revealed to

potentiate effects of anti-cancer agent vincristine on cell death in Jurkat and CEM-R cells (Evangelisti et al., 2014). A SK1 inhibitor BML-258 was also shown to have an inhibitory effect on cell growth in monocytic leukemia U937 and Jurkat cells (Paugh et al., 2008). Recently, SK2 inhibition by a SK2 inhibitor ABC294640 was shown to induce cell death in ALL cells (Wallington-Beddoe et al., 2014). Importantly, SK2 was uncovered to up-regulate Myc, suggesting that SK2 has an oncogenic role in ALL cells. SK inhibition by F-12509a overcame the multi-drug resistance of HL-60/Dox cells by leading the accumulation of ceramide with caspase-3 activation and inhibition of XIAP (Bonhoure et al., 2006). Those findings are believed to raise the potential involvement of SK2 in drug resistance. Overall, further investigation seems to be indispensable for the clinical use of SK inhibitor as a leukemia therapy.

SMS

Tricyclodecan-9-yl-xanthogenate (D609) was reported to inhibit SMS and to induce cell death of U-937 leukemia cells with an increase of ceramide and a decrease of diacylglycerol (Meng et al., 2004). The EC_{50} value of D609 for 48 h treatment was 125 μM. As this concentration was enough to inhibit other kind of enzymes such as GCS, the specificity of D609 should be taken an attention and caution should be exercised with its usage. In SMS-defected WR19L/Fas-SMS(-) lymphoid leukemia cells, overexpression of SMS1, but not SMS2, restored the deterioration of the uptake of transferrin/transferrin receptor complex through clathrin-coated pits, suggesting that SM content regulated by SMS1 in the microdomain plays a role in cell proliferation through transferrin/transferrin receptor system (Shakor et al., 2011). Overexpression of BCR-ABL in HL-60 cells up-regulated SMS activity, suggesting that SMS1 is a downstream signal of BCR-ABL (Burns et al., 2013).

Defects in both SMS1 and SMS2 showed the increase of migration though the activation of CXCL12/CXCR4 axis in mouse embryonic fibroblasts, suggesting an inhibitory effect of SM on cell motility (Asano et al., 2012). The similar activation of migration was detected in SM-deficient WR19L/Fas T leukemia cells (personal communication of S. Asano). These observations may suggest that SM plays a role in the regulatory mechanism of chemokine-induced migration.

SMase

Ceramide generation by nSMase was firstly reported as a mechanism to increase pro-apoptotic signal in leukemia cells (Hannun, 1994; Okazaki et al., 1989). Then, a diversity of experimental data has piled up to show the important function of nSMase as well as aSMase in cancer therapy (Savic and Schuchman, 2013; Shamseddine et al., 2015). Since aSMase deficiency is a key in the pathogenesis of Niemann-Pick disease, recombinant aSMase is available as a reagent for the clinical use. Recently it was shown that the combination of recombinant aSMase with a tyrosine kinase inhibitor sorafenib exhibited a synergistic effect on reducing the tumor volume and blood vessel density in Huh7 hepatocellular carcinoma xenografts (Savic et al., 2013). Unfortunately the clinical trial using recombinant aSMase or nSMase and the activator of SMases has not been performed because of the difficulty of the targeting to the malignant cells.

GCS

The inhibition of GCS has an anti-apoptotic effect because its enzyme metabolizes pro-apoptotic ceramide to glucosylceramide. GCS expression has been demonstrated to confer chemo-resistance in cancer cells (Liu et al., 1999; Senchenkov et al., 2001). Chemo-resistant leukemia blasts showed lower level of GCS as well as SMS as compared to chemo-sensitive leukemia cells (Itoh et al., 2003). As GCS is localized in cytosol portion of Golgi apparatus and MDR1 exists in the Golgi as well, the relation between MDR1 and GCS-related detoxification of ceramide in the overcome of the resistance should be considered. Inhibition of GCS by siRNA reduced the expression of MDR1 and increased rhodamine123 retention in K562/AO2 leukemia cells (Zhang et al., 2011). Those suggest that GCS expression is associated with MDR1 expression and function. In fact, GCS inhibition by 1-phenyl-2-palmitoylamino-3-morpholino-1-propanol overcame the resistance through the inhibition of MDR1 in TF-1 leukemia cells (Turzanski et al., 2005). In CML cells, GCS inhibition by PDMP promoted ceramide accumulation, resulting in the enhancement of cell death (Huang et al., 2011). It was also reported that ceramide increased by the inhibition of GCS is involved in the overcome of drug-resistance through Bcl-2 inhibition in CML cells (Baran et al., 2011; Wang et al., 2014). Although the precise mechanisms by which GCS is associated with MDR1 and the development of chemo-resistance are unclear, GCS is a possible therapeutic target to overcome the chemo-resistance in hematologic malignancies.

CDase

Interferon regulatory factor 8 (IRF8) is a key transcription factor for myeloid cell differentiation and its expression is down-regulated in CML cells. IRF8 was revealed to bind to the promoter region of aCDase to repress its transcription and the resistance to FasL was shown in IRF8-down-regulated CML cells probably due to the decrease of the ceramide level. Since the increase of ceramide through the inhibition of aCDase sensitized CML cells to FasL-induced apoptosis, the suppression of ceramide-induced apoptotic signal may be involved in FasL resistance of CML cells induced by the deficiency of IRF8 (Hu et al., 2011).

CerS and SPT

A tyrosine kinase inhibitor nilotinib promoted to kill human K562 and Meg-01 CML cells and its extent was correlated to the expression level of CerS genes, suggesting the involvement of nilotinib-induced inhibition of BCR-ABL in ceramide-related apoptotic signal (Camgoz et al., 2011). At present it is not known whether the activation of BCR-ABL directly inhibits ceramide generation through CerS. Recently BCR-ABL was reported to phosphorylate SPT long chain-1 (SPTLC1) at Tyr164 and repress SPT activity and ceramide synthesis (Taouji et al., 2013). Interestingly, over-expression of the mutant SPTLC1 (Tyr164 to Y164F) increased the activity of SPT and sensitized the induction of apoptosis in K562 CML cells. Those studies might suggest that BCR-ABL promotes oncogenic activity by suppressing ceramide synthesis. Consistent with that SPTLC1 is an endoplasmic reticulum-resident protein, ABL was also shown to localize in endoplasmic reticulum (Qi and Mochly-Rosen, 2008).

Clinical aspect of sphingolipids

Sphingolipidomics in human blood samples have been performed. It was shown that the blood contains a large amount of C_{16}-SM (64 μM), C_{24}-ceramide (4 μM) and S1P (0.68 μM in serum) (Hammad et al., 2010). Serum C_{18}-ceramide level was expected as a predictive marker for the chemotherapy with gemcitabine and doxorubicin in head and neck cancer (Saddoughi et al., 2011), but so far there is no report showing the clinical significance of sphingolipid changes in serum or plasma from leukemia patients.

Malignant lymphoma

Sphingosine is synthesized by the hydrolysis of ceramide by CDase and has not been recognized as the main precursor of ceramide synthesis because 3-keto sphinganine is a main precursor for *de novo* ceramide synthesis by CerSs. However, salvage ceramide synthesis pathway from sphingosine to ceramide by CerSs is taking attention as an alternative one for ceramide synthesis in programmed cell death (Mullen et al., 2011). In the mantle cell lymphoma cell line Rec-1, the stable endocannabinoid analogue R(+)-methanandamide increased the activities of the enzymes involved in *de novo* ceramide synthesis such as SPT, CerS and dihydroceramide desaturase, and inhibited ceramide metabolizing enzymes such as GCS, suggesting that cannabinoid induces cell death through pro-apoptotic ceramide signal (Gustafsson et al., 2009). However, the role for cannabinoid receptor types 1 and 2 in the increase of ceramide is controversial in mantle cell lymphoma cells (Gustafsson et al., 2006; 2009). Rituximab is an important reagent in immunotherapy for malignant B cell lymphoma. The mechanism by which rituximab exerts its cytotoxic effect after binding to CD20 molecule remains unknown. Rituximab, which is a chimeric human immunoglobulin G1 (IgG1) anti-CD20 monoclonal antibody, induced cell cycle arrest and growth inhibition by induction of p27Kip1 with an increase of ceramide through activation of aSMase in plasma membrane microdomains (Bezombes et al., 2004). The relation between accumulation of CD20 and sphingolipids in the microdomain after treatment with rituximab was investigated (Semac et al., 2003). The effect of rituximab on malignant lymphoma cells may correlate to the expression of CD20 in the membrane microdomain regulated by GM1. CLL showed low level of GM1 while marginal zone lymphoma expressed higher GM1 level. CLL patients with a higher expression of GM1 showed higher susceptibility to rituximab-based immunotherapy, suggesting the role of GM1 in the partitioning of CD20 molecules in the microdomain (Meyer zum Buschenfelde et al., 2008). So far, the role for ceramide and SM in the microdomain in the effectiveness of rituximab has not been reported in malignant lymphoma cells.

Multiple myeloma cells

In Gaucher disease it is well known that multiple myeloma and B cell malignancies frequently occurred (Ayto and Hughes, 2013). The GBA1-deficient mice showed the increase of glucosylceramide and glucosylsphingosine in plasma and induced the generation of B cell lymphomas (11of 21 mice) and monoclonal gammopathy (11 of 39 mice) (Pavlova et al., 2013). The precise cause of these malignancies remains to be clear, although the abnormal accumulation and deficiency of sphingolipids such as ceramide and sphingosine was pointed out.

S1P treatment up-regulated CXCL12-induced migration and adhesion of multiple myeloma cells by an increase of $\alpha4\beta1$ integrin. Through CXCR4 and S1PR1, CXCL12 and S1P, respectively, activated the DOCK2-Rac1 pathway, which was required for stimulation of myeloma cell adhesion involving $\alpha4\beta1$ integrin (Garcia-Bernal et al., 2013). S1P-transduced signaling inhibited dexamethasone-induced apoptosis by increasing Mcl-1 expression in multiple myeloma cell lines, which was blocked by pertussis toxin, a pan inhibitor of S1PRs (Li et al., 2008). Those studies suggest that S1P-S1P receptor signal transduces adhesion and migration of multiple myeloma cells.

Epigallocatechin-3-gallate, a polyphenol extracted from green tea, has cancer chemopreventive and chemotherapeutic activities. Epigallocatechin-3-gallate induces cell cycle arrest and subsequent apoptosis through 67 kDa laminin receptor in multiple myeloma cells (Shammas et al., 2006). Tsukamoto *et al*. (2012) trated that ceramide generated by aSMase activation was increased in a microdomain for clustering of laminin receptor in MM cells treated with epigallocatechin-3-gallate *in vitro* and *in vivo* xenograft mouse model. Importantly, cytotoxic effects of epigallocatechin-3-gallate were confined to multiple myeloma cells and the adverse effects did not appear to occur in normal peripheral lymphocytes.

ROLE OF MODULATOR OF SPHINGOLIPID FUNCTION IN HEMATOLOGICAL MALIGNANCIES

FTY720 (fingolimod)

FTY720 is an analogue of sphingosine and phosphorylated by SK2, but not SK1. FTY720 treatment induced cell death in AML-M2 type leukemia and Kasumi cells, and also suppressed *in vivo* tumor growth in a Kasumi-1 cell xenograft model. Interestingly, treatment of Kasumi cells with FTY720 increased ceramide and gene expression of ceramide-generating enzymes such as GBA1, DES1, aSMase and nSMase2 (Chen et al., 2014). Moreover, pharmacological inhibition of ceramide synthase or nSMase prevented Kasumi cells from FTY720-induced apoptosis. Therefore, FTY720 is suggested to induce apoptosis by promoting ceramide synthesis. Moreover, in FTY720-treated NK-cell leukemia, elevated levels of sphingosine were suggested to mediate apoptosis (Liao et al., 2011). As to the mechanism by which FTY720 treatment induces cell death in leukemia cells, the inhibition of protein phosphatase 2A (PP2A) is proposed in Kasumi-1 AML cells and Jurkat T cell leukemia cells (Matsuoka et al., 2003; Pippa et al., 2014). However, in ALL cell lines okadaic acid did not inhibit FTY-720-induced cell death regardless of inhibition of protein phosphatase 2A, suggesting the independent pathway of FTY-720 to induce cell death from ceramide-activated PP2A (Wallington-Beddoe et al., 2011).

FTY720 has been also implicated in autophagy induction. Wallington-Beddoe et al. (2011) demonstrated that FTY720 treatment induced cell death with an increase of LC3-II and reactive oxygen species but independently of caspases. Probably, FTY720 might serve as an inducer of autophagy-associated cell death. The pro-apoptotic proteins such as Bim and Bid were also involved in cell death of CML K562 cells with FTY720 treatment (Kiyota et al., 2013). Whereas FTY720 treatment decreased the number of malignant cells in a Bcl-2-independent manner and prolonged survival time in a xenograft mouse model of Raji B cells (Liu et al., 2008). Recently, FTY720 treatment was also shown to suppress tumor growth in a xenograft mice model of Ph(+) ALL cell lines, but not in that of Ph(–) ALL cells, showing selective effectiveness of FTY720 on tumor growth (Wallington-Beddoe et al., 2012). Moreover, treatment with FTY720 was shown to induce apoptosis in BCR-ABL-transformed myeloid precursor 32Dcl3 cells (Neviani et al., 2007). Those studies raise the potential of FTY720 as a therapeutic reagent for CML.

Taken together, accumulating evidence suggests that FTY720 potentially serves as a chemotherapeutic reagent for hematological malignancy and the further investigations as to the mechanisms and therapeutic efficacy should be required for clinical development.

S1P receptors

It is well known that S1P stimulates lymphoid cell migration through S1PR1. The activation by CXCL12 or BCR cross-linking reduced S1PR1 expression and migratory response

toward S1P of CLL cells isolated from patients, whereas the inhibitors of BCR-associated kinase such as Syk increased S1PR1 and migration toward S1P in CLL cells, suggesting the inhibitory effect of B cell activation on S1PR1 expression (Borge et al., 2014). S1PR2-deficient mice generated the tumor with the features of germinal center-derived diffuse large B cell lymphoma and 28 of 106 (26%) diffuse large B cell lymphoma patients harbored the multiple somatic mutations in the 5' sequences of the S1PR2 gene (Cattoretti et al., 2009). In the KM-H2 and SUP-HD1 Hodgkin lymphoma cells, the increase of their migration by S1P treatment was blocked by S1PR1 antagonists VPC44116 and FTY720-P. A subset of Hodgkin lymphoma cases (7/57, 12%) showed strong, membranous staining for S1PR1, suggesting the therapeutic potential of S1PR inhibitor for infiltration of Hodgkin lymphoma cells in some cases (Kluk et al., 2013). Immunohistochemistry of S1PR1 in clinical samples showed the strong expression in the lymph node (19 out of 19), gastrointestinal tract (10 out of 10), bone marrow (9 out of 9), and orbita (1 out of 1) of mantle cell lymphoma patients (Nishimura et al., 2010). The role of S1PR1 was opposite to that of S1PR2 in the regulation of lymphoid cell proliferation. The precise biological implications of S1PRs in survival/proliferation of lymphoid cells should be clarified in the future.

CONCLUSIONS

Sphingolipids such as ceramide and S1P have been recognized as bioactive lipids in cell death and proliferation/survival. In this review we overviewed role for sphingolipids in the induction of cell death in several types of hematological malignancies such as leukemia, malignant lymphoma and multiple myeloma. The main backbone molecule of sphingolipid network is ceramide. Ceramide is synthesized from serine and fatty acids with different kinds of carbon chains by *de novo* ceramide pathway in endoplasmic reticulum, and transferred to the Golgi apparatus by CERT for synthesis of SM by SMS while ceramide transfer protein for glucosylceramide to Golgi apparatus remains unknown. Sphingosine generated by CDase is phosphorylated and turns to S1P by SK1 or SK2. The complex sphingolipids such as glucosylceramide and GM3 are transferred to the micro-organelles to exert their functions and again returned to ceramide by salvage pathway. Likely, the catabolized sphingolipids such as sphingosine and sphingadienine are possibly resynthesized to ceramide by CerSs. Thus, it is critical to consider the difference of molecular species in active sphingolipids and the variation of time and localization in sphingolipid metabolism to understand the *bona fide* role of pathobiological sphingolipids in the cells. For example, curcumin-induced HL-60 apoptosis showed two phase-increases of ceramide accumulation by the activation of nSMase and subsequent inhibition of SMS, suggesting that each increase of ceramide plays a role in a different time course and localization in the process of cell death (Shakor et al., 2014). We have recently reported (1) the important role of SM in the microdomain of plasma membrane in transferrin-induced lymphoid cell proliferation and CXCL12-induced cell migration, (2) the positive role of ceramide in the nucleus in Fas-induced cell death even though the existence of sphingolipid metabolizing enzymes in the nucleus remains clarified and (3) the competitive role of ceramide and S1P in the cytosol in the regulation of mTOR in the process of autophagy (Taniguchi et al., 2012). In addition, although SK1 and SK2 both generate S1P, SK1 resides in the cytosol and moves to the near site of plasma membrane but SK2 locates in the nucleus (Siow and Wattenberg, 2011). SK1 is involved in cell proliferation while SK2 plays a role in apoptotic cell death. The lipids exist as a structurally different form in the cells such as a vesicular micelle, a component of the membrane microdomain and a protein-binding form. Thus, it remains to clarify the precise implications of the vesicular and non-vesicular trafficking, and the membrane topology of sphingolipids in the regulation of cell death and proliferation/survival. Taken together, these findings suggest that a diverse bioactive sphingolipids play a role in many cell functions at the different time, different form and intracellular components and that these dysregulation of sphingolipids may be closely related to the causes in human disorders.

To develop a novel therapeutics for hematological malignancies we have to investigate the role of sphingolipids: (1) as an intracellular signaling molecule (ceramide, S1P and FTY720) and a regulator of microdomain to control trans-membrane receptor (SM and S1P receptor), (2) as a homeostatic keeper of the bioactive vesicles such as recycling and degradation endosomes, autophagosomes and lysosomes (SM, ceramide and S1P) and (3) as an regulator of intercellular communicator like exosomes and ectosomes (SM, ceramide and S1P). In the future, we hope that the research, which clarifies the regulation of bioactive sphingolipid functions in not only the cells but also the animal models and human disorders, brings about a novel and effective sphingolipid-targeting therapy in hematological malignancies different from the recently on-going chemo-, radio- or immunotherapy.

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