

# We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

185,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index  
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?  
Contact [book.department@intechopen.com](mailto:book.department@intechopen.com)

Numbers displayed above are based on latest data collected.  
For more information visit [www.intechopen.com](http://www.intechopen.com)



## Bioactive Sphingolipids in Neuroblastoma

Mehrdad Rahmaniyan, Amr Qudeimat and Jacqueline M. Kraveka

*Division of Pediatric Hematology Oncology  
Hollings Cancer Center and Darby Children's Research Institute  
Medical University of South Carolina  
USA*

### 1. Introduction

Neuroblastoma is a solid tumor cancer that originates in the nerve tissue of the neck, chest, abdomen, or pelvis, but most commonly in the adrenal gland. It is the third most common pediatric cancer and accounts for ~15% of all childhood cancer deaths. Neuroblastoma has one of the lowest survival rates of all pediatric cancers. It is the most common solid tumor diagnosed during infancy. This tumor may involute spontaneously in infants, or it may sometimes mature to a benign ganglioneuroma. On the other hand, older children often present with advanced stage disease at the time of diagnosis. While survival in patients with favorable biological features may exceed 90%; the survival rates for children with high risk neuroblastoma have historically been under 40%. However, advances made in recent years in understanding the biology of neuroblastoma have led to the identification of patient groups with higher risk disease and to the rise of new modalities for combating this disease. Patients with high-risk neuroblastoma undergo aggressive multi-modal therapies including, chemotherapy, immunotherapy, surgery, stem cell transplantation, and radiation. Survivors also experience many treatment related toxicities and long term side effects. Therefore, new approaches are needed for these patients (Maris, 2010)

One of the molecules that can stimulate differentiation of neuroblastoma cells is Ceramide (Fig. 1), a biologically active effector molecule composed of a fatty acyl chain bound to a sphingoid backbone via an amide linkage (Kraveka & Hannun, 2009). The study and modulation of sphingolipids and their effects has emerged as an attractive therapeutic target due to their potent anti-proliferative effects (Hannun & Obeid, 2008). Ceramide is the central building block for sphingolipids. It serves as a precursor for the synthesis of more complex sphingolipids, and is generated by multiple pathways (Fig. 2). Sphingolipids comprise a class of lipids that share the presence of a sphingosine (or related sphingoid) base in the backbone of their structures. Research in the past two decades has shown that sphingolipids, in addition to their roles as structural components of cell membranes, play important roles as regulators of signal transduction in cell differentiation, cell proliferation, inflammation and apoptosis. Ceramide mediates important cellular activities such as induction of cell differentiation, growth arrest, senescence, and apoptosis. Sphingosine-1-phosphate has the opposite effect of ceramide stimulating cell proliferation and is involved in angiogenesis and inflammation (Kolesnick, 2002). In this chapter we will review the roles

these bioactive sphingolipids and their related enzymes play in cancer pathogenesis and how their regulation is a novel target for neuroblastoma therapy.

## 2. Sphingolipids

### 2.1 Background

Sphingolipids were first discovered in the late 1800s. Sphingolipids such as cerebroside and sphingomyelin were first isolated from brain tissues by J. L. W. Thudichum (van Echten-Deckert & Herget, 2006). He named these compounds after the Sphinx because of their enigmatic properties. For a long time, sphingolipids had been identified as biologically inert components of cell membranes. However, advances in biochemistry and molecular biology have demonstrated that these lipids play key roles in the regulation of several fundamental biological processes such as signal transduction, cell proliferation, migration, and apoptosis (Ogretmen & Hannun, 2004). These regulations are mediated by many enzymes involved in sphingolipid metabolism (Hannun & Luberto, 2000).

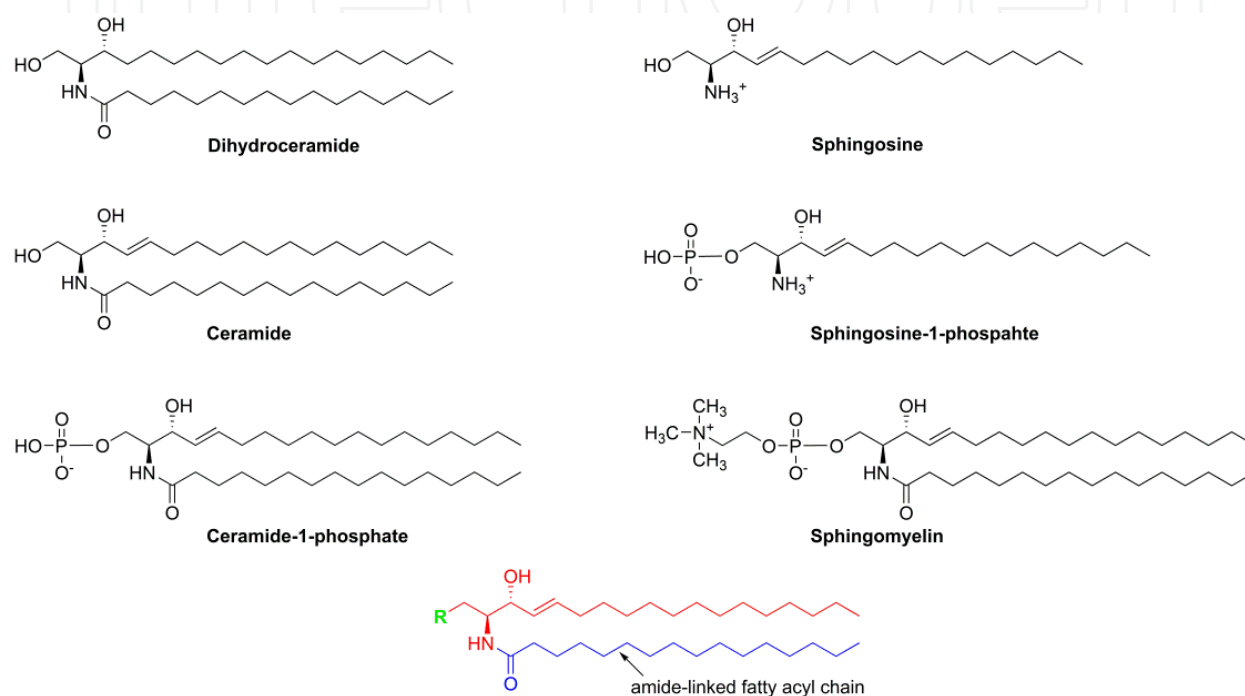
### 2.2 Structure and metabolism of sphingolipids

Sphingolipids are composed of a long chain sphingoid base that is acylated with a fatty acid via an amide linkage, and a polar head group (Fig 1.). In contrast to ceramide, complex sphingolipids contain hydrophilic groups such as phosphate, phosphocholine, or sugar residues. Based on the head group, sphingolipids may be divided into three groups: ceramides, sphingomyelins, and glycosphingolipids. Ceramide is the central building block for sphingolipids and has a hydroxyl group at position 1, sphingomyelin has phosphocholine, and glycosphingolipids have carbohydrate groups. The latter are further subdivided into cerebroside, gangliosides, and sulfatides (Jana & Pahan, 2010; Sietsma et al., 2002). Gangliosides, such as GD2, are composed of a glycosphingolipid (ceramide and oligosaccharide) with one or more sialic acids such as n-acetylneuraminic acid linked to the sugar.

The metabolic pathways of sphingolipids are very complex (Fig 2.). Sphingolipid metabolism is regulated at several different levels such as the expression of regulating enzymes, post-translational modifications, or allosteric mechanisms (Futerman & Hannun, 2004). There are multiple pathways for the generation and regulation of bioactive Sphingolipids (Figure 2). Many of these pathways reside in specific sub-cellular compartments and respond to various extra- and intra-cellular stimuli. Most enzymes of sphingolipid metabolism have specific sub-cellular localization(s), thereby exerting profound effects on the signaling and regulatory functions of the generated sphingolipid in a specific compartment. The best studied of these pathways are the *de-novo* pathway, the sphingomyelinase pathway, and the salvage/recycling pathway (Kravetska & Hannun, 2009).

The *de-novo* pathway of ceramide synthesis starts at the cytosolic surface of the endoplasmic reticulum (ER) where a series of enzymes generate ceramides with different acyl chain lengths from non-sphingolipid precursors (Futerman & Hannun, 2004; Gault et al., 2010; Kok et al., 1997; Merrill, 2002). This pathway is initiated by condensation of serine with palmitoyl-CoA, and catalyzed by the rate-limiting enzyme serine palmitoyl transferase, resulting in the formation of 3-ketosphinganine. The latter is reduced in an NADPH-dependent reaction by the enzyme 3-ketosphinganine reductase to sphinganine, which is then acylated into dihydroceramide by (dihydro)-ceramide synthases (Fig. 3) (Saddoughi et

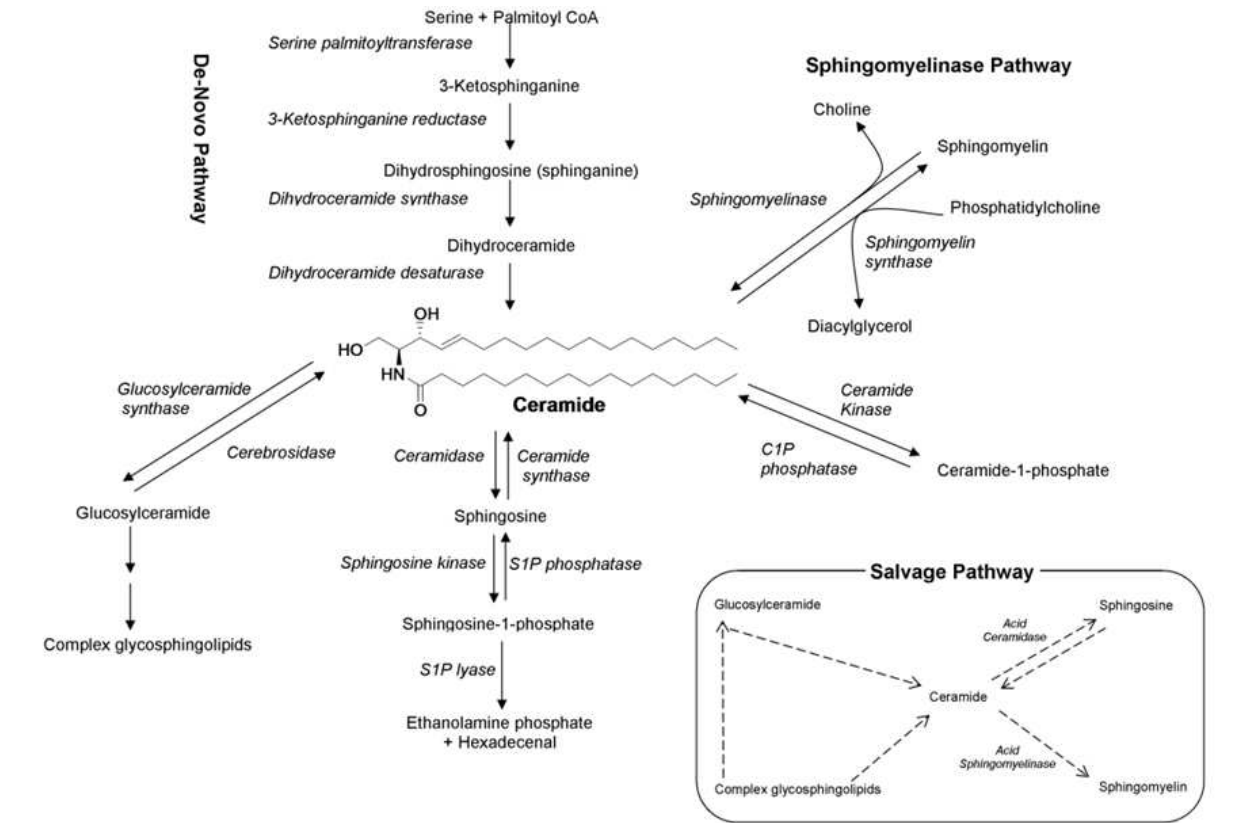
al., 2008, van Echten-Deckert, 2006). A desaturation follows by the enzyme dihydroceramide desaturase (DES-1/DEGS-1) to insert a double bond between carbons 4-5 in the sphingosine backbone of dihydroceramide to generate ceramide (Michel et al., 1997). Ceramide synthases (Fig. 3) are also referred to as Lass (longevity-assurance homologue) family members. Six mammalian ceramide synthases have been identified molecularly, and each ceramide synthase protein exerts specificity for the generation of distinct endogenous dihydroceramides and ceramides with fatty-acid chain length specificity (Ogretmen, 2006; Pewzner-Jung et al., 2006).



Ceramide is composed of a sphingoid base (18 carbons) and an amide linked fatty acid (14-26 carbons). Complex sphingolipids are composed of a hydrophilic head group (R) attached to the lipophilic ceramide backbone. These head groups may be phosphocholine in sphingomyelin or sugars in glycosphingolipids.

Fig. 1. Bioactive sphingolipids.

The *de novo* pathway is localized to the ER and continues in the Golgi apparatus where the enzymes glucosylceramide synthase and sphingomyelin synthase are localized (Pettus et al., 2002). Once ceramide is generated, it can be glycosylated by glucosylceramide synthase to form glucosylceramide on the cytoplasmic surface of Golgi. Glucosylceramide then serves as the precursor for glycosphingolipids. Ceramide may also be galactosylated to galactosylceramide by galactosylceramide synthase in the ER. Sulfatides and Gala-series glycosphingolipids are formed from galactosylceramide synthase. In turn, glycosphingolipids are hydrolyzed by  $\beta$ -glucosidases and galactosidases to regenerate ceramide (Tettamanti, 2004). Gangliosides such as GD-2, are structurally and biochemically derived from lactosylceramide which is formed by transfer of a galactosyl residue to glucosylceramide. Sequential addition of one, two or three sialic acids to lactosylceramide results in formation of GM3, GD3 and GT3 (Bektas & Spiegel, 2004). In addition, ceramide can be converted into a number of other bioactive sphingolipids, including ceramide-1-phosphate, sphingosine, and sphingosine-1-phosphate.



Ceramide is the central building block for complex sphingolipids. Enzymes are shown in italics.

Fig. 2. Sphingolipid Metabolism.

In the sphingomyelinase pathway, ceramide is generated from hydrolysis of sphingomyelin through the action of either acid or neutral sphingomyelinases (Clarke & Hannun, 2006; Marchesini & Hannun, 2004). These enzymes break down sphingomyelin to produce ceramide and phosphocholine, and are stimulated in response to TNF- $\alpha$  (Luberto et al., 2002; Schwandner et al., 1998), Fas ligand (Lin et al., 2000), or oxidative stress (Goldkorn et al., 1998). The sphingomyelinase mediated hydrolysis of sphingomyelin has emerged as a major pathway of stress-induced ceramide generation. Conversely, sphingomyelin synthase transfers the headgroup of phosphatidylcholine to ceramide and generates sphingomyelin and diacylglycerol in the process. This pathway has been suggested to regulate the levels of not only sphingomyelin and ceramide, but also diacylglycerol (Villani et al., 2008) as well as the activation of NF $\kappa$ B (Hailemariam et al., 2008; Luberto et al., 2000). The sphingolipid recycling or salvage pathway refers to the various mechanisms of ceramide generation from the catabolism of complex sphingolipids which are broken down into sphingosine, which is then reused through reacylation to produce ceramide (Kitatani et al., 2008). This pathway involves a number of key enzymes that include sphingomyelinases, cerebrosidases, ceramidases, and ceramide synthases. It has been estimated to contribute from 50% to 90% of sphingolipid biosynthesis. Degradation of sphingolipids and glycosphingolipids takes place mostly in the acidic subcellular compartments, the late endosomes and the lysosomes. Sphingomyelin is converted to ceramide by acid sphingomyelinases. Ceramide can be deacylated with loss of the fatty acid from the amide bond through the action of acid ceramidases to yield sphingosine. Sphingosine can then translocate across the lysosome where it can be either re-acylated to ceramide or



phosphorylated by sphingosine kinase 1 or 2 to generate sphingosine-1-phosphate. Sphingosine-1-phosphate can be cleared by the action of specific phosphatases that regenerate sphingosine or by the action of a lyase that cleaves sphingosine-1-phosphate into ethanolamine-1-phosphate and a C<sub>16</sub>-fatty-aldehyde.

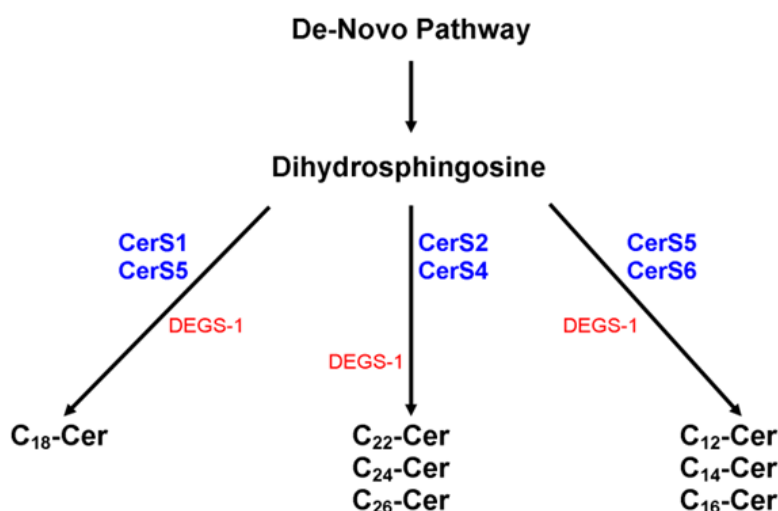


Fig. 3. Ceramide Synthases.

Ceramide synthases (CerS) acylate dihydrosphingosine to form dihydroceramide. In mammalian cells, each CerS protein exerts specificity for the generation of endogenous ceramides with distinct fatty-acid chain lengths. (Adapted from Ogretmen B, *FEBS Lett*, 2006)

### 3. Role of sphingolipids in cancer cell proliferation, cell differentiation, senescence and apoptosis

Sphingolipids play essential roles in cell growth, survival, and death (Birbes et al., 2002; Ogretmen & Hannun, 2004). These regulatory properties of sphingolipids allowed them to be extensively studied in cancer pathogenesis, cancer treatment, and multidrug resistance. In sphingolipid metabolism the precursors and products are biologically not inert, but they are effectors to mediate specific cell regulatory actions. These regulatory actions are mediated by interaction of sphingolipids with cell surface receptors, modulation of lipid rafts, and intracellular actions (Billich & Baumruker, 2008). The most studied bioactive sphingolipids are ceramide, ceramide-1-phosphate, sphingosine, and sphingosine-1-phosphate.

#### 3.1 Ceramide

Ceramide exerts its downstream effects via activation of ceramide-activated serine-threonine phosphatases such as protein phosphatases 1 and 2 (PP1 and PP2A) (Dobrowsky et al., 1993; Wolff et al., 1994). In addition, ceramide has been shown to activate PKC $\zeta$ , the kinase KSR, and cathepsin D (Conway et al., 2000; Heinrich et al., 1999; Lozano et al., 1994). Different stimuli can activate ceramide formation. Chemotherapeutic agents such as daunorubicin, etoposide, camptothecin, fludarabine, and gemcitabine activate the *de-novo* pathway of ceramide generation. Other agents such as cytarabine, actinomycin D, etoposide, cisplatin (Biswal et al., 2000; Bose et al., 1995; Chalfant et al., 2002; Gomez del Pulgar et al., 2002; Lacour et al., 2004;

Perry et al., 2000; Strum et al., 1994; Suzuki et al., 1997) activate the sphingomyelinase pathways. TNF- $\alpha$ , UV, and  $\gamma$  radiation have been shown to activate sphingomyelinases (Haimovitz-Friedman et al., 1994; Kim et al., 1991; Liu et al., 1998; Zhang et al., 2001)

Ceramide has been widely implicated in the regulation of programmed cell death via numerous stimuli. Ceramide triggers apoptosis via mitochondrial, ER-stress and lysosomal pathways (Hannun & Obeid, 2011; Huang et al.; Mullen et al., 2011). Ceramide is able to induce G0/G1 cell cycle arrest by acting on different targets. It induces dephosphorylation of retinoblastoma (Rb) protein by the activation of protein phosphatase 1 (PP1) (Dbaiibo et al., 1995; Jayadev et al., 1995; Kraveka et al., 2007). Additionally, ceramide specifically inactivates cyclin-dependent kinase 2 (CDK2) through dephosphorylation (Lee et al., 2000), or by up-regulation of CDK inhibitors p21 and p27 through activation of protein phosphatase 2A (PP2A) (Adibhatla & Hatcher, 2010).

Ceramide also can be increased by administration of short chain cell-permeable ceramides (Huang *et al.*, 2011; Ryland et al., 2011). However, the effect of systemic application of these exogenous ceramides is reduced due to precipitation and minimal membrane transport. Nanotechnology has been developed to encapsulate short-chain ceramides for systemic delivery. The nanotechnology includes nanoliposomes, nanocolloids, and nanodendrimers (Ryland *et al.*, 2011). Encapsulation of the exogenous ceramides into nanoliposomes reduces the systemic side effects when applied via intravenous or intraperitoneal routes. Exogenous C<sub>2</sub>-ceramide treatment of neuroblastoma cell line SH-SY5Y induced apoptosis by inactivation of Akt and translocation of apoptosis-inducing factor (AIF) to the nucleus and neuronal cell death by a mitochondrial pathway (Kim et al., 2007).

The involvement of ceramide in senescence is supported by the fact that ceramide levels increased in human fibroblasts as they became senescent. Additional studies showed that treatment of low-passage-number human fibroblasts with exogenous short ceramide was able to induce morphological and biological changes associated with senescence such as dephosphorylation of Rb protein and inhibition of cyclin dependent kinases. The underlying mechanisms for these changes were the inhibition of phospholipase D (PLD), diacylglycerol generation, and PKC activity (Venable et al., 1995).

Our group has provided another direct link between ceramide and senescence by involvement of ceramide in the regulation of telomerase activity and telomere length (Kraveka et al., 2003). Telomeres contain long stretches of tandemly repeated 5'-TTAGGG-3' sequences found at chromosome ends in mammals to protect chromosomes. Progressive shortening of telomeres during cell division triggers the onset of events leading to senescence and/or cell death. However, telomeres are more stable in immortalized and cancer cells. This stabilization is achieved by the activity of telomerase, an RNA-dependent DNA polymerase, whose activity is detected in most cancer cells, allowing them to escape senescence and acquire immortality. Treatment of human neuroblastoma cell lines, SK-N-SH and SK-N-AS, with all trans retinoic acid (ATRA) inhibited telomerase activity and increased the endogenous levels of C<sub>24:0</sub> and C<sub>24:1</sub> ceramides). The relation between ceramide and telomerase was supported by the fact that treatment of cells with ATRA in the presence of myriocin, a serine palmitoyl transferase inhibitor, significantly blocked the accumulation of ceramide, and presence of myriocin prevented in part the inhibition of telomerase. Mechanistically, inhibition of telomerase by endogenous ceramide in response to ATRA treatment involved, at least in part, down-regulation of the expression of telomerase reverse transcriptase (hTERT) mRNA. The regulation of telomerase expression by ceramide involves the inactivation of c-MYC transcription factor through increased ubiquitin-proteasome-

mediated proteolysis (Ogretmen et al., 2001). Additionally, ceramide mediates rapid shortening of telomeres, involving the inhibition of nuclear localization and the telomere-binding function of glyceraldehydes-3-phosphate dehydrogenase (Sundararaj et al., 2004). These data suggest that ceramide is one of the upstream regulators of telomerase activity and telomere length and has a key role in induction of senescence.

### 3.2 Dihydroceramide

Dihydroceramide, an intermediate in *de novo* pathway, is synthesized from sphinganine. Dihydroceramides were thought to be biologically inactive. Previous studies on the biological activity of dihydroceramides using their short chain analogs concluded that dihydroceramides were inactive in inducing cell death and apoptosis (Ahn & Schroeder, 2002; Bielawska et al., 1993; Sugiki et al., 2000). However, recent studies have shown dihydroceramides to be involved in important cellular responses such as cell cycle arrest, apoptosis, ceramide channel formation, autophagy, and oxidative stress (Idkowiak-Baldys et al., 2010; Kraveka et al., 2007; Signorelli et al., 2009; Stiban et al., 2006; Wang et al., 2008; Zheng et al., 2006). These studies indicate the significance of endogenous levels of dihydroceramides in cell regulatory processes, and they suggest that the enzyme dihydroceramide desaturase has the potential to regulate cell function through both ceramide and dihydroceramide their respective sphingolipid metabolites. We will discuss dihydroceramides more in depth later in the chapter.

### 3.3 Sphingosine and sphingosine-1-phosphate

Sphingosine, was the first bioactive sphingolipid to be identified (Hannun et al., 1986). It is the product of ceramide degradation by ceramidases, acts like ceramide to exerting apoptotic effects in multiple cell lines (Ekiz & Baran, 2010). Other studies also showed that sphingosine interferes with multidrug resistance mechanisms in cancer cells. These mechanisms were ineffective against sphingosine-induced cell death. Sphingosine interacts with protein kinase C (PKC), ERK, akt/Protein Kinase B (akt/PKB) to induce apoptosis (Chang et al., 2001; Jarvis et al., 1997). Additionally, sphingosine causes the release of cytochrome c from mitochondrial membrane followed by activation of caspases (Cuvillier et al., 2001). In murine Neuro-2A neuroblastoma cells, sphingosine induced differentiation (Riboni, et al., 1998).

Sphingosine-1-phosphate acts not only as anti-apoptotic agent, but also is implicated in angiogenesis, adhesion, migration, and inflammation (Chalfant & Spiegel, 2005; van Echten-Deckert & Herget, 2006). It can function as an extracellular first messenger and also as intracellular second messenger. Intracellularly, it serves as second messenger regulating calcium mobilization, cell proliferation and survival (Ling et al., 2011). Extracellularly, sphingosine-1-phosphate exerts most of its actions as a specific ligand for a family of nine G-protein-coupled receptors (GPCRs) called sphingosine-1-phosphate  $\text{-R}_{1-5}$  and Gpr63 (Argraves et al., 2010), contributing to physiological and pathological processes such as angiogenesis which is a critical factor for tumor progression (Ling *et al.*, 2011). Mechanistically, the proliferative effect of sphingosine-1-phosphate may be mediated by mitogen-activated protein kinase (MAPK) pathway (Shu et al. 2002). It also stimulates cell survival pathways, such as nuclear factor  $\kappa\text{B}$  (NF- $\kappa\text{B}$ ) (Xia et al., 2002) and the Akt/PI3K (Phosphatidylinositol 3-kinase) pathway (Banno et al., 2001). Growth factors and cytokines



including vascular endothelial growth factor (VEGF) and platelet derived growth factor (PDGF) induce sphingosine kinase leading to elevation of sphingosine-1-phosphate.

Sphingosine kinase-1 inhibition can sensitize cells toward the action of radiation, to ceramide, and to cytotoxic agents such as doxorubicin, etoposide, imatinib, and cisplatin (Billich & Baumruker, 2008). There are currently several sphingosine kinase-1 and 2 inhibitors available and in development. Sphingosine kinase is an attractive target in cancer treatment because blockage of its product, sphingosine-1-phosphate inhibits proliferation and induces apoptosis in cancer cells (French et al., 2006). Another approach is the use of the anti-sphingosine-1-phosphate receptor monoclonal antibodies. These antibodies can induce tumor progression in murine xenograft and allograft models (Saddoughi et al., 2008). They also can prevent sphingosine-1-phosphate induced cell proliferation and the release of pro-angiogenic cytokines. This pathway presents an important target for cancer treatment.

### 3.4 Ceramide-1-phosphate

Ceramide-1-phosphate, another product of ceramide by phosphorylation ceramide kinase (CERK), shows pro-survival effects by inducing DNA synthesis, blocking caspases, suppressing acid sphingomyelinase, and promoting phagosome formation (Chalfant & Spiegel, 2005; Gomez-Munoz, 2004; Gomez-Munoz et al., 1995; Gomez-Munoz et al., 2004; Hinkovska-Galcheva et al., 2005). It is involved in mast cell degranulation (Mitsutake et al., 2004). It has been shown to also serve as an activator of cytosolic phospholipase A2 (cPLA2a) (Pettus et al., 2004), and thus may function as a key regulator of eicosanoid synthesis. ATRA has been reported to inhibit CERK transcription in SH-SY-5Y neuroblastoma cells (Murakami et al., 2010).

### 3.5 Glucosylceramide

Glucosylceramides have proliferative effects and are implicated in the development of drug resistance in cancer cells (Lucci et al., 1998; Messner & Cabot, 2010; Ogretmen & Hannun, 2004). Numerous studies showed that glucosylceramide synthase overexpression is associated with multidrug resistance in many cancers including breast cancer (Zhang et al., 2011), leukemia (Xie et al., 2008) colon cancer (Liu et al., 2010b), and glioblastoma (Barth et al., 2010). One study showed that glucosylceramide synthase regulates MDR1 expression through cSrc and  $\beta$ -catenin signaling (Liu et al., 2010b). In leukemia cells overexpression glucosylceramide synthase gene is correlated with Bcl-2 signal transduction (Liu et al., 2010a). In glioblastoma and neuroblastoma cells, glucosylceramide synthase mediated drug resistance is via inhibition of NADPH oxidase (NOX), thus blocking ROS generation and limiting apoptosis (Barth et al., 2010). A new mixed-backbone oligonucleotide (MBO-asGCS), that specifically suppresses overexpressed human glucosylceramide synthase gene, can restore drug sensitivity in multidrug resistant cancer cells leading to apoptosis (Patwardhan et al., 2009). Additionally, co-Inhibition of MDR1 gene and glucosylceramide synthase by siRNA can restore sensitivity in multidrug resistant breast cancer cells (Zhang et al., 2011). Inhibition of glucosylceramide synthase by 1-Phenyl-2-decanolyamino-3-morpholino-1-propanol (PDMP) inhibited cell growth in murine neuroblastoma cells (Uemura et al., 1991). Taken together, these data suggest the involvement of glucosylceramide synthase in multidrug resistance and pharmacological targeting of glucosylceramide synthase is an important goal to restore sensitivity of multidrug resistant cancer cells to cytotoxic agents and improve their efficacy.

#### 4. Ceramide levels and sphingolipid expression in cancer

Total ceramide levels and the amounts of individual ceramide species have been shown to be altered in tumor samples. Total ceramide content was decreased in ovarian tumors compared to normal ovarian tissue (Rylova et al., 1998). High grade astrocytomas also had lower levels of ceramide (Riboni et al., 2002). Utilizing liquid chromatography/mass spectrometry (LC/MS) to identify individual ceramide species has yielded some interesting results. Defects in the ceramide synthase 1 dependent generation of C<sub>18</sub>-ceramide (Figure 3) have been implicated in the pathogenesis of squamous cell carcinomas of the head and neck (HNSCC) (Koybasi et al., 2004). The data showed that the levels of C<sub>16</sub>-, C<sub>24</sub>-, C<sub>24:1</sub>-ceramides were significantly elevated in the majority of tumor tissues compared to their normal tissues, while the levels of only C<sub>18</sub>-ceramide were significantly decreased. Further experiments showed that overexpression of ceramide synthase 1, which is responsible for C<sub>18</sub>- generation, resulted in the inhibition of HNSCC cell growth, and enhanced chemotherapy-induced apoptosis in UMSCC22A HNSCC cells *in situ*, and in HNSCC xenografts *in vivo* (Senkal et al., 2007). More recent work from Dr. Ogretmen's group showed that knockdown of ceramide synthase 6/C<sub>16</sub>-ceramide induced endoplasmic reticulum stress-mediated apoptosis (Senkal et al., 2010). A study by Schiffmann (Schiffmann et al., 2009) measured endogenous ceramide levels in 43 malignant breast tumors and 21 benign breast biopsies via LC/MS. The levels of C<sub>16</sub>-, C<sub>24:1</sub>- and C<sub>24:0</sub>-ceramides were significantly elevated in malignant tumors as compared to benign and normal tissue. As expected, increased mRNA expression of ceramide synthases 2, 4, and 6 was detected. In breast cancer elevated levels of C<sub>16</sub> ceramide were associated with metastatic disease.

In summary, these recent reports appear contradictory to many of the older cell-based studies in which *in-vitro* treatments with ceramide or agents that stimulate ceramide generation induced apoptosis. However, these newer studies suggest that the levels of specific ceramide species and/or the activity and expression enzymes of sphingolipid metabolism play an important role in cancer pathogenesis. These studies suggest a relationship between certain long-chain ceramides and malignant transformation (Ponnusamy et al., 2010; Ryland et al., 2011). Studies also suggest that *de novo* generated ceramides differing in their fatty acid chain length may have opposing roles in promotion/suppression of tumors. These facts show that the biological function of ceramide may vary by its carbon chain length. Studies are currently underway in our laboratory examining sphingolipid species content and expression of sphingolipid enzymes in "low/intermediate risk" and "high risk" neuroblastoma tumor samples.

The cellular ratio of ceramide/sphingosine-1-phosphate determines the cell fate and response to chemotherapy. Several studies suggest that sphingosine-1-phosphate regulates survival, migration, and proliferation of cancer cells. Elevated sphingosine-1-phosphate levels are associated with resistance to apoptosis and poor prognosis in cancer (Ryland et al., 2011). Lung, colon, kidney, breast, ovary, stomach, and uterine cancers showed increased levels of sphingosine kinase 1 protein and mRNA (Ogretmen & Hannun, 2004). Sphingosine kinase 2 overexpression has been reported in neuroblastoma (Li et al., 2011).

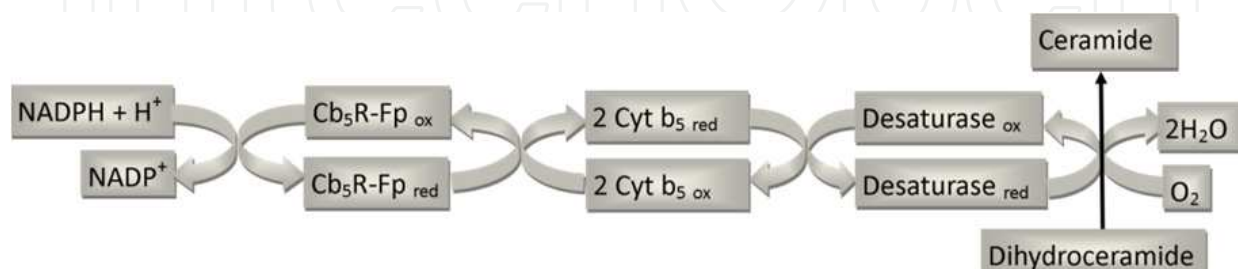
Acid ceramidase, another enzyme in sphingolipid metabolism that hydrolyzes ceramide into sphingosine, may be dysregulated in some cancers. Acid ceramidase was overexpressed in leukemic T-cell large granular lymphocyte leukemia and its inhibition induced apoptosis

in these cells (Shah et al., 2008). One study showed that acid ceramidase was induced by genistein (a phytoestrogen isoflavone) contributing to growth in MCF-7 breast cancer cells (Lucki & Sewer, 2011). Daunorubicin treatment in hepatoma cell line increased acid ceramidase levels leading to protection of these cells from daunorubicin-induced apoptosis (Morales et al., 2007). Acid ceramidase overexpression lead to resistance of prostate cancer cells to chemotherapy and radiation. In these cells acid ceramidase overexpression was associated with increased autophagy and lysosomal density leading to higher resistance by rapidly metabolizing ceramide (Mahdy et al., 2009; Turner et al., 2011). These results demonstrate that inhibitors to sphingolipid pathway enzymes have great potential in the treatment of malignancies.

## 5. The dihydroceramide desaturase enzyme: Implications for neuroblastoma therapy

### 5.1 Desaturase enzymes

Dihydroceramide desaturase family members, consisting of DES1 and DES2, belong to the desaturase/hydroxylase superfamily. Dihydroceramide desaturase 1, is encoded by DES1 (degenerative spermatocyte homologue 1) gene, and it is myristoylated at N-terminus (Beauchamp et al., 2007)). A family of sphingolipid  $\Delta^4$ -desaturases (homologues of the *Drosophila melanogaster* degenerative spermatocyte gene-1 (*des-1*)) were identified via a bioinformatics approach (Ternes et al., 2002). These proteins contain three His-containing consensus motifs that are characteristic of a group of membrane fatty acid desaturases. The human homologue of *des-1* is now referred to as *DEGS-1*, although it was first cloned in 1997 and named as Membrane Lipid Desaturase (MLD) since its physiologic substrate was not determined at the time (Cadena et al., 1997). *DEGS-1* is the only dihydroceramide desaturase reported to be present in human cells, and its mouse homologue (mDES1) was shown to have desaturase activity (Omae et al., 2004). hDES2, the human homologue of the mouse DES2 (mDes2) gene, like mDES2 has dihydroceramide hydroxylase activity (Mizutani et al., 2004). While mDES2 has been reported to have both desaturase and hydroxylase activity, no desaturase activity was detected in HEK 293 human embryonic kidney cells overexpressing hDES2 (Mizutani et al., 2004). DES2 is responsible for biosynthesis of phytosphingoglycolipids in the microvilli of intestinal epithelial cells, kidney, and skin (Omae et al., 2004).



Dihydroceramide synthesized by de novo pathway is desaturated by the multi-enzyme complex consisting of a flavoprotein-containing cytochrome b5 reductase (Cb5R-Fp), a cytochrome (cyt) b5, and a desaturase. (Adapted from Geeraert L, et al., Biochem J 1997)

Fig. 4. Dihydroceramide Desaturase Complex.

The dihydroceramide desaturase complex consists of flavin-containing cytochrome b<sub>5</sub> reductase, a heme-containing cytochrome b<sub>5</sub>, and a non heme-containing desaturase (Causeret et al., 2000; Geeraert et al., 1997).

## 5.2 Role of dihydroceramide desaturase on cell growth, cell cycle, and endogenous ceramide and dihydroceramide levels in neuroblastoma

We investigated the role of dihydroceramide desaturase as a key enzyme in the *de novo* pathway of ceramide generation using human neuroblastoma SMS-KCNR cells (Kravka et al., 2007). This study included the use of a novel *in situ* assay for desaturase activity using pyridinium conjugated water-soluble dihydroceramide analogues. The dihydroceramidoid (C<sub>12</sub>-dhCCPS) was as substrate to measure the activity of the enzyme by monitoring the conversion of C<sub>12</sub>-dhCCPS to C<sub>12</sub>-CCPS via LC/MS. Our results showed that dihydroceramide desaturase was an active enzyme in neuroblastoma cells and the assay provided a powerful method to study the dihydroceramide desaturase activity and the effects of related inhibitors on dihydroceramide desaturase activity.

The effect of dihydroceramide desaturase inhibition by siRNA on endogenous ceramide and dihydroceramides was studied. There was approximately a 13-fold increase of endogenous dihydroceramides whereas endogenous ceramides decreased by 25% compared to untreated cells. No significant changes were seen in other sphingolipids. Desaturase inhibition resulted in growth inhibition and cell cycle arrest at G<sub>0</sub>/G<sub>1</sub>. Phosphorylated Rb (pRb) is critical for cell cycle progression by regulating the G<sub>1</sub>/S phase restriction point, thus controlling entry into the S phase. There was more than 50% decrease in pRb in cells treated with dihydroceramide desaturase siRNA, whereas no change in total Rb level was seen. To determine the involvement of protein phosphate 1 or 2A (PP1 and PP2A) in Rb dephosphorylation, cells were pre-treated with either okadaic acid, a specific PP2A inhibitor, or tautomycin, a specific PP1 inhibitor. Tautomycin was able to inhibit Rb hypophosphorylation whereas okadaic acid showed minimal effect, indicating involvement of PP1.

Next, the effect of the synthetic retinoid, *N*-(4-hydroxyphenyl)retinamide (4-HPR), also known as fenretinide, on dihydroceramide desaturase activity was studied. This retinoid had been reported to increase ceramide levels via *de-novo* synthesis within 6 hours of treatment (Wang et al., 2001). We therefore initially aimed at testing if the inhibition of DEGS-1 by siRNA would block the 4-HPR induced ceramide generation and the anti-tumor effects of 4-HPR. Previously, 4-HPR had been reported to generate ceramide. However, some of the limitations of these earlier studies were due to the method used for the quantitation of ceramide levels in which ceramide was measured by enzymatic or labeling methods where it was difficult to differentiate ceramide from dihydroceramide species. Sphingolipid levels were measured by LC/MS. Increasing concentrations of 4-HPR were directly proportional to increases in endogenous dihydroceramide levels. There was no elevation but rather a modest decrease in endogenous ceramide levels. All-trans-retinoic acid had no effect on desaturase activity. The *in situ* assay for desaturase activity showed inhibition of desaturase activity in cells treated with 4-HPR. There was no change in mRNA as well as protein levels of dihydroceramide desaturase indicating that 4-HPR may be a direct and/or posttranslational inhibitor of dihydroceramide desaturase.

Indeed, we recently demonstrated that dihydroceramide desaturase is a direct *in vitro* target for fenretinide (see below) (Rahmaniyan et al., 2011). Taken together, these observations reveal that 4-HPR is a potent and rapid inhibitor of dihydroceramide desaturase which



induces dihydroceramide generation. In agreement with our study, it was recently reported that inhibition of dihydroceramide desaturase in cultured keratinocytes contributes to increases in dihydroceramide and phytoceramides while ceramide levels decrease (Brodesser & Kolter, 2011). Additionally, Wang et al showed that 4-HPR works synergistically with *D*-erythro-*N,N*-dimethylsphingosine (DMS), a sphingosine kinase inhibitor, resulting in increased intracellular sphinganine and dihydroceramide (Wang *et al.*, 2008).

Furthermore, treatment of 4-HPR with the synthetic sphingolipid protein kinase C inhibitor, *l*-threo-dihydrosphingosine (safingol) was synergistic in neuroblastoma, melanoma, prostate, ewings sarcoma, colon, breast, and lung cancer cell lines (Batra et al., 2004; Maurer et al., 2000). Of importance for neuroblastoma is that neuroblastoma cell lines resistant to cis-retinoic acid are sensitive to 4-HPR (Reynolds et al., 2000). Together with our findings, these data suggest that dihydroceramides have a novel biological function involving cell growth and cell cycle, and they may play a role as targets for cytotoxic agents in cancer cells.

## 6. Dihydroceramide desaturase inhibitors

### 6.1 Background on enzyme Inhibition

Before discussing the inhibitors for dihydroceramide desaturase, it is helpful to review the basic biochemistry of enzyme kinetics and types of inhibition. In general, enzyme inhibitors mediate their inhibitory actions either via affecting the gene expression of the enzyme of interest or via posttranslational modification of the enzyme, which are an indirect effect. Another mode of inhibition is to act directly on the enzyme itself, for instance binding to the active center of the enzyme and thus blocking the activity. Direct acting inhibitors may inhibit the enzyme activity reversibly or irreversibly. The reversible inhibition is divided into competitive, non-competitive, and uncompetitive inhibition. In competitive inhibition the inhibitor and the substrate compete to bind to the pools of free enzyme molecules (Fig. 5). While most likely inhibitor and substrate compete for a common binding pocket (i.e. active site), they could bind to separate sites on the enzyme molecule exerting a negative regulation on each other. Thus, the apparent  $K_m$  value increases with increasing inhibitor concentrations, but the  $V_{max}$  value stays constant at all inhibitor concentrations (Copeland, 2005). The dissociation constant  $K_m$  represents the substrate's affinity for the enzyme and  $K_i$  represents the inhibitor's affinity for the enzyme. The smaller these values, the higher the binding's affinity for the enzyme. A non-competitive inhibitor binds to both the free enzyme and the enzyme-substrate (ES) complex (Fig. 5). This type of inhibitor has two different dissociation constants, one for binary enzyme-inhibitor (EI) complex ( $K_i$ ) and one for the ternary enzyme-substrate-inhibitor (ESI) complex ( $\alpha K_i$ ). Thus,  $V_{max}$  decreases with increasing inhibitor concentrations, but  $K_m$  value depends on the  $\alpha$  values. If  $\alpha > 1$ , the inhibitor binds preferentially to the free enzyme, and  $K_m$  increases with increasing inhibitor concentrations. If  $\alpha < 1$  the inhibitor binds preferentially to the ES complex, and  $K_m$  value decreases with increasing inhibitor concentrations. If  $\alpha = 1$ , the inhibitor binds with equal affinity to both the free enzyme and the ES complex, thus the  $K_m$  values is constant. Unlike competitive inhibitors, non-competitive inhibitors can't be overcome by high substrate concentrations.

An uncompetitive inhibitor binds exclusively to the ES complex (Fig. 5). The formation of ES complex augments the inhibitor affinity for the ES complex contributing to decreased apparent values of both  $K_m$  and  $V_{max}$  with increasing substrate concentrations. Irreversible inhibitors or inactivators are characterized by the formation of covalent bonds with enzyme



molecules. The rate of covalent bond formation (rate of enzyme inactivation) will be slow (Copeland, 2005). Thus, irreversible inhibitors display time-dependent inhibition. This is because the amount of active enzyme at a given concentration of irreversible inhibitor depends on pre-incubation time of the inhibitor with the enzyme. The inactivation rate of the system is determined by the pseudo-first-order rate constant  $K_{obs}$  (Adam et al., 2001; Copeland, 2005). Irreversible inhibitors form initially a reversible non-covalent complex with the enzyme (EI or ESI) and this then reacts to produce the covalently modified "dead-end complex" EI\*. The rate at which EI\* is formed is called the inactivation rate or  $k_{inact}$ . The binding and inactivation steps of this reaction are investigated by incubating the enzyme with inhibitor and assaying the amount of activity remaining over time. The activity will decrease in a time-dependent manner (Maurer & Fung, 2000). In this section some of the dihydroceramide desaturase inhibitors will be discussed which act directly or indirectly on the enzyme.

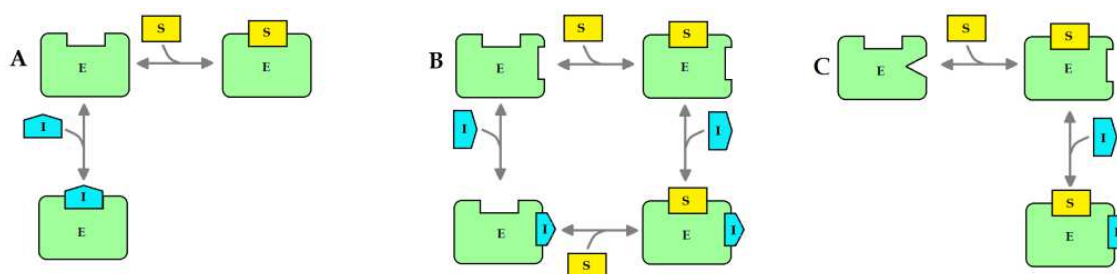


Fig. 5. Types of Enzyme Inhibition.

(A) Competitive inhibition, the enzyme (E) can bind either the substrate (S) or the inhibitor (I); (B) Non-competitive inhibition, the inhibitor can bind to both the free enzyme and ES complex; (C) Uncompetitive inhibition, the inhibitor binds only to the ES complex.

## 6.2 Direct acting agents: Retinoids and ceramide analogues

Retinoids are natural and synthetic derivatives of vitamin A which include all-trans retinoic acid (ATRA), 13-cis retinoic acid, (13-cis-RA), and 4-HPR. They have been used in neuroblastoma treatment due to their effects on cell differentiation, proliferation, and apoptosis (Formelli & Cleris, 2000; Reynolds, 2000; Reynolds & Lemons, 2001). Treatment with 13-cis retinoic acid is now standard of care for patients with high risk neuroblastoma following stem cell transplant (Matthay et al., 2009; Matthay et al., 1999). Some of retinoids mediate their effects through the binding to retinoic acid receptors (RAR) and retinoid X receptors (RXR). Their toxicity has limited their clinical use. For this reason, a large number of synthetic analogues of retinoids has been developed and used in pre-clinical and clinical settings (Villani et al., 2006). ATRA has been shown to modulate sphingolipid metabolizing enzymes. In a recent study, it has been shown that ATRA induces growth arrest in estrogen receptor-positive MCF-7 cells by increased ceramide levels through neutral sphingomyelinase-2. P70 ribosomal S6 kinase (S6K) was identified as downstream effector of ATRA and neutral sphingomyelinase-2. In the same study, ATRA contributed to 3-fold upregulation of dihydroceramide desaturase mRNA (Clarke et al., 2011). 4-HPR is a synthetic retinoid with low toxicity in humans compared to its parent compound ATRA. This difference is believed to be a result of the substitution of an amide-linked 4-hydroxyphenyl moiety for the carbonyl functional group of ATRA (Fig 6). In addition, this

change markedly reduces 4-HPR's binding affinity for nuclear retinoid receptors, supporting the fact that 4-HPR's anticancer activity is independent of retinoid receptors (Hail et al., 2010). The hydroxyl functional group of 4-HPR has been demonstrated to have essential roles in 4-HPR-promoting cytotoxic effects. This functional group also mediates its uptake in cancer cells. In addition, the hydroxyl functional group of 4-HPR triggers ROS production by a mechanism requiring the mitochondrial enzyme dihydroorotate dehydrogenase, an enzyme associated with the mitochondrial electron transport chain and required for *de novo* pyrimidine synthesis.

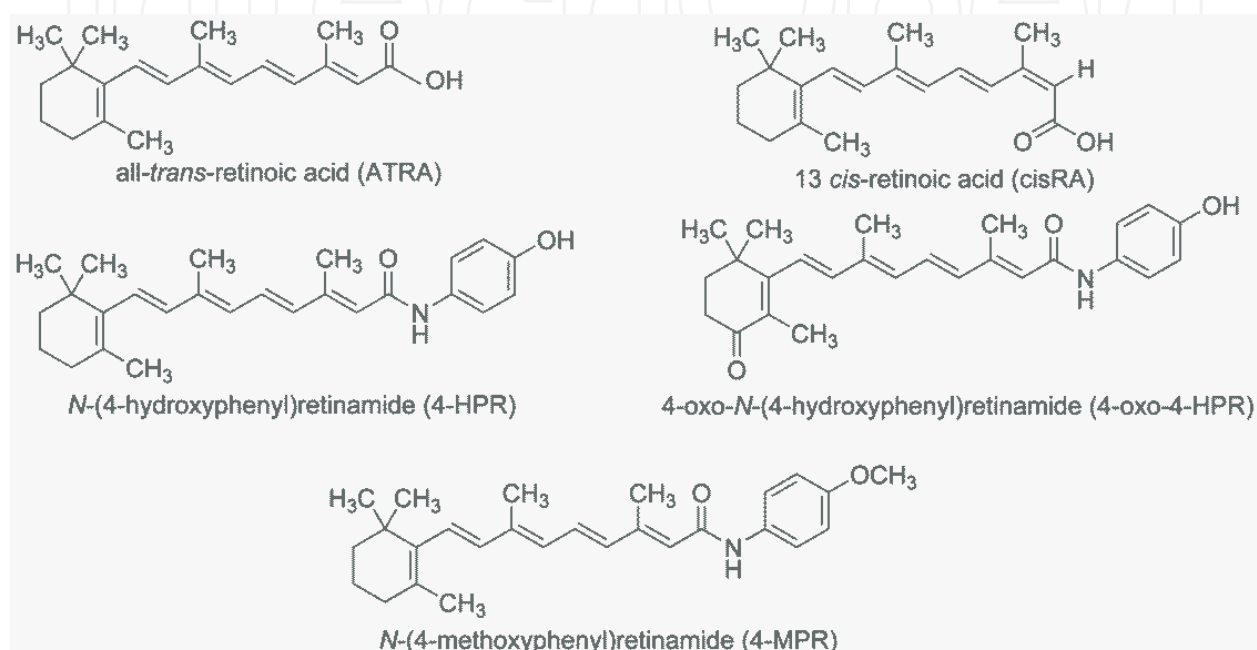


Fig. 6. The Chemical Structures of ATRA, cisRA, 4-HPR and its metabolites 4-oxo-4-HPR and 4-MPR.

4-HPR has been used in clinical trials as chemopreventive and chemotherapeutic agent for various cancers including breast, prostate, head and neck, pancreas, oral leukoplakia, and neuroblastoma. 4-HPR reduced the incidence of contralateral breast cancer and ipsilateral breast cancer recurrence (Torrise et al., 2001). At low levels, 4-HPR induces apoptosis; at higher levels it shows necrosis as well (Lovat et al., 2004). The precise mechanism of its apoptotic action is not fully understood. Unlike most retinoids, 4-HPR has low affinity for retinoid receptors and its mediated apoptosis is RAR independent (Mershon et al., 2007). 4-HPR induces alkaline ceramidase 2 leading to increased dihydrosphingosine (sphinganine) levels (Mao et al., 2010). Besides its ability to generate ROS (Jiang et al., 2011; Lovat et al., 2005), 4-HPR may increase ceramide levels via induction of ceramide synthase (Fig. 7) (Jiang et al., 2004; Wang et al., 2001) and acidic sphingomyelinase (Corazzari et al., 2005; Lovat et al., 2004). 4-HPR-enhanced ceramide is subsequently metabolized via glycosphingolipids and GD3 synthase to the ganglioside GD3 which activates 12-lipoxygenase (12-LOX). 12-LOX then mediates ROS generation with subsequent growth arrest and DNA damage-inducible transcription factor GADD153 and the Bcl-2 related protein BAK (Fig. 7) (Lovat et al., 2005) resulting in release of cytochrome c and activation of caspases-9 and-3 and ultimately apoptosis. In addition, in neuroblastoma cells 4-HPR induces the proapoptotic gene BBC3, a BH3-only family member of Bcl-2, which initiates the mitochondrial apoptotic pathway (Wei

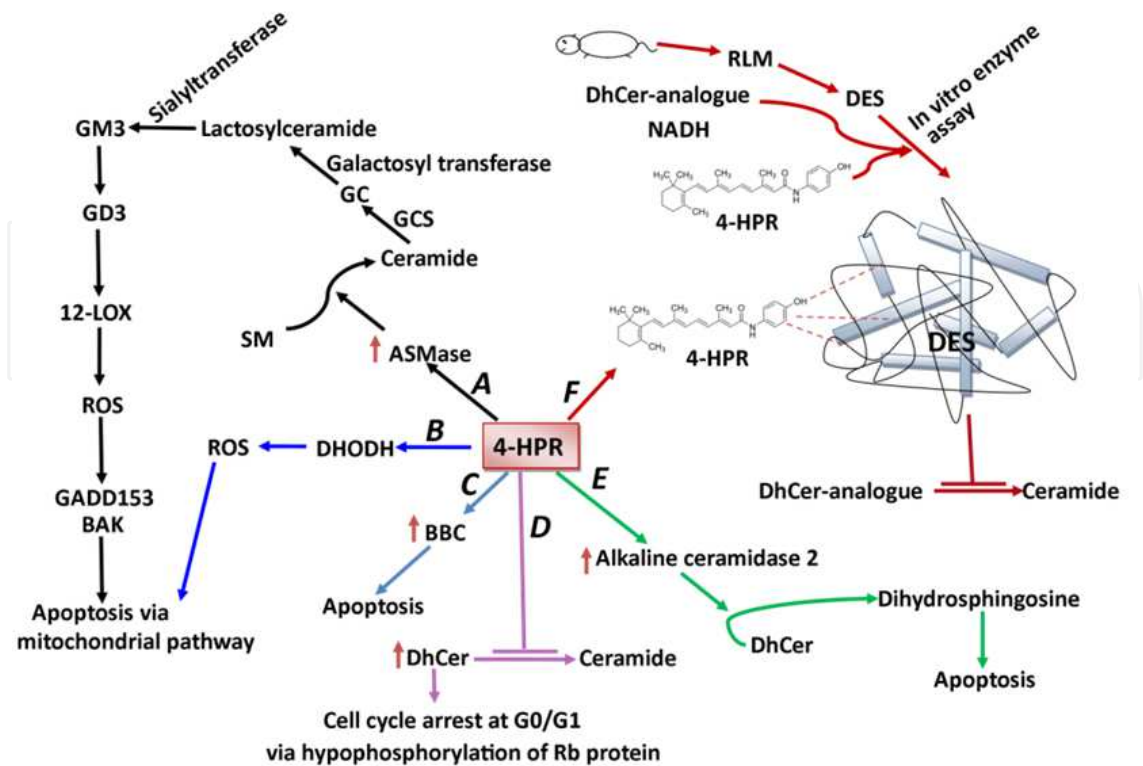
et al., 2005). All mentioned mechanisms are p53-independent, indicating that all cancers with p53 mutation are sensitive to cytotoxic effects of 4-HPR.

4-HPR has a very broad range (0.7- 10  $\mu\text{M}$ ) of cytotoxicity and may have different effects dependent on the concentration and type of cancer cell used (Formelli et al., 2008). In neuroblastoma cells, high concentrations of 4-HPR ( $>5 \mu\text{M}$ ) have been shown to induce apoptosis and necrosis (Wang et al., 2001a) while lower concentrations ( $< 3 \mu\text{M}$ ) of 4-HPR have been reported to induce G<sub>1</sub>-S arrest and hypophosphorylation of Rb (DiPietrantonio et al., 1998; Kravaka et al., 2007; Wu et al., 2001). For neuroblastoma patients enrolled on a Phase I pediatric trial the mean plasma steady-state concentration on day 7 was as 9.9  $\mu\text{mol/L}$  with the maximal tolerated dosage of 2,475 mg/m<sup>2</sup> per day (Villablanca et al., 2006). One of the difficulties with 4-HPR treatment in patients is its low absorption. Another more recent pediatric Phase I study using a new formulation of 4-HPR to improve bioavailability (4HPR/Lym-X-Sorb oral powder), reported the day 7 mean peak plasma concentrations to be 19.7  $\mu\text{M}$  at the recommended dose of 1700 mg/m<sup>2</sup> per day (Marachelian et al., 2009). These doses safely achieved levels active against neuroblastoma in vivo with minimal toxicity.

We recently determined that the enzyme dihydroceramide desaturase is a direct target for 4-HPR *in-vitro* using rat liver microsomes as enzyme source (Rahmaniyan et al., 2011). 4-HPR inhibited the enzyme dihydroceramide desaturase in a time-dependent manner and a dilution experiment showed almost no recovery of the enzyme activity suggesting that 4-HPR may inhibit the dihydroceramide desaturase activity irreversibly (Rahmaniyan et al., 2011).

Two major metabolites of 4-HPR have been identified: N-(4-methoxyphenyl)retinamide (4-MPR) and 4-oxo-N-(4-hydroxyphenyl)retinamide (4-oxo-4-HPR). 4-MPR is an inactive metabolite (Fig 6) with no apoptotic effects that can be used in cancer cells as a biomarker to predict response of cells to 4-HPR (Mehta et al., 1998). 4-MPR has longer plasma half-life than 4-HPR indicating that 4-MPR and 4-HPR have distinct tissue uptake and metabolism (Hail et al., 2010). 4-oxo-4-HPR, a very active and polar metabolite, is able to inhibit 4-HPR resistant cell growth and to act synergistically with the parent drug (Tiberio et al., 2010). In ovarian carcinoma cells 4-oxo-4-HPR is formed by induction of CYP26A1 (Villani et al., 2004). Mechanistically, 4-oxo-4-HPR induces cell cycle arrest at G<sub>2</sub>/M, increases ceramide levels, and caspase-dependent apoptosis (Villani et al., 2006). It generates ROS and causes mitotic arrest via inhibition of tubulin polymerization. ROS generation is independent from mitotic arrest and occurs earlier (Tiberio et al., 2010). In our *in vitro* model, we showed that 4-oxo-4-HPR is also a direct inhibitor of dihydroceramide desaturase as well, exhibiting lower  $K_i$  and  $IC_{50}$  than 4-HPR, indicating that 4-oxo-4-HPR is more potent than the parent drug. 4-MPR showed only very little inhibitory effect on dihydroceramide desaturase activity (Rahmaniyan et al., 2011).

Another direct inhibitor of dihydroceramide desaturase is ceramide analogue, C<sub>8</sub>-cyclopropenyl-ceramide (C<sub>8</sub>CPPC or GT11) (Triola et al., 2001) which is a competitive inhibitor of dihydroceramide desaturase. It was used in our *in vitro* studies and served as positive control to validate the assay (Rahmaniyan et al., 2011). At higher concentrations, GT11 loses its specificity where it also inhibits serine palmitoyl transferase in cells but not in vitro. In addition, it inhibits sphingosine lyase activity contributing to sphingosine-1-phosphate accumulation (Triola et al., 2004). Together, these data indicate that the enzyme dihydroceramide desaturase may be a direct target for chemotherapeutic agents and exploring the type of bonds in enzyme-inhibitor complex could be of further interest.



(A) Enhanced ceramide by activation of acid sphingomyelinase (ASMase) can lead to formation of gangliosides GM3 and GD3 resulting in reactive oxygen species (ROS) generation via 12-Lipoxygenase (12-LOX). ROS induces the transcription factor GADD153 and BAK protein resulting in mitochondrial apoptosis; (B) Dihydroorotate dehydrogenase (DHODH) is a required component for 4-HPR-mediated ROS generation in certain tissues resulting in mitochondrial apoptosis; (C) The proapoptotic gene BBC3, a BH-3 only protein member of Bcl-2 family, can mediate apoptosis in response to 4-HPR; (D) inhibition of dihydroceramide desaturase (DES) by 4-HPR accumulates dihydroceramides (dhCer) in cells resulting in cell cycle arrest at G0/G1 by a mechanism involving pRb; (E) Increased dihydrosphingosine resulting from activation of alkaline ceramidase-2 by 4-HPR can initiate apoptosis; (F) In addition to above mentioned *in vivo* mechanisms, 4-HPR also has an *in vitro* effects. *In vitro* enzyme assay using rat liver microsomes (RLM) as protein source for DES, dhCer-analogue as substrate, and NADH as cofactor demonstrates that 4-HPR inhibits the conversion of dhCer-analogue into ceramide supporting the fact that 4-HPR binds directly to the DES enzyme itself.

Fig. 7. Spingolipid Mediated Mechanisms of Action of Fenretinide (4-HPR):

6.3 Indirect acting agents: Oxidative stress, celecoxib, and resveratrol

Dihydroceramide desaturase activity can be modulated by oxidative stress contributing to accumulation of dihydroceramides (Idkowiak-Baldys *et al.*, 2010). We have reported that H<sub>2</sub>O<sub>2</sub>, tert-butylhydroperoxide, and the intracellular ROS inducer menadione contribute to dihydroceramide desaturase inhibition in breast and lung cancers as well as neuroblastoma. In these cells, accumulation of dihydroceramides was observed with little change in ceramide levels. The *in vitro* assay using cell lysates obtained from cells treated with H<sub>2</sub>O<sub>2</sub> showed inactivation of dihydroceramide desaturase. However, in the direct *in vitro* assay using rat liver microsomes or cell homogenates, H<sub>2</sub>O<sub>2</sub> did not show a significant effect on dihydroceramide desaturase activity indicating that H<sub>2</sub>O<sub>2</sub> inhibits dihydroceramide desaturase indirectly. The multi-enzyme complex of dihydroceramide desaturase (Fig. 4) is



involved in coupled reactions responsible for electron transport from NADPH to terminal desaturase that reduces oxygen. However, NADPH is added to the reaction and is present in both RLMs and cell homogenates. These results suggest that dihydroceramide desaturase inhibition by  $H_2O_2$  is mediated by modulating the redox status of the cell.

Another indirect-acting dihydroceramide desaturase inhibitor is the COX-2 inhibitor celecoxib. Celecoxib has been reported to inhibit neuroblastoma growth and induce apoptosis (Chen et al., 2011; Ponthan et al., 2007). One study showed that treatment of various cancer cells with celecoxib has increased dihydroceramide species such as  $C_{16}$ ,  $C_{24:0}$  and  $C_{24:1}$ - dihydroceramide by dihydroceramide desaturase inhibition, whereas very long chain ceramides such as  $C_{24:0}$  and  $C_{24:1}$ -ceramides were decreased. The anti-proliferative effect of celecoxib were related to dihydroceramide desaturase inhibition which was COX-2-independent (Schiffmann et al., 2009).

Resveratrol is a natural product found in grape skin, red wine, cranberries, blueberries, and peanuts. It is a phytochemical with anti-oxidant, anti-inflammatory and cardioprotective properties. It also causes apoptotic effects on cancer cells, including neuroblastoma (Soto et al., 2011; van Ginkel et al., 2007). In leukemia cell line HL-60 the apoptotic effect is mediated by ceramide accumulation via induction of CerS/LASS genes and down-regulation of SK1 and GCS genes (Cakir et al., 2011). Additionally, it induces autophagy in HGC-27 gastric cancer cells without apoptosis. In this study, autophagy was mediated by the inhibition of dihydroceramide desaturase (Signorelli et al., 2009) by resveratrol. However, our *in vitro* assay neither celecoxib or resveratrol inhibited dihydroceramide desaturase. They had some inhibition at higher concentrations, supporting the fact that both agents may act indirectly on the enzyme (unpublished observations). Additionally, photodynamic therapy (Separovic et al., 2009),  $\gamma$ -tocopherol (Jiang et al., 2004), and XM462 (Munoz-Olaya et al., 2008) may function as indirect inhibitors of dihydroceramide desaturase by modulating the redox status of the cells as in case of hydrogen peroxide.

## 7. Conclusions and future directions

In cancer cells there is an underlying defect in apoptosis due to either overexpression of anti-apoptotic genes, or mutations in pro-apoptotic genes (Ogretmen & Hannun, 2004) leading to escape of apoptotic signaling and ultimately tumorigenesis (Huang et al., 2011). Thus, cancer is a disease characterized by imbalance between cell division and cell death. (Corazzari et al., 2005). Ceramide, the central molecule of sphingolipids, has been identified as mediator for differentiation, cell cycle arrest, cellular senescence, and apoptosis. Dysregulated enzymes and metabolites in sphingolipid metabolism, are associated with development and pathogenesis of various types of cancers. Sphingolipid metabolism is characterized by interconversion of one metabolite into another. For instance, ceramide can be degraded by ceramidase into sphingosine, which is further phosphorylated into sphingosine-1-phosphate. This and other pathways in sphingolipid metabolism create a network regulating individual molecules. A dysregulation in this network by altered levels of bioactive metabolites and enzymes in cancer indicates the importance of sphingolipids in pathogenesis and progression of cancer (Ogretmen & Hannun, 2004).

As discussed before, accumulating evidence shows involvement of sphingolipid signaling in development of human cancers. Angiogenesis is an essential factor for tumor growth and metastasis. The process of angiogenesis is regulated by many angiogenic factors, such as



VEGF (Eggert et al., 2000). In neuroblastoma, tumor vascularity correlates with an aggressive phenotype. It has been demonstrated that VEGF is highly overexpressed in neuroblastoma, which is associated with poor prognosis (Li et al., 2011). Sphingosine-1-phosphate signaling pathway is known to be involved in angiogenesis, which shows a close interaction with VEGF (Heo et al., 2009). Li and colleagues found that sphingosine kinase 2 is highly expressed in neuroblastoma cells and tissues. The product of this enzyme, sphingosine-1-phosphate, induces VEGF expression in neuroblastoma cells via HIF-1- $\alpha$  independent pathway. The same study showed that among sphingosine-1-phosphate receptors, S1P-receptor 2 (S1P<sub>2</sub>) correlates with VEGF mRNA expression suggesting that S1P/S1P<sub>2</sub>/VEGF signaling pathway may promote neuroblastoma growth and angiogenesis. This observation was supported by the fact that blockade of sphingosine-1-phosphate<sub>2</sub> by sphingosine-1-phosphate antagonist JTE-013 resulted in inhibition of tumor growth and angiogenesis. Taken together, these data suggest that sphingolipid signaling pathway is involved in angiogenesis and poor prognosis in neuroblastoma and modulation of sphingolipid signaling pathway may provide an effective approach to control neuroblastoma.

Neuroblastomas originate from embryonic neural crest tissue, and are characterized by failure in differentiation. Spontaneous regression may result from maturation of neoplastic cells into terminally differentiated ganglion cells. Neuroblastoma is known to undergo spontaneous regression by differentiation and apoptosis to a benign phenotype, almost half of cases are aggressive with tendency to metastasize. The ability of neuroblastoma to spontaneously regress through differentiation has generated considerable interest in agents to induce this important biological process (Lovat et al., 2000). Previous studies demonstrated that ceramide positively influences neurite outgrowth, which is one aspect of neuronal differentiation, either as a mediator of nerve growth factor signaling through p75 in hippocampal neurons (Schultz & Larsson, 2004), or upon direct application to neuroblastoma cells in culture such as Neuro2a cells (Prinetti et al., 1997; Riboni et al., 1998; Riboni et al., 1995). Riboni and colleagues showed that agents such as ATRA, or conditions leading to increase in cellular ceramide levels contribute to neuroblastoma differentiation. ATRA induced ceramide increase was also augmented by exogenous administration of sphingomyelin, sphingosine, or L-serine. Additionally, ATRA contributed to activation of neutral sphingomyelinase. The resulting increase in ceramide content contributed to differentiation in neuroblastoma cells. Mechanistically, ceramide-induced differentiation has been mediated by PP2A. An isoform of protein kinase C (PKC), namely PKC $\epsilon$ , was also shown to be involved in both ATRA and growth factor induced morphological changes during neuroblastoma differentiation (Schultz & Larsson, 2004). It was also shown that in Neuro2a cells, exogenously administered sphingosine can be rapidly metabolized by degradation or N-acylation, where N-acylation largely dominates over degradation. This results in increase cellular levels of ceramide and, consequently, in differentiation of neuroblastoma cells. These data suggest that ceramide is involved in the regulation of neuroblastoma differentiation, which may offer a new strategy in neuroblastoma treatment. Spontaneous regression may also result from programmed cell death or apoptosis. Schaefer and colleagues showed that ceramide treatment of five neuroblastoma cell lines results in apoptosis. Although these neuroblastoma cells express CD95 receptors (a TNF receptor family member), they are resistant to apoptosis stimulated by anti-CD95 antibody or by CD95 ligand. However, ceramide treatment of these cells leads to apoptosis, suggesting that ceramide may act downstream of the CD95-death inducing signaling complex (DISC)

(Schaefer et al., 2000). Ceramide is also able to induce apoptosis in neuroblastoma cells by inactivation of Akt pathway and translocation of apoptotic-inducing factor (AIF) from the mitochondria (Kim et al., 2007). Administration of short chain C<sub>2</sub> ceramide to cultured neuroblastoma cells causes apoptosis by activation of intrinsic pathway (Movsesyan et al., 2002). As reported before, dihydroceramide accumulation in neuroblastoma cells causes cell cycle arrest as a result of dihydroceramide desaturase inhibition (Kravetska et al., 2007). Taken together, these data provide evidence in support of a role of sphingolipids in neuronal apoptosis, which might result in spontaneous regression in neuroblastoma, and this will open a new avenue in the therapeutic treatment of neuroblastoma.

A dynamic sphingolipid equilibrium has been described. This equilibrium is characterized by a balance between pro-apoptotic and pro-survival sphingolipids (Cuvillier et al., 1996; Fox et al., 2006), for example the balance between ceramide and sphingosine-1-phosphate. If this balance shifts to ceramide, it can lead to cellular death or cell cycle arrest. If it shifts to sphingosine-1-phosphate, it can result in cell proliferation. The regulation of ceramidases and sphingosine kinases may help to regulate the dynamic balance between these two metabolites. However, this may not be sufficient because there are other sphingolipid metabolites such as ceramide-1-phosphate and glycosphingolipids that are also in balance with ceramide. Thus, it is necessary to study all sphingolipid metabolites and their regulating enzymes. Processes, which enhance the intracellular accumulation of ceramide, will provide a favorable pro-apoptotic outcome in chemotherapy. Yet another way to increase ceramide levels is to administer exogenous short chain ceramides and metabolic precursors of ceramide (palmitate, sphingosine, and C<sub>6</sub>-ceramide). These can be used in combination with chemotherapeutics to augment their effects.

Dihydroceramide desaturase is an important enzyme that has the potential to modulate the dihydroceramide /ceramide ratio in the cell. However, the regulation of dihydroceramide desaturase activity has not been well characterized. Dihydroceramide has been considered primarily as an inactive precursor of ceramide. However, recent studies including our work have demonstrated that dihydroceramides have novel biological functions including cell cycle arrest, apoptosis, ceramide channel formation, autophagy and oxidative stress. These studies indicate the significance of endogenous levels of dihydroceramides in cell regulatory processes, and they suggest that dihydroceramide desaturase has the potential to regulate cell function through both ceramide and dihydroceramide (Kravetska et al., 2007, Rahmaniyan et al., 2011). These observations have implications for the mechanism of action of 4-HPR and for a potential role for dihydroceramide desaturase as a target for chemotherapeutic agents (Rahmaniyan et al., 2011). Thus, the development of new drugs which augment ceramide and dihydroceramide levels will induce cell toxicity and apoptosis in cancer cells. Inhibition of dihydroceramide desaturase is a novel target for cancer therapy. The balance of ceramides and dihydroceramides within a cell may be key to cancer cell proliferation.

## 8. Acknowledgements

We dedicate this chapter to our patients and their families. We would like to thank the members of the Kravetska Laboratory, Dr. Li Li, Dr. Leslie Wooten-Blanks and Dr. Heather Escoto. We would like to thank Dr. Lina Obeid, Dr. Yusuf Hannun, and Dr. Besim Ogretmen for their mentorship, support, and helpful discussions. We thank the Lipidomics Core Facility in the Department of Biochemistry and Molecular Biology at the Medical University of South Carolina, especially Dr. Alicja Bielawska, Dr. Jacek Bielawski, Dr. Zdzislaw M.

Szulc, Barbara Rembiesa and Jason Pierce. We thank the Children's Oncology Group Neuroblastoma Committee and the Children's Oncology Group Neuroblastoma Tumor Bank for providing us with neuroblastoma tumor samples. This work was supported by National Institutes of Health Grants: K01-CA100767 and P20-RR17677, a CureSearch Research Fellowship Award, and by grants from the Rally Foundation for Childhood Cancer Research, St. Baldrick's Foundation, National Philoptochos Children's Medical Fund, Hyundai Hope on Wheels, Monica Kreber Golf Tournament, and Chase After a Cure Foundation.

## 9. References

- Adam, G.C.; Cravatt, B.F. & Sorensen, E.J. (2001) Profiling the specific reactivity of the proteome with non-directed activity-based probes. *Chem Biol*, Vol.8, No.1, pp.81-95
- Adibhatla, R.M. & Hatcher, J.F. (2010) Protection by d609 through cell-cycle regulation after stroke. *Mol Neurobiol*, Vol.41, No.2-3, pp.206-17
- Ahn, E.H. & Schroeder, J.J. (2002) Sphingoid bases and ceramide induce apoptosis in ht-29 and hct-116 human colon cancer cells. *Exp Biol Med (Maywood)*, Vol.227, No.5, pp.345-53
- Argraves, K.M.; Wilkerson, B.A. & Argraves, W.S. (2010) Sphingosine-1-phosphate signaling in vasculogenesis and angiogenesis. *World J Biol Chem*, Vol.1, No.10, pp.291-7
- Barth, B.M.; Gustafson, S.J.; Young, M.M.; Fox, T.E.; Shanmugavelandy, S.S.; Kaiser, J.M.; Cabot, M.C.; Kester, M. & Kuhn, T.B. (2010) Inhibition of nadph oxidase by glucosylceramide confers chemoresistance. *Cancer biology & therapy*, Vol.10, No.11, pp.1126-36
- Batra, S.; Reynolds, C.P. & Maurer, B.J. (2004) Fenretinide cytotoxicity for ewing's sarcoma and primitive neuroectodermal tumor cell lines is decreased by hypoxia and synergistically enhanced by ceramide modulators. *Cancer research*, Vol.64, No.15, pp.5415-24
- Beauchamp, E.; Goenaga, D.; Le Bloc'h, J.; Catheline, D.; Legrand, P. & Rioux, V. (2007) Myristic acid increases the activity of dihydroceramide delta4-desaturase 1 through its n-terminal myristoylation. *Biochimie*, Vol.89, No.12, pp.1553-61
- Bektas, M. & Spiegel, S. (2004) Glycosphingolipids and cell death. *Glycoconj J*, Vol.20, No.1, pp.39-47
- Bielawska, A.; Crane, H.M.; Liotta, D.; Obeid, L.M. & Hannun, Y.A. (1993) Selectivity of ceramide-mediated biology. Lack of activity of erythro-dihydroceramide. *J Biol Chem*, Vol.268, No.35, pp.26226-32
- Billich, A. & Baumruker, T. (2008) Sphingolipid metabolizing enzymes as novel therapeutic targets. *Subcell Biochem*, Vol.49, 487-522
- Birbes, H.; El Bawab, S.; Obeid, L.M. & Hannun, Y.A. (2002) Mitochondria and ceramide: Intertwined roles in regulation of apoptosis. *Adv Enzyme Regul*, Vol.42, 113-29
- Biswal, S.S.; Datta, K.; Acquaah-Mensah, G.K. & Kehrer, J.P. (2000) Changes in ceramide and sphingomyelin following fludarabine treatment of human chronic b-cell leukemia cells. *Toxicology*, Vol.154, No.1-3, pp.45-53
- Bose, R.; Verheij, M.; Haimovitz-Friedman, A.; Scotto, K.; Fuks, Z. & Kolesnick, R. (1995) Ceramide synthase mediates daunorubicin-induced apoptosis: An alternative mechanism for generating death signals. *Cell*, Vol.82, No.3, pp.405-14

- Brodesser, S. & Kolter, T. (2011) Dihydroceramide desaturase inhibition by a cyclopropanated dihydroceramide analog in cultured keratinocytes. *Journal of lipids*, Vol.2011, 724015
- Cadena, D.L.; Kurten, R.C. & Gill, G.N. (1997) The product of the mld gene is a member of the membrane fatty acid desaturase family: Overexpression of mld inhibits egf receptor biosynthesis. *Biochemistry*, Vol.36, No.23, pp.6960-7
- Cakir, Z.; Saydam, G.; Sahin, F. & Baran, Y. (2011) The roles of bioactive sphingolipids in resveratrol-induced apoptosis in hl60: Acute myeloid leukemia cells. *Journal of cancer research and clinical oncology*, Vol.137, No.2, pp.279-86
- Causeret, C.; Geeraert, L.; Van Der Hoeven, G.; Mannaerts, G.P. & Van Veldhoven, P.P. (2000) Further characterization of rat dihydroceramide desaturase: Tissue distribution, subcellular localization, and substrate specificity. *Lipids*, Vol.35, No.10, pp.1117-25
- Chalfant, C.E.; Rathman, K.; Pinkerman, R.L.; Wood, R.E.; Obeid, L.M.; Ogretmen, B. & Hannun, Y.A. (2002) De novo ceramide regulates the alternative splicing of caspase 9 and bcl-x in a549 lung adenocarcinoma cells. Dependence on protein phosphatase-1. *J Biol Chem*, Vol.277, No.15, pp.12587-95
- Chalfant, C.E. & Spiegel, S. (2005) Sphingosine 1-phosphate and ceramide 1-phosphate: Expanding roles in cell signaling. *J Cell Sci*, Vol.118, No.Pt 20, pp.4605-12
- Chang, H.C.; Tsai, L.H.; Chuang, L.Y. & Hung, W.C. (2001) Role of akt kinase in sphingosine-induced apoptosis in human hepatoma cells. *J Cell Physiol*, Vol.188, No.2, pp.188-93
- Chen, Y.; Tsai, Y.H. & Tseng, S.H. (2011) Combined valproic acid and celecoxib treatment induced synergistic cytotoxicity and apoptosis in neuroblastoma cells. *Anticancer research*, Vol.31, No.6, pp.2231-9
- Clarke, C.J. & Hannun, Y.A. (2006) Neutral sphingomyelinases and nsmase2: Bridging the gaps. *Biochim Biophys Acta*, Vol.1758, No.12, pp.1893-901
- Clarke, C.J.; Mediwala, K.; Jenkins, R.W.; Sutton, C.A.; Tholanikunnel, B.G. & Hannun, Y.A. (2011) Neutral sphingomyelinase-2 mediates growth arrest by retinoic acid through modulation of ribosomal s6 kinase. *The Journal of biological chemistry*, Vol.286, No.24, pp.21565-76
- Conway, A.; Pyne, N.J. & Pyne, S. (2000) Ceramide-dependent regulation of p42/p44 mitogen-activated protein kinase and c-jun n-terminal-directed protein kinase in cultured airway smooth muscle cells. *Cell Signal*, Vol.12, No.11-12, pp.737-43
- Copeland, R.A. (2005) *Evaluation of enzyme inhibitors in drug discovery: A guide for medicinal chemists and pharmacologists* (0471686964 edn) (Hoboken, Wiley intersciences).
- Corazzari, M.; Lovat, P.E.; Oliverio, S.; Di Sano, F.; Donnorso, R.P.; Redfern, C.P. & Piacentini, M. (2005) Fenretinide: A p53-independent way to kill cancer cells. *Biochemical and biophysical research communications*, Vol.331, No.3, pp.810-5
- Cuvillier, O.; Nava, V.E.; Murthy, S.K.; Edsall, L.C.; Levade, T.; Milstien, S. & Spiegel, S. (2001) Sphingosine generation, cytochrome c release, and activation of caspase-7 in doxorubicin-induced apoptosis of mcf7 breast adenocarcinoma cells. *Cell Death Differ*, Vol.8, No.2, pp.162-71
- Cuvillier, O.; Pirianov, G.; Kleuser, B.; Vanek, P.G.; Coso, O.A.; Gutkind, S. & Spiegel, S. (1996) Suppression of ceramide-mediated programmed cell death by sphingosine-1-phosphate. *Nature*, Vol.381, No.6585, pp.800-3



- Dbaibo, G.S.; Pushkareva, M.Y.; Jayadev, S.; Schwarz, J.K.; Horowitz, J.M.; Obeid, L.M. & Hannun, Y.A. (1995) Retinoblastoma gene product as a downstream target for a ceramide-dependent pathway of growth arrest. *Proceedings of the National Academy of Sciences of the United States of America*, Vol.92, No.5, pp.1347-51
- Dipietrantonio, A.M.; Hsieh, T.C.; Olson, S.C. & Wu, J.M. (1998) Regulation of g1/s transition and induction of apoptosis in hl-60 leukemia cells by fenretinide (4hpr). *Int J Cancer*, Vol.78, No.1, pp.53-61
- Dobrowsky, R.T.; Kamibayashi, C.; Mumby, M.C. & Hannun, Y.A. (1993) Ceramide activates heterotrimeric protein phosphatase 2a. *J Biol Chem*, Vol.268, No.21, pp.15523-30
- Eggert, A.; Sieverts, H.; Ikegaki, N. & Brodeur, G.M. (2000) P75 mediated apoptosis in neuroblastoma cells is inhibited by expression of trka. *Med Pediatr Oncol*, Vol.35, No.6, pp.573-6
- Ekiz, H.A. & Baran, Y. (2010) Therapeutic applications of bioactive sphingolipids in hematological malignancies. *Int J Cancer*, Vol.127, No.7, pp.1497-506
- Formelli, F.; Cavadini, E.; Luksch, R.; Garaventa, A.; Villani, M.G.; Appierto, V. & Persiani, S. (2008) Pharmacokinetics of oral fenretinide in neuroblastoma patients: Indications for optimal dose and dosing schedule also with respect to the active metabolite 4-oxo-fenretinide. *Cancer Chemother Pharmacol*, Vol.62, No.4, pp.655-65
- Formelli, F. & Cleris, L. (2000) Therapeutic effects of the combination of fenretinide and all-trans-retinoic acid and of the two retinoids with cisplatin in a human ovarian carcinoma xenograft and in a cisplatin-resistant sub-line. *Eur J Cancer*, Vol.36, No.18, pp.2411-9
- Fox, T.E.; Finnegan, C.M.; Blumenthal, R. & Kester, M. (2006) The clinical potential of sphingolipid-based therapeutics. *Cellular and molecular life sciences : CMLS*, Vol.63, No.9, pp.1017-23
- French, K.J.; Upson, J.J.; Keller, S.N.; Zhuang, Y.; Yun, J.K. & Smith, C.D. (2006) Antitumor activity of sphingosine kinase inhibitors. *J Pharmacol Exp Ther*, Vol.318, No.2, pp.596-603
- Futerman, A.H. & Hannun, Y.A. (2004) The complex life of simple sphingolipids. *EMBO Rep*, Vol.5, No.8, pp.777-82
- Gault, C.R.; Obeid, L.M. & Hannun, Y.A. (2010) An overview of sphingolipid metabolism: From synthesis to breakdown. *Adv Exp Med Biol*, Vol.688, 1-23
- Geeraert, L.; Mannaerts, G.P. & Van Veldhoven, P.P. (1997) Conversion of dihydroceramide into ceramide: Involvement of a desaturase. *Biochem J*, Vol.327 ( Pt 1), 125-32
- Goldkorn, T.; Balaban, N.; Shannon, M.; Chea, V.; Matsukuma, K.; Gilchrist, D.; Wang, H. & Chan, C. (1998) H<sub>2</sub>O<sub>2</sub> acts on cellular membranes to generate ceramide signaling and initiate apoptosis in tracheobronchial epithelial cells. *J Cell Sci*, Vol.111 ( Pt 21), 3209-20
- Gomez Del Pulgar, T.; Velasco, G.; Sanchez, C.; Haro, A. & Guzman, M. (2002) De novo-synthesized ceramide is involved in cannabinoid-induced apoptosis. *Biochem J*, Vol.363, No.Pt 1, pp.183-8
- Gomez-Munoz, A. (2004) Ceramide-1-phosphate: A novel regulator of cell activation. *FEBS Lett*, Vol.562, No.1-3, pp.5-10
- Gomez-Munoz, A.; Duffy, P.A.; Martin, A.; O'brien, L.; Byun, H.S.; Bittman, R. & Brindley, D.N. (1995) Short-chain ceramide-1-phosphates are novel stimulators of DNA



- synthesis and cell division: Antagonism by cell-permeable ceramides. *Mol Pharmacol*, Vol.47, No.5, pp.833-9
- Gomez-Munoz, A.; Kong, J.Y.; Sallh, B. & Steinbrecher, U.P. (2004) Ceramide-1-phosphate blocks apoptosis through inhibition of acid sphingomyelinase in macrophages. *J Lipid Res*, Vol.45, No.1, pp.99-105
- Hail, N., Jr.; Chen, P.; Kepa, J.J.; Bushman, L.R. & Shearn, C. (2010) Dihydroorotate dehydrogenase is required for n-(4-hydroxyphenyl)retinamide-induced reactive oxygen species production and apoptosis. *Free radical biology & medicine*, Vol.49, No.1, pp.109-16
- Hailemariam, T.K.; Huan, C.; Liu, J.; Li, Z.; Roman, C.; Kalbfisch, M.; Bui, H.H.; Peake, D.A.; Kuo, M.S.; Cao, G.; Wadgaonkar, R. & Jiang, X.C. (2008) Sphingomyelin synthase 2 deficiency attenuates nf[ $\kappa$ ]b activation. *Arterioscler Thromb Vasc Biol*,
- Haimovitz-Friedman, A.; Kan, C.C.; Ehleiter, D.; Persaud, R.S.; Mcloughlin, M.; Fuks, Z. & Kolesnick, R.N. (1994) Ionizing radiation acts on cellular membranes to generate ceramide and initiate apoptosis. *J Exp Med*, Vol.180, No.2, pp.525-35
- Hannun, Y.A.; Loomis, C.R.; Merrill, A.H., Jr. & Bell, R.M. (1986) Sphingosine inhibition of protein kinase c activity and of phorbol dibutyrate binding in vitro and in human platelets. *J Biol Chem*, Vol.261, No.27, pp.12604-9
- Hannun, Y.A. & Luberto, C. (2000) Ceramide in the eukaryotic stress response. *Trends Cell Biol*, Vol.10, No.2, pp.73-80
- Hannun, Y.A. & Obeid, L.M. (2008) Principles of bioactive lipid signalling: Lessons from sphingolipids. *Nat Rev Mol Cell Biol*, Vol.9, No.2, pp.139-50
- Hannun, Y.A. & Obeid, L.M. (2011) Many ceramides. *The Journal of biological chemistry*, Vol.286, No.32, pp.27855-62
- Heinrich, M.; Wickel, M.; Schneider-Brachert, W.; Sandberg, C.; Gahr, J.; Schwandner, R.; Weber, T.; Saftig, P.; Peters, C.; Brunner, J.; Kronke, M. & Schutze, S. (1999) Cathepsin d targeted by acid sphingomyelinase-derived ceramide. *Embo J*, Vol.18, No.19, pp.5252-63
- Heo, K.; Park, K.A.; Kim, Y.H.; Kim, S.H.; Oh, Y.S.; Kim, I.H.; Ryu, S.H. & Suh, P.G. (2009) Sphingosine 1-phosphate induces vascular endothelial growth factor expression in endothelial cells. *BMB reports*, Vol.42, No.10, pp.685-90
- Hinkovska-Galcheva, V.; Boxer, L.A.; Kindzelskii, A.; Hiraoka, M.; Abe, A.; Goparaju, S.; Spiegel, S.; Petty, H.R. & Shayman, J.A. (2005) Ceramide 1-phosphate, a mediator of phagocytosis. *J Biol Chem*, Vol.280, No.28, pp.26612-21
- Huang, W.C.; Chen, C.L.; Lin, Y.S. & Lin, C.F. (2011) Apoptotic sphingolipid ceramide in cancer therapy. *J Lipids*, Vol.2011, 565316
- Idkowiak-Baldys, J.; Apraiz, A.; Li, L.; Rahmaniyan, M.; Clarke, C.J.; Kraveka, J.M.; Asumendi, A. & Hannun, Y.A. (2010) Dihydroceramide desaturase activity is modulated by oxidative stress. *Biochem J*, Vol.427, No.2, pp.265-74
- Ijana, A. & Pahan, K. (2010) Sphingolipids in multiple sclerosis. *Neuromolecular Med*, Vol.12, No.4, pp.351-61
- Jarvis, W.D.; Fornari, F.A., Jr.; Auer, K.L.; Freemerman, A.J.; Szabo, E.; Birrer, M.J.; Johnson, C.R.; Barbour, S.E.; Dent, P. & Grant, S. (1997) Coordinate regulation of stress- and mitogen-activated protein kinases in the apoptotic actions of ceramide and sphingosine. *Mol Pharmacol*, Vol.52, No.6, pp.935-47

- Jayadev, S.; Liu, B.; Bielawska, A.E.; Lee, J.Y.; Nazaire, F.; Pushkareva, M.; Obeid, L.M. & Hannun, Y.A. (1995) Role for ceramide in cell cycle arrest. *The Journal of biological chemistry*, Vol.270, No.5, pp.2047-52
- Jiang, Q.; Wong, J.; Fyrst, H.; Saba, J.D. & Ames, B.N. (2004) Gamma-tocopherol or combinations of vitamin e forms induce cell death in human prostate cancer cells by interrupting sphingolipid synthesis. *Proc Natl Acad Sci U S A*, Vol.101, No.51, pp.17825-30
- Kim, M.Y.; Linardic, C.; Obeid, L. & Hannun, Y. (1991) Identification of sphingomyelin turnover as an effector mechanism for the action of tumor necrosis factor alpha and gamma-interferon. Specific role in cell differentiation. *J Biol Chem*, Vol.266, No.1, pp.484-9
- Kim, N.H.; Kim, K.; Park, W.S.; Son, H.S. & Bae, Y. (2007) Pkb/akt inhibits ceramide-induced apoptosis in neuroblastoma cells by blocking apoptosis-inducing factor (aif) translocation. *J Cell Biochem*, Vol.102, No.5, pp.1160-70
- Kitatani, K.; Idkowiak-Baldys, J. & Hannun, Y.A. (2008) The sphingolipid salvage pathway in ceramide metabolism and signaling. *Cell Signal*, Vol.20, No.6, pp.1010-8
- Kok, J.W.; Nikolova-Karakashian, M.; Klappe, K.; Alexander, C. & Merrill, A.H., Jr. (1997) Dihydroceramide biology. Structure-specific metabolism and intracellular localization. *J Biol Chem*, Vol.272, No.34, pp.21128-36
- Kolesnick, R. (2002) The therapeutic potential of modulating the ceramide/sphingomyelin pathway. *J Clin Invest*, Vol.110, No.1, pp.3-8
- Koybasi, S.; Senkal, C.E.; Sundararaj, K.; Spassieva, S.; Bielawski, J.; Osta, W.; Day, T.A.; Jiang, J.C.; Jazwinski, S.M.; Hannun, Y.A.; Obeid, L.M. & Ogretmen, B. (2004) Defects in cell growth regulation by c18:0-ceramide and longevity assurance gene 1 in human head and neck squamous cell carcinomas. *J Biol Chem*, Vol.279, No.43, pp.44311-9
- Kraveka, J.M. & Hannun, Y.A. (2009) Bioactive sphingolipids: An overview on ceramide, ceramide 1-phosphate dihydroceramide, sphingosine, sphingosine 1-phosphate, in: A. Lajtha (Ed.) *Handbook of neurochemistry and molecular neurobiology*. (vol. XVI) 3rd ed. (New York, NY, Springer), 373-384.
- Kraveka, J.M.; Li, L.; Bielawski, J.; Obeid, L.M. & Ogretmen, B. (2003) Involvement of endogenous ceramide in the inhibition of telomerase activity and induction of morphologic differentiation in response to all-trans-retinoic acid in human neuroblastoma cells. *Arch Biochem Biophys*, Vol.419, No.2, pp.110-9
- Kraveka, J.M.; Li, L.; Szulc, Z.M.; Bielawski, J.; Ogretmen, B.; Hannun, Y.A.; Obeid, L.M. & Bielawska, A. (2007) Involvement of dihydroceramide desaturase in cell cycle progression in human neuroblastoma cells. *J Biol Chem*, Vol.282, No.23, pp.16718-28
- Lacour, S.; Hammann, A.; Grazide, S.; Lagadic-Gossmann, D.; Athias, A.; Sergent, O.; Laurent, G.; Gambert, P.; Solary, E. & Dimanche-Boitrel, M.T. (2004) Cisplatin-induced cd95 redistribution into membrane lipid rafts of ht29 human colon cancer cells. *Cancer Res*, Vol.64, No.10, pp.3593-8
- Lee, J.Y.; Bielawska, A.E. & Obeid, L.M. (2000) Regulation of cyclin-dependent kinase 2 activity by ceramide. *Experimental cell research*, Vol.261, No.2, pp.303-11
- Li, M.H.; Hla, T. & Ferrer, F. (2011) Sphingolipid modulation of angiogenic factor expression in neuroblastoma. *Cancer Prev Res (Phila)*,

- Lin, T.; Genestier, L.; Pinkoski, M.J.; Castro, A.; Nicholas, S.; Mogil, R.; Paris, F.; Fuks, Z.; Schuchman, E.H.; Kolesnick, R.N. & Green, D.R. (2000) Role of acidic sphingomyelinase in fas/cd95-mediated cell death. *J Biol Chem*, Vol.275, No.12, pp.8657-63
- Ling, B.; Chen, L.; Alcorn, J.; Ma, B. & Yang, J. (2011) Sphingosine-1-phosphate: A potential therapeutic agent against human breast cancer. *Invest New Drugs*, Vol.29, No.2, pp.396-9
- Liu, B.; Andrieu-Abadie, N.; Levade, T.; Zhang, P.; Obeid, L.M. & Hannun, Y.A. (1998) Glutathione regulation of neutral sphingomyelinase in tumor necrosis factor-alpha-induced cell death. *J Biol Chem*, Vol.273, No.18, pp.11313-20
- Liu, Y.; Xie, K.M.; Yang, G.Q.; Bai, X.M.; Shi, Y.P.; Mu, H.J.; Qiao, W.Z.; Zhang, B. & Xie, P. (2010a) Gcs induces multidrug resistance by regulating apoptosis-related genes in k562/ao2 cell line. *Cancer chemotherapy and pharmacology*, Vol.66, No.3, pp.433-9
- Liu, Y.Y.; Gupta, V.; Patwardhan, G.A.; Bhinge, K.; Zhao, Y.; Bao, J.; Mehendale, H.; Cabot, M.C.; Li, Y.T. & Jazwinski, S.M. (2010b) Glucosylceramide synthase upregulates mdrl expression in the regulation of cancer drug resistance through csrc and beta-catenin signaling. *Molecular cancer*, Vol.9, 145
- Lovat, P.E.; Corazzari, M.; Di Sano, F.; Piacentini, M. & Redfern, C.P. (2005) The role of gangliosides in fenretinide-induced apoptosis of neuroblastoma. *Cancer letters*, Vol.228, No.1-2, pp.105-10
- Lovat, P.E.; Di Sano, F.; Corazzari, M.; Fazi, B.; Donnorso, R.P.; Pearson, A.D.; Hall, A.G.; Redfern, C.P. & Piacentini, M. (2004) Gangliosides link the acidic sphingomyelinase-mediated induction of ceramide to 12-lipoxygenase-dependent apoptosis of neuroblastoma in response to fenretinide. *J Natl Cancer Inst*, Vol.96, No.17, pp.1288-99
- Lovat, P.E.; Ranalli, M.; Annichiarico-Petruzzelli, M.; Bernassola, F.; Piacentini, M.; Malcolm, A.J.; Pearson, A.D.; Melino, G. & Redfern, C.P. (2000) Effector mechanisms of fenretinide-induced apoptosis in neuroblastoma. *Exp Cell Res*, Vol.260, No.1, pp.50-60
- Lozano, J.; Berra, E.; Municio, M.M.; Diaz-Meco, M.T.; Dominguez, I.; Sanz, L. & Moscat, J. (1994) Protein kinase c zeta isoform is critical for kappa b-dependent promoter activation by sphingomyelinase. *J Biol Chem*, Vol.269, No.30, pp.19200-2
- Luberto, C.; Hassler, D.F.; Signorelli, P.; Okamoto, Y.; Sawai, H.; Boros, E.; Hazen-Martin, D.J.; Obeid, L.M.; Hannun, Y.A. & Smith, G.K. (2002) Inhibition of tumor necrosis factor-induced cell death in mcf7 by a novel inhibitor of neutral sphingomyelinase. *J Biol Chem*, Vol.277, No.43, pp.41128-39
- Luberto, C.; Yoo, D.S.; Suidan, H.S.; Bartoli, G.M. & Hannun, Y.A. (2000) Differential effects of sphingomyelin hydrolysis and resynthesis on the activation of nf-kappa b in normal and sv40-transformed human fibroblasts. *J Biol Chem*, Vol.275, No.19, pp.14760-6
- Lucci, A.; Cho, W.I.; Han, T.Y.; Giuliano, A.E.; Morton, D.L. & Cabot, M.C. (1998) Glucosylceramide: A marker for multiple-drug resistant cancers. *Anticancer research*, Vol.18, No.1B, pp.475-80
- Lucki, N.C. & Sewer, M.B. (2011) Genistein stimulates mcf-7 breast cancer cell growth by inducing acid ceramidase (asah1) gene expression. *The Journal of biological chemistry*, Vol.286, No.22, pp.19399-409

- Mahdy, A.E.; Cheng, J.C.; Li, J.; Elojeimy, S.; Meacham, W.D.; Turner, L.S.; Bai, A.; Gault, C.R.; Mcpherson, A.S.; Garcia, N.; Beckham, T.H.; Saad, A.; Bielawska, A.; Bielawski, J.; Hannun, Y.A.; Keane, T.E.; Taha, M.I.; Hammouda, H.M.; Norris, J.S. & Liu, X. (2009) Acid ceramidase upregulation in prostate cancer cells confers resistance to radiation: Ac inhibition, a potential radiosensitizer. *Molecular therapy : the journal of the American Society of Gene Therapy*, Vol.17, No.3, pp.430-8
- Mao, Z.; Sun, W.; Xu, R.; Novgorodov, S.; Szulc, Z.M.; Bielawski, J.; Obeid, L.M. & Mao, C. (2010) Alkaline ceramidase 2 (acer2) and its product dihydrosphingosine mediate the cytotoxicity of n-(4-hydroxyphenyl)retinamide in tumor cells. *The Journal of biological chemistry*, Vol.285, No.38, pp.29078-90
- Marachelian, A.; Kang, M.H.; Hwang, K.; Villablanca, J.G.; Groshen, S.; Matthay, K.K.; Maris, J.; Desantes, K.B.; Reynolds, C.P. & Maurer, B.J. (2009) Phase i study of fenretinide (4-hpr) oral powder in patients with recurrent or resistant neuroblastoma: New approaches to neuroblastoma therapy (nant) consortium trial. *J Clin Oncol*. 27:15s, Abstract 10009.
- Marchesini, N. & Hannun, Y.A. (2004) Acid and neutral sphingomyelinases: Roles and mechanisms of regulation. *Biochem Cell Biol*, Vol.82, No.1, pp.27-44
- Maris, J.M. (2010) Recent advances in neuroblastoma. *The New England journal of medicine*, Vol.362, No.23, pp.2202-11
- Matthay, K.K.; Reynolds, C.P.; Seeger, R.C.; Shimada, H.; Adkins, E.S.; Haas-Kogan, D.; Gerbing, R.B.; London, W.B. & Villablanca, J.G. (2009) Long-term results for children with high-risk neuroblastoma treated on a randomized trial of myeloablative therapy followed by 13-cis-retinoic acid: A children's oncology group study. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*, Vol.27, No.7, pp.1007-13
- Matthay, K.K.; Villablanca, J.G.; Seeger, R.C.; Stram, D.O.; Harris, R.E.; Ramsay, N.K.; Swift, P.; Shimada, H.; Black, C.T.; Brodeur, G.M.; Gerbing, R.B. & Reynolds, C.P. (1999) Treatment of high-risk neuroblastoma with intensive chemotherapy, radiotherapy, autologous bone marrow transplantation, and 13-cis-retinoic acid. Children's cancer group. *The New England journal of medicine*, Vol.341, No.16, pp.1165-73
- Maurer, B.J.; Melton, L.; Billups, C.; Cabot, M.C. & Reynolds, C.P. (2000) Synergistic cytotoxicity in solid tumor cell lines between n-(4-hydroxyphenyl)retinamide and modulators of ceramide metabolism. *Journal of the National Cancer Institute*, Vol.92, No.23, pp.1897-909
- Maurer, T. & Fung, H.L. (2000) Comparison of methods for analyzing kinetic data from mechanism-based enzyme inactivation: Application to nitric oxide synthase. *AAPS PharmSci*, Vol.2, No.1, pp.E8
- Mehta, R.R.; Hawthorne, M.E.; Graves, J.M. & Mehta, R.G. (1998) Metabolism of n-[4-hydroxyphenyl]retinamide (4-hpr) to n-[4-methoxyphenyl]retinamide (4-mpr) may serve as a biomarker for its efficacy against human breast cancer and melanoma cells. *European journal of cancer*, Vol.34, No.6, pp.902-7
- Merrill, A.H., Jr. (2002) De novo sphingolipid biosynthesis: A necessary, but dangerous, pathway. *J Biol Chem*, Vol.277, No.29, pp.25843-6
- Mershon, S.M.; Anding, A.L.; Chapman, J.S.; Clagett-Dame, M.; Stonerock, L.A. & Curley, R.W., Jr. (2007) Solid phase-assisted synthesis and screening of a small library of n-



- (4-hydroxyphenyl)retinamide (4-hpr) analogs. *Bioorg Med Chem Lett*, Vol.17, No.3, pp.836-40
- Messner, M.C. & Cabot, M.C. (2010) Glucosylceramide in humans. *Advances in experimental medicine and biology*, Vol.688, 156-64
- Michel, C.; Van Echten-Deckert, G.; Rother, J.; Sandhoff, K.; Wang, E. & Merrill, A.H., Jr. (1997) Characterization of ceramide synthesis. A dihydroceramide desaturase introduces the 4,5-trans-double bond of sphingosine at the level of dihydroceramide. *J Biol Chem*, Vol.272, No.36, pp.22432-7
- Mitsutake, S.; Kim, T.J.; Inagaki, Y.; Kato, M.; Yamashita, T. & Igarashi, Y. (2004) Ceramide kinase is a mediator of calcium-dependent degranulation in mast cells. *J Biol Chem*, Vol.279, No.17, pp.17570-7
- Mizutani, Y.; Kihara, A. & Igarashi, Y. (2004) Identification of the human sphingolipid c4-hydroxylase, hdes2, and its up-regulation during keratinocyte differentiation. *FEBS Lett*, Vol.563, No.1-3, pp.93-7
- Morales, A.; Paris, R.; Villanueva, A.; Llacuna, L.; Garcia-Ruiz, C. & Fernandez-Checa, J.C. (2007) Pharmacological inhibition or small interfering rna targeting acid ceramidase sensitizes hepatoma cells to chemotherapy and reduces tumor growth in vivo. *Oncogene*, Vol.26, No.6, pp.905-16
- Movsesyan, V.A.; Yakovlev, A.G.; Dabaghyan, E.A.; Stoica, B.A. & Faden, A.I. (2002) Ceramide induces neuronal apoptosis through the caspase-9/caspase-3 pathway. *Biochem Biophys Res Commun*, Vol.299, No.2, pp.201-7
- Mullen, T.D.; Jenkins, R.W.; Clarke, C.J.; Bielawski, J.; Hannun, Y.A. & Obeid, L.M. (2011) Ceramide synthase-dependent ceramide generation and programmed cell death: Involvement of salvage pathway in regulating postmitochondrial events. *J Biol Chem*, Vol.286, No.18, pp.15929-42
- Munoz-Olaya, J.M.; Matabosch, X.; Bedia, C.; Egido-Gabas, M.; Casas, J.; Llebaria, A.; Delgado, A. & Fabrias, G. (2008) Synthesis and biological activity of a novel inhibitor of dihydroceramide desaturase. *ChemMedChem*, Vol.3, No.6, pp.946-53
- Murakami, M.; Ito, H.; Hagiwara, K.; Yoshida, K.; Sobue, S.; Ichihara, M.; Takagi, A.; Kojima, T.; Tanaka, K.; Tamiya-Koizumi, K.; Kyogashima, M.; Suzuki, M.; Banno, Y.; Nozawa, Y. & Murate, T. (2010) ATRA inhibits ceramide kinase transcription in a human neuroblastoma cell line, sh-sy5y cells: The role of coup-tfi. *Journal of neurochemistry*, Vol.112, No.2, pp.511-20
- Ogretmen, B. (2006) Sphingolipids in cancer: Regulation of pathogenesis and therapy. *FEBS Lett*, Vol.580, No.23, pp.5467-76
- Ogretmen, B. & Hannun, Y.A. (2004) Biologically active sphingolipids in cancer pathogenesis and treatment. *Nat Rev Cancer*, Vol.4, No.8, pp.604-16
- Ogretmen, B.; Kravets, J.M.; Schady, D.; Usta, J.; Hannun, Y.A. & Obeid, L.M. (2001) Molecular mechanisms of ceramide-mediated telomerase inhibition in the A549 human lung adenocarcinoma cell line. *The Journal of biological chemistry*, Vol.276, No.35, pp.32506-14
- Omae, F.; Miyazaki, M.; Enomoto, A.; Suzuki, M.; Suzuki, Y. & Suzuki, A. (2004) Des2 protein is responsible for phytoceramide biosynthesis in the mouse small intestine. *Biochem J*, Vol.379, No. Pt 3, pp.687-95
- Patwardhan, G.A.; Zhang, Q.J.; Yin, D.; Gupta, V.; Bao, J.; Senkal, C.E.; Ogretmen, B.; Cabot, M.C.; Shah, G.V.; Sylvester, P.W.; Jazwinski, S.M. & Liu, Y.Y. (2009) A new mixed-



- backbone oligonucleotide against glucosylceramide synthase sensitizes multidrug-resistant tumors to apoptosis. *PloS one*, Vol.4, No.9, pp.e6938
- Perry, D.K.; Carton, J.; Shah, A.K.; Meredith, F.; Uhlinger, D.J. & Hannun, Y.A. (2000) Serine palmitoyltransferase regulates de novo ceramide generation during etoposide-induced apoptosis. *J Biol Chem*, Vol.275, No.12, pp.9078-84
- Pettus, B.J.; Bielawska, A.; Subramanian, P.; Wijesinghe, D.S.; Maceyka, M.; Leslie, C.C.; Evans, J.H.; Freiberg, J.; Roddy, P.; Hannun, Y.A. & Chalfant, C.E. (2004) Ceramide 1-phosphate is a direct activator of cytosolic phospholipase a2. *J Biol Chem*, Vol.279, No.12, pp.11320-6
- Pettus, B.J.; Chalfant, C.E. & Hannun, Y.A. (2002) Ceramide in apoptosis: An overview and current perspectives. *Biochim Biophys Acta*, Vol.1585, No.2-3, pp.114-25
- Pewzner-Jung, Y.; Ben-Dor, S. & Futerman, A.H. (2006) When do lasses (longevity assurance genes) become cers (ceramide synthases)? Insights into the regulation of ceramide synthesis. *J Biol Chem*, Vol.281, No.35, pp.25001-5
- Ponnusamy, S.; Meyers-Needham, M.; Senkal, C.E.; Saddoughi, S.A.; Sentelle, D.; Selvam, S.P.; Salas, A. & Ogretmen, B. (2010) Sphingolipids and cancer: Ceramide and sphingosine-1-phosphate in the regulation of cell death and drug resistance. *Future oncology*, Vol.6, No.10, pp.1603-24
- Ponthan, F.; Wickstrom, M.; Gleissman, H.; Fuskevag, O.M.; Segerstrom, L.; Sveinbjornsson, B.; Redfern, C.P.; Eksborg, S.; Kogner, P. & Johnsen, J.I. (2007) Celecoxib prevents neuroblastoma tumor development and potentiates the effect of chemotherapeutic drugs in vitro and in vivo. *Clinical cancer research : an official journal of the American Association for Cancer Research*, Vol.13, No.3, pp.1036-44
- Prinetti, A.; Bassi, R.; Riboni, L. & Tettamanti, G. (1997) Involvement of a ceramide activated protein phosphatase in the differentiation of neuroblastoma neuro2a cells. *FEBS Lett*, Vol.414, No.2, pp.475-9
- Rahmaniyan, M.; Curley, R.W., Jr.; Obeid, L.M.; Hannun, Y.A. & Kraveka, J.M. (2011) Identification of dihydroceramide desaturase as a direct in vitro target for fenretinide. *The Journal of biological chemistry*,
- Reynolds, C.P. (2000) Differentiating agents in pediatric malignancies: Retinoids in neuroblastoma. *Current oncology reports*, Vol.2, No.6, pp.511-8
- Reynolds, C.P. & Lemons, R.S. (2001) Retinoid therapy of childhood cancer. *Hematology/oncology clinics of North America*, Vol.15, No.5, pp.867-910
- Reynolds, C.P.; Wang, Y.; Melton, L.J.; Einhorn, P.A.; Slamon, D.J. & Maurer, B.J. (2000) Retinoic-acid-resistant neuroblastoma cell lines show altered myc regulation and high sensitivity to fenretinide. *Medical and pediatric oncology*, Vol.35, No.6, pp.597-602
- Riboni, L.; Bassi, R.; Caminiti, A.; Prinetti, A.; Viani, P. & Tettamanti, G. (1998) Metabolic fate of exogenous sphingosine in neuroblastoma neuro2a cells. Dose-dependence and biological effects. *Ann N Y Acad Sci*, Vol.845, 46-56
- Riboni, L.; Campanella, R.; Bassi, R.; Villani, R.; Gaini, S.M.; Martinelli-Boneschi, F.; Viani, P. & Tettamanti, G. (2002) Ceramide levels are inversely associated with malignant progression of human glial tumors. *Glia*, Vol.39, No.2, pp.105-13
- Riboni, L.; Prinetti, A.; Bassi, R.; Caminiti, A. & Tettamanti, G. (1995) A mediator role of ceramide in the regulation of neuroblastoma neuro2a cell differentiation. *J Biol Chem*, Vol.270, No.45, pp.26868-75

- Ryland, L.K.; Fox, T.E.; Liu, X.; Loughran, T.P. & Kester, M. (2011) Dysregulation of sphingolipid metabolism in cancer. *Cancer Biol Ther*, Vol.11, No.2, pp.138-49
- Rylova, S.N.; Somova, O.G. & Dyatlovitskaya, E.V. (1998) Comparative investigation of sphingoid bases and fatty acids in ceramides and sphingomyelins from human ovarian malignant tumors and normal ovary. *Biochemistry. Biokhimiia*, Vol.63, No.9, pp.1057-60
- Saddoughi, S.A.; Song, P. & Ogretmen, B. (2008) Roles of bioactive sphingolipids in cancer biology and therapeutics. *Subcell Biochem*, Vol.49, 413-40
- Schaefer, J.T.; Barthlen, W. & Schweizer, P. (2000) Ceramide induces apoptosis in neuroblastoma cell cultures resistant to cd95 (fas/apo-1)-mediated apoptosis. *Journal of pediatric surgery*, Vol.35, No.3, pp.473-9
- Schiffmann, S.; Sandner, J.; Birod, K.; Wobst, I.; Angioni, C.; Ruckhaberle, E.; Kaufmann, M.; Ackermann, H.; Lotsch, J.; Schmidt, H.; Geisslinger, G. & Grosch, S. (2009) Ceramide synthases and ceramide levels are increased in breast cancer tissue. *Carcinogenesis*,
- Schultz, A. & Larsson, C. (2004) Ceramide influences neurite outgrowth and neuroblastoma cell apoptosis regulated by novel protein kinase c isoforms. *J Neurochem*, Vol.89, No.6, pp.1427-35
- Schwandner, R.; Wiegmann, K.; Bernardo, K.; Kreder, D. & Kronke, M. (1998) Tnf receptor death domain-associated proteins tradd and fadd signal activation of acid sphingomyelinase. *J Biol Chem*, Vol.273, No.10, pp.5916-22
- Senkal, C.E.; Ponnusamy, S.; Bielawski, J.; Hannun, Y.A. & Ogretmen, B. (2010) Antiapoptotic roles of ceramide-synthase-6-generated c16-ceramide via selective regulation of the atf6/chop arm of er-stress-response pathways. *The FASEB journal : official publication of the Federation of American Societies for Experimental Biology*, Vol.24, No.1, pp.296-308
- Senkal, C.E.; Ponnusamy, S.; Rossi, M.J.; Bialewski, J.; Sinha, D.; Jiang, J.C.; Jazwinski, S.M.; Hannun, Y.A. & Ogretmen, B. (2007) Role of human longevity assurance gene 1 and c18-ceramide in chemotherapy-induced cell death in human head and neck squamous cell carcinomas. *Mol Cancer Ther*, Vol.6, No.2, pp.712-22
- Separovic, D.; Bielawski, J.; Pierce, J.S.; Merchant, S.; Tarca, A.L.; Ogretmen, B. & Korbelik, M. (2009) Increased tumour dihydroceramide production after photofrin-pdt alone and improved tumour response after the combination with the ceramide analogue lcl29. Evidence from mouse squamous cell carcinomas. *Br J Cancer*, Vol.100, No.4, pp.626-32
- Shah, M.V.; Zhang, R.; Irby, R.; Kothapalli, R.; Liu, X.; Arrington, T.; Frank, B.; Lee, N.H. & Loughran, T.P., Jr. (2008) Molecular profiling of lgl leukemia reveals role of sphingolipid signaling in survival of cytotoxic lymphocytes. *Blood*, Vol.112, No.3, pp.770-81
- Sietsma, H.; Dijkhuis, A.J.; Kamps, W. & Kok, J.W. (2002) Sphingolipids in neuroblastoma: Their role in drug resistance mechanisms. *Neurochem Res*, Vol.27, No.7-8, pp.665-74
- Signorelli, P.; Munoz-Olaya, J.M.; Gagliostro, V.; Casas, J.; Ghidoni, R. & Fabrias, G. (2009) Dihydroceramide intracellular increase in response to resveratrol treatment mediates autophagy in gastric cancer cells. *Cancer Lett*, Vol.282, No.2, pp.238-43
- Soto, B.L.; Hank, J.A.; Van De Voort, T.J.; Subramanian, L.; Polans, A.S.; Rakhmilevich, A.L.; Yang, R.K.; Seo, S.; Kim, K.; Reisfeld, R.A.; Gillies, S.D. & Sondel, P.M. (2011) The

- anti-tumor effect of resveratrol alone or in combination with immunotherapy in a neuroblastoma model. *Cancer immunology, immunotherapy* : CII, Vol.60, No.5, pp.731-8
- Stiban, J.; Fistere, D. & Colombini, M. (2006) Dihydroceramide hinders ceramide channel formation: Implications on apoptosis. *Apoptosis*, Vol.11, No.5, pp.773-80
- Strum, J.C.; Small, G.W.; Pauig, S.B. & Daniel, L.W. (1994) 1-beta-d-arabinofuranosylcytosine stimulates ceramide and diglyceride formation in hl-60 cells. *J Biol Chem*, Vol.269, No.22, pp.15493-7
- Sugiki, H.; Hozumi, Y.; Maeshima, H.; Katagata, Y.; Mitsuhashi, Y. & Kondo, S. (2000) C2-ceramide induces apoptosis in a human squamous cell carcinoma cell line. *Br J Dermatol*, Vol.143, No.6, pp.1154-63
- Sundararaj, K.P.; Wood, R.E.; Ponnusamy, S.; Salas, A.M.; Szulc, Z.; Bielawska, A.; Obeid, L.M.; Hannun, Y.A. & Ogretmen, B. (2004) Rapid shortening of telomere length in response to ceramide involves the inhibition of telomere binding activity of nuclear glyceraldehyde-3-phosphate dehydrogenase. *J Biol Chem*, Vol.279, No.7, pp.6152-62
- Suzuki, A.; Iwasaki, M.; Kato, M. & Wagai, N. (1997) Sequential operation of ceramide synthesis and ice cascade in cpt-11-initiated apoptotic death signaling. *Exp Cell Res*, Vol.233, No.1, pp.41-7
- Ternes, P.; Franke, S.; Zahringer, U.; Sperling, P. & Heinz, E. (2002) Identification and characterization of a sphingolipid delta 4-desaturase family. *J Biol Chem*, Vol.277, No.28, pp.25512-8
- Tettamanti, G. (2004) Ganglioside/glycosphingolipid turnover: New concepts. *Glycoconj J*, Vol.20, No.5, pp.301-17
- Tiberio, P.; Cavadini, E.; Abolafio, G.; Formelli, F. & Appierto, V. (2010) 4-oxo-n-(4-hydroxyphenyl)retinamide: Two independent ways to kill cancer cells. *PloS one*, Vol.5, No.10, pp.e13362
- Torrisi, R.; Decensi, A.; Formelli, F.; Camerini, T. & De Palo, G. (2001) Chemoprevention of breast cancer with fenretinide. *Drugs*, Vol.61, No.7, pp.909-18
- Triola, G.; Fabrias, G.; Dragusin, M.; Niederhausen, L.; Broere, R.; Llebaria, A. & Van Echten-Deckert, G. (2004) Specificity of the dihydroceramide desaturase inhibitor n-[(1r,2s)-2-hydroxy-1-hydroxymethyl-2-(2-tridecyl-1-cyclopropenyl)ethyl]o ctanamide (gt11) in primary cultured cerebellar neurons. *Molecular pharmacology*, Vol.66, No.6, pp.1671-8
- Triola, G.; Fabrias, G. & Llebaria, A. (2001) Synthesis of a cyclopropene analogue of ceramide, a potent inhibitor of dihydroceramide desaturase *Angew Chem Int Ed Engl*, Vol.40, No.10, pp.1960-1962
- Turner, L.S.; Cheng, J.C.; Beckham, T.H.; Keane, T.E.; Norris, J.S. & Liu, X. (2011) Autophagy is increased in prostate cancer cells overexpressing acid ceramidase and enhances resistance to c6 ceramide. *Prostate cancer and prostatic diseases*, Vol.14, No.1, pp.30-7
- Uemura, K.; Sugiyama, E. & Taketomi, T. (1991) Effects of an inhibitor of glucosylceramide synthase on glycosphingolipid synthesis and neurite outgrowth in murine neuroblastoma cell lines. *Journal of biochemistry*, Vol.110, No.1, pp.96-102
- Van Echten-Deckert, G. & Herget, T. (2006) Sphingolipid metabolism in neural cells. *Biochim Biophys Acta*, Vol.1758, No.12, pp.1978-94
- Van Ginkel, P.R.; Sareen, D.; Subramanian, L.; Walker, Q.; Darjatmoko, S.R.; Lindstrom, M.J.; Kulkarni, A.; Albert, D.M. & Polans, A.S. (2007) Resveratrol inhibits tumor

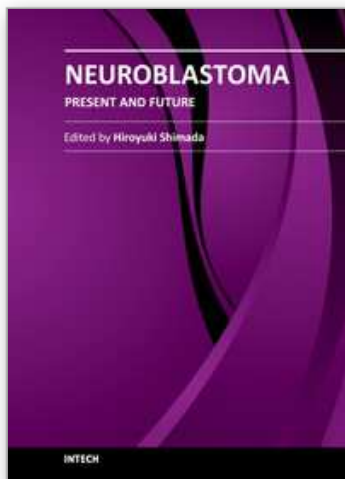
- growth of human neuroblastoma and mediates apoptosis by directly targeting mitochondria. *Clinical cancer research : an official journal of the American Association for Cancer Research*, Vol.13, No.17, pp.5162-9
- Venable, M.E.; Lee, J.Y.; Smyth, M.J.; Bielawska, A. & Obeid, L.M. (1995) Role of ceramide in cellular senescence. *J Biol Chem*, Vol.270, No.51, pp.30701-8
- Villablanca, J.G.; Krailo, M.D.; Ames, M.M.; Reid, J.M.; Reaman, G.H. & Reynolds, C.P. (2006) Phase i trial of oral fenretinide in children with high-risk solid tumors: A report from the children's oncology group (ccg 09709). *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*, Vol.24, No.21, pp.3423-30
- Villani, M.; Subathra, M.; Im, Y.B.; Choi, Y.; Signorelli, P.; Del Poeta, M. & Luberto, C. (2008) Sphingomyelin synthases regulate production of diacylglycerol at the golgi. *Biochem J*,
- Villani, M.G.; Appierto, V.; Cavadini, E.; Bettiga, A.; Prinetti, A.; Clagett-Dame, M.; Curley, R.W. & Formelli, F. (2006) 4-oxo-fenretinide, a recently identified fenretinide metabolite, induces marked g2-m cell cycle arrest and apoptosis in fenretinide-sensitive and fenretinide-resistant cell lines. *Cancer Res*, Vol.66, No.6, pp.3238-47
- Villani, M.G.; Appierto, V.; Cavadini, E.; Valsecchi, M.; Sonnino, S.; Curley, R.W. & Formelli, F. (2004) Identification of the fenretinide metabolite 4-oxo-fenretinide present in human plasma and formed in human ovarian carcinoma cells through induction of cytochrome p450 26a1. *Clin Cancer Res*, Vol.10, No.18 Pt 1, pp.6265-75
- Wang, H.; Maurer, B.J.; Liu, Y.Y.; Wang, E.; Allegood, J.C.; Kelly, S.; Symolon, H.; Liu, Y.; Merrill, A.H., Jr.; Gouaze-Andersson, V.; Yu, J.Y.; Giuliano, A.E. & Cabot, M.C. (2008) N-(4-hydroxyphenyl)retinamide increases dihydroceramide and synergizes with dimethylsphingosine to enhance cancer cell killing. *Mol Cancer Ther*, Vol.7, No.9, pp.2967-76
- Wang, H.; Maurer, B.J.; Reynolds, C.P. & Cabot, M.C. (2001) N-(4-hydroxyphenyl)retinamide elevates ceramide in neuroblastoma cell lines by coordinate activation of serine palmitoyltransferase and ceramide synthase. *Cancer research*, Vol.61, No.13, pp.5102-5
- Wei, J.S.; Whiteford, C.C.; Cenacchi, N.; Son, C.G. & Khan, J. (2005) Bbc3 mediates fenretinide-induced cell death in neuroblastoma. *Oncogene*, Vol.24, No.54, pp.7976-83
- Wolff, R.A.; Dobrowsky, R.T.; Bielawska, A.; Obeid, L.M. & Hannun, Y.A. (1994) Role of ceramide-activated protein phosphatase in ceramide-mediated signal transduction. *J Biol Chem*, Vol.269, No.30, pp.19605-9
- Wu, J.M.; Dipietrantonio, A.M. & Hsieh, T.C. (2001) Mechanism of fenretinide (4-hpr)-induced cell death. *Apoptosis*, Vol.6, No.5, pp.377-88
- Xie, P.; Shen, Y.F.; Shi, Y.P.; Ge, S.M.; Gu, Z.H.; Wang, J.; Mu, H.J.; Zhang, B.; Qiao, W.Z. & Xie, K.M. (2008) Overexpression of glucosylceramide synthase in associated with multidrug resistance of leukemia cells. *Leukemia research*, Vol.32, No.3, pp.475-80
- Zhang, X.; Wu, X.; Li, J.; Sun, Y.; Gao, P.; Zhang, C.; Zhang, H. & Zhou, G. (2011) Mdr1 (multidrug resistance 1) can regulate gcs (glucosylceramide synthase) in breast cancer cells. *J Surg Oncol*,
- Zhang, Y.; Mattjus, P.; Schmid, P.C.; Dong, Z.; Zhong, S.; Ma, W.Y.; Brown, R.E.; Bode, A.M.; Schmid, H.H. & Dong, Z. (2001) Involvement of the acid sphingomyelinase pathway in uva-induced apoptosis. *J Biol Chem*, Vol.276, No.15, pp.11775-82



Zheng, W.; Kollmeyer, J.; Symolon, H.; Momin, A.; Munter, E.; Wang, E.; Kelly, S.; Allegood, J.C.; Liu, Y.; Peng, Q.; Ramaraju, H.; Sullards, M.C.; Cabot, M. & Merrill, A.H., Jr. (2006) Ceramides and other bioactive sphingolipid backbones in health and disease: Lipidomic analysis, metabolism and roles in membrane structure, dynamics, signaling and autophagy. *Biochim Biophys Acta*, Vol.1758, No.12, pp.1864-84

IntechOpen

IntechOpen



## **Neuroblastoma - Present and Future**

Edited by Prof. Hiroyuki Shimada

ISBN 978-953-307-016-2

Hard cover, 366 pages

**Publisher** InTech

**Published online** 08, February, 2012

**Published in print edition** February, 2012

Neuroblastoma, once called "enigmatic", due to "unpredictable" clinical behaviors, is composed of biologically diverse tumors. Molecular/genomic properties unique to the individual tumors closely link to the clinical outcomes of patients. Establishing risk stratification models after analyzing biologic characteristics of each case has made a great success in patient management. However, the trend of improving survival rates in neuroblastoma over the last 30 years has started to level off, and currently available treatment modalities have almost reached to their maximized intensity. Furthermore, aggressive treatment causes significant long-term morbidities to the survivors. We really need to make the next step to the level of personalized medicine with more precise understanding of neuroblastoma biology. This book includes useful data and insights from the world's experts in this field. I believe this book can make an excellent contribution to all the investigators working hard and fighting for the children stricken by this disease.

### **How to reference**

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Mehrdad Rahmaniyan, Amr Qudeimat and Jacqueline M. Kravaka (2012). Bioactive Sphingolipids in Neuroblastoma, Neuroblastoma - Present and Future, Prof. Hiroyuki Shimada (Ed.), ISBN: 978-953-307-016-2, InTech, Available from: <http://www.intechopen.com/books/neuroblastoma-present-and-future/bioactive-sphingolipids-in-neuroblastoma>

**INTech**  
open science | open minds

### **InTech Europe**

University Campus STeP Ri  
Slavka Krautzeka 83/A  
51000 Rijeka, Croatia  
Phone: +385 (51) 770 447  
Fax: +385 (51) 686 166  
[www.intechopen.com](http://www.intechopen.com)

### **InTech China**

Unit 405, Office Block, Hotel Equatorial Shanghai  
No.65, Yan An Road (West), Shanghai, 200040, China  
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元  
Phone: +86-21-62489820  
Fax: +86-21-62489821

© 2012 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the [Creative Commons Attribution 3.0 License](https://creativecommons.org/licenses/by/3.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

IntechOpen

IntechOpen