Published in final edited form as:

Expert Opin Ther Targets. 2010 May ; 14(5): 529–539. doi:10.1517/14728221003752768.

Targeting sphingolipid metabolism in head and neck cancer: rational therapeutic potentials

Thomas H Beckham,

Medical University of South Carolina, Charleston, South Carolina, USA

Saeed Elojeimy,

Carolinas Medical Center, Charlotte, North Carolina, USA

Joseph C Cheng,

Medical University of South Carolina, Charleston, South Carolina, USA

Lorianne S. Turner,

Francis Marion University, South Carolina, USA

Stanley Hoffman,

Medical University of South Carolina, Charleston, South Carolina, USA

James S Norris, and

Medical University of South Carolina, Charleston, South Carolina, USA

Xiang Liu

Medical University of South Carolina, Charleston, South Carolina, USA

Abstract

Importance of the field—Ceramide accumulation has been shown to be a conserved mechanism of apoptosis initiation in normal physiological processes as well as in response to cancer treatments such as radiation and chemotherapy. Therefore, it is unsurprising that many cancers develop aberrations of sphingolipid metabolism that prevent the accumulation of ceramide, whether by reduction of ceramide generation or by enhanced ceramide catabolism, particularly dangerous when catabolism leads to generation of pro-tumor sphingosine-1-phosphate and ceramide-1-phosphate. Numerous studies have now implicated dysregulation of sphingolipid metabolism in head and neck cancers.

What the reader will gain—The roles of sphingolipids and sphingolipid metabolism in cancer are reviewed and the reader will be brought up to date with discoveries in the field of sphingolipid metabolism in head and neck cancer.

Areas covered in this review—This review seeks to highlight the importance of sphingolipid metabolism and to bring sphingolipid metabolism to the forefront in the investigation of novel therapies for head and neck cancer. Further, it will review sphingolipid-centric therapies under investigation in preclinical and clinical trials of cancers of the head and neck.

Take home message—As treatments for head and neck cancers are currently limited, the potentials of targeting sphingolipid metabolism should be taken into consideration as we seek novel ways to combat this dangerous group of tumors.

Keywords

Head and neck cancer; sphingolipids; ceramide; lipid metabolism; novel chemotherapeutic targets

1. Introduction

Therapeutic management of local, locally advanced, recurrent and metastatic head and neck cancer is often limited by resistance to chemotherapy and radiation therapy as well as unacceptable toxicity and side effect profiles. Therefore, novel agents and treatment approaches are needed to improve outcomes of patients with this disease. With increased understanding of bioactive sphingolipids and their role in cancer biology, more and more innovative research has placed emphasis on incorporating these new concepts into clinic protocols. Investigation of sphingolipid signaling in head and neck cancers has shown a number of metabolic aberrations, making sphingolipid metabolism an attractive molecular target for treatment of head and neck cancer. This review discusses the rationale for exploring sphingolipid-based therapies and describes the potential opportunities of novel sphingolipid-based agents in the management of head and neck cancers.

1.1 Head and neck cancer

Head and neck cancers account for approximately 6% of malignancies diagnosed in the United States with an estimated 35,720 new incidents and 7,600 deaths in 2009 [1]. Treatment options for patients with head and neck cancers are limited. The anatomic density of the region often complicates radiotherapy and effective tumor resection, and current chemotherapeutics are usually reserved for palliation, as they are not generally considered curative. Early diagnosis and treatment of premalignant lesions is presently the most effective way to improve survival. Unfortunately, despite significant advances in diagnosis and treatment of head and neck cancer, 5 year survival has not appreciably improved over the past 20 years [2], highlighting the need for identification of new therapeutic targets.

1.2 Sphingolipids and sphingolipid metabolism

Sphingolipids are major structural components of cells and, as has become evident over the past two decades, are important signaling molecules in diverse cellular processes. Sphingolipids such as ceramide, ceramide-1-phosphate (C1P), sphingosine, and sphingosine-1-phosphate (S1P) function as bioactive signaling lipids in various processes including proliferation, senescence, apoptosis, inflammation, and cell cycle arrest [3,4]. Central to sphingolipid signaling is ceramide, the basic building block of complex sphingolipids. Ceramide species consist of a fatty acid of variable chain length (predominate endogenous species range from 12–26 carbons) attached to a sphingosine backbone. Ceramide is well recognized as an important mediator of cell death in response to stress such as radiation, chemotherapy, hypoxia, and nutrient deprivation [5–7]. When cells are exposed to stressful conditions or treatment, endogenous levels of ceramide are elevated either by *de novo* synthesis from serine and palmitoyl CoA or by hydrolysis of sphingomyelin or cerebroside [4]. Generation of ceramide is critical as ceramide has a number of important downstream targets [8], including, among others, ceramide-activated protein kinase (CAPK) [9], ceramide-activated protein phosphatase (CAPP) [10,11], protein kinase C (PKC) [12], cathepsin D [13], and the autophagy-associated proteins Beclin-1 and BNIP3 [14–16]. Cell death via ceramide signaling occurs through two main signaling pathways. First, through the mitochondrial pathway, increased ceramide levels activate protein phosphatase 2A (PP2A). Activated PP2A dephosphorylates the pro-apoptotic proteins Bak and Bax, resulting in conformational change and activation [17], and the anti-apoptotic protein Bcl-2, resulting in proteasomal degradation [18]. The second mechanism by which ceramide induces apoptosis is activation of the stress-activated protein kinase (SAPK/p38MAPK) pathway [19–21]. Importantly, functional signaling through both pathways has been shown to be required for induction of apoptosis in response to ceramide accumulation [20,22].

Antagonistic to the apoptotic function of ceramide accumulation are multiple mechanisms of ceramide catabolism. Ceramide can be deacylated to form sphingosine, phosphorylated to form C1P, glycosylated to form glucosylceramide, or incorporated into sphingomyelin by sphingomyelin synthase. Particularly relevant to cancer development is the conversion of ceramide to S1P. Ceramide can be metabolized by ceramidases to sphingosine, which is quickly converted to S1P, a molecule known to promote cancer in several ways including inhibition of apoptosis, enhancing proliferation, transformation, and angiogenesis as well as contributing to inflammation [4]. Because S1P favors cell survival and proliferation, the advantage to the cell is not only prevention of cell death via reduction of ceramide levels but also tumor promotion through S1P signaling. The balance of ceramide and S1P is critical to cell fate, and is recognized as an important target for cancer therapy [23–26]. While conversion of ceramide to sphingosine is the most widely recognized and studied aspect of sphingolipid metabolism as it pertains to cancer, other mediators of ceramide trafficking and metabolism can play important roles. Ceramide transport protein (CERT) transports newly formed ceramide from the ER to the Golgi [27]. This protein has been found to be upregulated in cancer and its upregulation has been observed to mediate multidrug resistance [28]. Inhibition of CERT causes accumulation of ceramide in the ER and sensitizes cancer cells to multiple chemotherapeutics, seemingly through potentiation of ER stress. Glucosylceramide synthase (GCS), which glycosylates ceramide thereby reducing its concentration in the cell, attenuates ceramide-mediated death signals. Increased expression of GCS in cells has been demonstrated to cause multidrug resistance in a number of models [29–31], whereas inhibition of GCS or reduction in expression with RNAi has reversed multidrug resistance [32–35]. Phosphorylation of ceramide by ceramide kinase produces biologically active C1P. C1P is inflammatory, interacting directly with cPLA₂ causing liberation of arachidonic acid [36]. Additionally, it has been shown that C1P positively impacts cell survival by activating the PI3K-Akt pathway [37]. Increased PI3K-Akt signaling is implicated in a wide variety of cancers [38].

1.3 Bioactive sphingolipids and cancer

Alterations in ceramide signaling have been observed in multiple human cancers, implicating ceramide dysregulation as an important determinant of tumor development and progression. Pro-apoptotic ceramide signaling can be stifled by defects in ceramide generation, increased ceramide metabolism, and increased levels of the pro-survival sphingolipid S1P. Our group has observed overexpression of acid ceramidase, which converts ceramide into sphingosine, in over 60% of prostate cancers and 70% of head and neck cancers, with an increased incidence of overexpression in higher grade tumors [39]. Kim *et al* have observed mutations in the gene for neutral sphingomyelinase, a ceramide-generating enzyme, in mouse osteosarcomas and human leukemias, providing evidence that failure of ceramide accumulation predisposes a cancerous phenotype [40]. Alterations in expression of sphingolipid metabolizing enzymes have also been observed in breast cancer. Through microarray analysis, Ruckhaberle *et al* observed multiple changes in expression of genes for sphingolipid-metabolizing enzymes in estrogen receptor positive and negative tumors, with the most consistent and significant change being an upregulation of sphingosine kinase 1 (SK1) [41]. SK1 overexpression was strongly correlated with poor clinical outcome. These studies represent just a few of the many examples of dysregulation of ceramide metabolism in human cancers and illustrate the importance of sphingolipid homeostasis in the occurrence and aggressiveness of malignancy.

Critical to the investigation of sphingolipid metabolism as a potential pharmacologic target for cancer treatment is whether or not the pathway can be manipulated to kill cancers or improve response to treatment. Fortunately, many experimental models provide convincing evidence that modulation of sphingolipid metabolism can reduce cancer cell viability [42,43], decrease tumor size [44,45], and sensitize cancers to conventional treatments [33,46]. Our group has focused on the role of acid ceramidase in tumor promotion and aggression, finding that the

aggressive phenotype of acid ceramidase overexpressing cells and tumors can be reversed with siRNA and small molecule inhibitors that target acid ceramidase [47–49]. Others have found that inhibition of GCS whether by siRNA or inhibition can restore chemotherapy and radiation sensitivity to resistant cells [29,33]. Likewise, inhibition of SK1 with novel inhibitors has led to reductions in cell proliferation and tumor size [50], and the dual SK1/protein kinase C (PKC) inhibitor safinol has even progressed to clinical trials [51]. These studies are merely demonstrative of a large body of work that establishes the legitimacy of targeting sphingolipid metabolism as a new chemotherapeutic approach against human cancers.

2. Sphingolipids in Head and Neck Cancer: Occurrence and Functional Consequences

2.1 Aberrant sphingolipid metabolism in head and neck cancer

Alterations in sphingolipid levels as well as dysregulated sphingolipid signaling have been shown in a wide array of cancers, with increasing literature focusing on sphingolipid changes in head and neck cancer. Chi and Yuan *et al* compared ceramide expression in laryngeal cancer tissues with leukoplakia tissues and healthy mucosa [52,53]. Ceramide levels were evaluated by immunohistochemical staining of tumor tissues with a ceramide specific antibody. The authors reported progressive decrease in ceramide levels as normal tissues progressed into leukoplakia and a further decrease in ceramide levels in laryngeal carcinoma. Aneuploid cells also had less ceramide compared to diploid cells strengthening the association of decreased ceramide levels with indicators of aggressive cancer. Similar results were reported by Young *et al* who found reduced ceramide staining in premalignant cells compared with normal keratinocytes and further reduction in ceramide levels in oral squamous cell carcinoma (SCC) as compared with premalignant and normal keratinocytes [54]. Furthermore, these decreased ceramide levels were associated with decreased PP2A signaling and enhanced tumor cell motility. Treatment of cancer cells with ceramide analogues restored PP2A activity and decreased motility confirming that the observed changes in cell motility were consequences of altered sphingolipid metabolism.

In an attempt to better quantify ceramide levels in head and neck tumor tissues, Koybasi *et al* used mass spectrometry which provides accurate quantification of total ceramide and specific ceramide species based on the length and nature of the carbon chain of the fatty acid moiety [55]. Interestingly, the results of these analyses differed between squamous and non-squamous head and neck cancers. In non-squamous head and neck cancer, the authors found decreased total ceramide levels. However, in head and neck squamous cell carcinoma (HNSCC) tissues, there was a selective downregulation of C18-ceramide (18 carbon acyl chain) while the levels of other ceramide species and total ceramide remained unchanged or were even increased in tumor tissues. This study revealed for the first time that changes in specific ceramide species rather than total ceramide might also be important in the development and progression of head and neck cancer, specifically HNSCC. This hypothesis was further supported by another study from the same group which showed that decreased C18-ceramide levels in HNSCC tumor samples correlated with higher overall tumor stages, increased incidence of lymphovascular invasion, and nodal metastasis [56].

The decrease in ceramide levels in head and neck cancer tissues may be a result of decreased expression of ceramide generating enzymes (sphingomyelinases, galactocerebrosidase, ceramide synthases) or increased expression of ceramide metabolizing enzymes (ceramidases). Several studies have found increased levels of ceramide precursors such as glycosphingolipids in head and neck carcinoma as compared with normal mucosa [57–59]. This substrate buildup is evidence of insufficient ceramide generating enzyme activity in head and neck cancers. By contrast, studies from our group have reported overexpression of the ceramide metabolizing

enzyme acid ceramidase in multiple tumor types including HNSCC [39]. Interestingly, our unpublished data also indicated that cell lines generated from head and neck tumor metastases had higher acid ceramidase levels than cell lines generated from the primary head and neck tumor from which the metastasis originated. These data provide evidence that acid ceramidase expression level correlates with degree of malignancy. Increases in activity and expression of acid ceramidase are particularly important as there is ample evidence that acid ceramidase overexpression facilitates the metabolism of ceramide into sphingosine, which is phosphorylated by sphingosine kinase into S1P. This shifts the sphingolipid balance from a ceramide dominant apoptotic signal to an S1P prevailing tumor-promoting signal, the importance of which has been demonstrated in head and neck cancers. Miller *et al* investigated S1P in esophageal cancer, a notoriously deadly disease with less than 20% overall survival. They found that the increases in cellular invasion and migration observed with TGF β treatment can be recapitulated with S1P treatment through the activation of the pro-proliferative MAPK family members ERK1/2 [60]. Inhibition of sphingosine kinases 1 and 2 with isozyme specific siRNA reduced invasion and migration and reduced the activation of ERK1/2 in response to TGF β treatment. Blocking ERK1/2 function by treatment with the MEK inhibitor PD98059 abrogated TGF β and S1P-induced invasion and migration. This study showed that TGF β 's tumor promoting functions are dependent on S1P, and that the proliferative effect of S1P is at least partially through MAPK activation. In a related study, Bergelin *et al* established the importance of sphingosine kinase in thyroid carcinoma. They found that follicular thyroid carcinoma cells overexpressing SK1 were more prone to serum-induced migration. SK1 overexpression resulted in increases in secreted S1P, and the observed increase in migration was through stimulation of extracellular S1P receptors in an autocrine fashion. Bergelin also found ERK1/2 activation to be important in S1P-induced migration, confirming Miller's observations [61].

2.2 Dysregulated sphingolipid signaling in treatment response

In addition to the role of altered sphingolipid metabolism in cancer development and progression, sphingolipid metabolism is relevant in the response of cancer to chemotherapy. A diverse group of chemotherapeutic compounds have been shown to cause cell death by elevation of ceramide [62], indicating that aberrant sphingolipid metabolism may cause multidrug resistance. Our group has shown that elevated levels of acid ceramidase conferred resistance to doxorubicin, cisplatin, etoposide, gemcitabine, and exogenous C6-ceramide therapy in prostate cancer cells, whereas knockdown of acid ceramidase with siRNA sensitized cells to therapy, indicating a role for acid ceramidase inhibition in sensitizing tumors to conventional therapy [49]. The consequences of acid ceramidase overexpression and therefore altered metabolism of intracellular ceramide were also investigated in HNSCC cells with respect to FasL induced cell death [39]. HNSCC cells expressing higher levels of acid ceramidase demonstrated reduced sensitivity to adenoviral FasL delivery. Similarly, cells in which acid ceramidase had been knocked down with siRNA were considerably more susceptible to the FasR agonist CH11. Dumitru *et al* have recently shown that gemcitabine-resistant glioma cells did not accumulate ceramide after therapy. When ceramide glucosyltransferases were inhibited using siRNA, ceramide accumulation occurred and gemcitabine sensitivity was restored [63]. Rath *et al* recently showed that follicular thyroid carcinoma cells' response to chemotherapeutics camptothecin and doxorubicin is dependent on *de novo* ceramide generation [64]. In their study, apoptosis was enhanced when the GCS inhibitor PDMP was used. Additionally, drug resistance was increased when GCS was overexpressed, providing another example of how cancer cells can develop resistance to therapy by upregulating ceramide metabolizing enzymes.

Further evidence suggests that in addition to improving the response of cancers to chemotherapy, targeting ceramide metabolism could improve tumor response to radiation of

head and neck cancers. The role of ceramide in radiation-induced apoptosis has been well studied [65]. Defects in ceramide generation [66,67] as well as increased ceramide metabolism [45] have been implicated in cancer cell resistance to radiation. Alphonse *et al* demonstrated a remarkable increase in radiation-induced apoptosis in radiation resistant SQ20B and SCC61 head and neck cancer cells using a sphingolipid metabolism-inhibiting drug cocktail [68]. They treated radiation resistant cells with DL-PDMP, an inhibitor of GCS; D-MAPP, a ceramidase inhibitor; and imipramine, which disturbs lipid turnover in biological membranes. Cells were then treated with ionizing radiation, and the effects of the inhibitor cocktail on ceramide levels and sensitivity to radiation were observed. Interestingly, the drug cocktail increased ceramide levels 340% over cells receiving only radiation treatment. The substantial increase in ceramide corresponded with a dramatic effect on radiation sensitivity, with significantly decreased clonogenic survival as well as upwards of 80% increases in apoptosis. This study clearly shows the potential for pharmacologic inhibition of sphingolipid metabolizing enzymes in improving tumor response to conventional radiotherapy.

Taken together, these studies establish an important role for sphingolipid metabolism in head and neck cancer. Sphingolipid homeostasis has been shown to be aberrant in several head and neck cancers, and modulation of sphingolipid metabolism has been shown to improve response to chemotherapies and radiation. The demonstrated efficacy of targeting sphingolipid metabolism to favor ceramide accumulation in experimental models makes the sphingolipid pathway an attractive target as we seek to advance treatments in head and neck cancer.

3. Challenges and Potential Opportunities of Sphingolipid-based Therapy

The challenges of sphingolipid-based therapies are well reviewed [69]. Briefly, the utility of exogenous ceramide and sphingolipid analogs is limited by low solubility, low cell permeability, and lack of specificity. While these challenges have slowed the progress of sphingolipid-based therapies, advances have been made in developing modified analogs, new delivery techniques, and non-lipid inhibitors as sphingolipid researchers turn obstacles into opportunities.

3.1 Direct administration of ceramide and ceramide analogs

An ostensibly simple approach to increasing the level of ceramide in the cell is treatment with exogenous ceramide. While this approach works *in vitro*, *in vivo* application of systemic ceramide has been limited by inherent hydrophobicity. One approach to overcoming ceramide's hydrophobic properties is through the incorporation of short chain ceramides into synthetic liposomes. Stover *et al* demonstrated the efficacy of systemic administration of liposomal C6-ceramide in breast adenocarcinoma xenografts [70]. Mice treated with C6-ceramide had decreased tumor volume, cancer cell proliferation, microvessel formation and increased apoptosis. The treatment did not impact animal weight and appeared to have minimal toxicity. Similarly, Shabbits and Mayer correlated the lower cytotoxicity of exogenous treatment with longer chain ceramides with the reduction of cellular uptake as acyl chain length increases [71]. When they incorporated C16-ceramide into liposomal lipid bilayers they noted a dramatic decrease in IC₅₀ from over 100 μ M for free C16-ceramide to 36.1 μ M in the liposomal preparation. The increased effectiveness of liposomal C16-ceramide approached that of free C6-ceramide, indicating that liposomal delivery is effectively able to overcome the problem of cellular uptake of long chain ceramides. With multiple groups investigating tumor-targeted liposomal delivery of ceramides and conventional chemotherapeutics, liposomal delivery of ceramide is becoming a realistic possibility.

The Bielawska and Ogretmen groups have addressed the issue of ceramide solubility and low cellular uptake by developing cationic water-soluble pyridinium ceramide analogs [44]. These compounds possess a positive charge that increases their solubility and favors accumulation

in negatively charged compartments, such as the interior of cells, particularly negatively charged organelles such as mitochondria and nuclei. One such analog, L-threo-C6-pyridinium-ceramide-bromide (L-t-C6-Pyr-Cer), exhibited substantial suppression of tumor growth in a HNSCC xenograft, suppressing tumor growth 2.5 better than non-cationic L-t-C6-Cer. They also tested L-t-C6-Pyr-Cer in combination with gemcitabine, preventing tumor growth almost entirely. These studies show that while ceramide therapy has limitations, liposomal delivery and chemical modification offer two promising means of making systemic ceramide therapy a reality for head and neck cancer patients.

3.2. Inhibition of acid ceramidase

Another approach that has been used to target sphingolipids in cancer is inhibition of ceramide metabolizing enzymes. One particularly attractive target is acid ceramidase, which catabolizes ceramide into sphingosine shifting the sphingolipid balance towards tumor promotion through S1P. Our group has observed overexpression of acid ceramidase in the majority of prostate and head and neck tumor tissues sampled when compared to adjacent normal tissue, legitimizing acid ceramidase as a therapeutic target [72]. Selzner *et al* utilized B13, a ceramide analog which affects potent acid ceramidase inhibition, showing that intraperitoneal delivery greatly decreased tumor formation and volume in hepatic cancer xenografts [73]. No adverse effects were observed. Following the experimental success of B13, our group has developed novel analogs of B13 with improved chemotherapeutic effects [74]. One such compound is LCL204, an acid ceramidase inhibitor with tropism to the lysosome, where acid ceramidase primarily functions. LCL204 was shown to improve survival alone and synergistically in combination with apoptin gene therapy in a prostate xenograft model [75]. We also investigated inhibition of acid ceramidase with LCL204 in HNSCC in combination with FasL gene therapy [39]. In this study, cells pretreated with LCL204 experienced significantly greater cell death after treatment with the activating FasR agonist CH11 to cells not treated with the acid ceramidase inhibitor. We then tested the combination of FasL gene therapy and LCL204 treatment in a mouse xenograft model of head and neck cancer, finding that the combination therapy dramatically reduced tumor size and resulted in 100% survival as compared to treatment with FasL or LCL204 alone, with survival rates of 50% and 40%, respectively. While a detailed discussion of these results is beyond the scope of this review, it is worth mentioning that we have also published work showing the efficacy of another inhibitor of acid ceramidase, LCL385, in inhibiting prostate cancer xenograft growth [45]. These applications of small molecule acid ceramidase inhibitors show that blocking ceramide catabolism can sensitize resistant tumors to therapy. Since we have previously observed that tumor cells with higher acid ceramidase expression are more sensitive to pharmaceutical inhibition of acid ceramidase but less sensitive to treatment with exogenous ceramide, inhibition of acid ceramidase may be particularly useful in head and neck cancer where we have shown overexpression of acid ceramidase in 70% of tumors sampled [72].

3.3. Inhibition of glucosylceramide synthase and sphingosine kinase 1

As discussed previously, elevations of GCS have been implicated in multidrug resistance in a variety of cancers. Studies have been published utilizing inhibitors of GCS in *in vitro* and *in vivo* models of cancer, but these agents have not yet been investigated in head and neck cancer [35,63,76]. Great interest has also been generated in the inhibition of SK1 in cancer treatment due to its status as an oncogene, its overexpression in a number of cancers, and the anti-cancer functional effects of downregulating SK1 in *in vitro* models [41,61,77–79]. A number of sphingosine isomers have been investigated as SK1 inhibitors with the most interest thus far being in DL-threo-dihydrosphingosine, also known as safingol. Safingol was developed as an inhibitor of PKC, but has since been found to also inhibit SK1 [42]. In addition to studies showing the efficacy of safingol in non head and neck cancers [42,76,80], Hamada *et al* have shown that safingol reduces cell adhesion and induces apoptosis in oral SCC cells [43,81].

Safingol has been included as a co-therapy with cisplatin in a Phase I trial, which will be discussed in a following section. French *et al* developed a screening assay for novel non-lipid SK1 inhibitors and found a number of compounds which demonstrate potent inhibition of SK1 [82]. Though none of these compounds have been investigated in head and neck cancers, three of the identified compounds, referred to as SKI-I, SKI-II, and SKI-V, have been studied in detail with encouraging results [50]. They decreased formation of S1P, decreased signaling through the PI3K-Akt survival and proliferation pathway, and inhibited JC mammary adenocarcinoma xenograft tumor growth 55–79% without observed adverse effects on the animals' health. SKI-II was shown to be orally bioavailable and capable of inhibiting tumor growth up to 79% when given by mouth. While still early in investigation, these compounds offer a glimpse into the bright future of sphingolipid analogs as well as non-lipid inhibitors of the sphingolipid pathway as cancer therapies.

4. Integration of Novel Strategies into the Treatment of Head and Neck Cancer

With a growing number of diverse sphingolipid-based therapies being developed, rational utilization of our increasing knowledge of sphingolipid signaling, metabolism and dysregulation is becoming a novel clinical approach. Sphingolipid-based therapies are already under clinical investigation in head and neck cancer in addition to sphingolipid-based clinical trials in other tumors. These trials represent imminent possibilities for new therapies in medical treatment of many cancers.

4.1 Sphingolipids in clinical trials of head and neck cancer

To date there have been few clinical trials targeting sphingolipid metabolism in head and neck cancer. This largely reflects the relative infancy of sphingolipid biology and the inherent challenges of lipid-based therapeutics. Nevertheless, we are beginning to see sphingolipid biology being tested in clinical investigation of treatments for head and neck cancers. One such trial is a phase II study investigating the response rate produced by the combination of gemcitabine and doxorubicin chemotherapies in patients with recurrent or progressive head and neck cancer. This trial, currently underway, is based on research by the Ogretmen group that shows the combination of gemcitabine and doxorubicin, both inducers of ceramide generation, synergistically inhibits the growth of head and neck squamous cell carcinomas *in vitro* [83]. While not a direct sphingolipid therapy, that chemotherapies are being combined with a goal being rational elevation of ceramide speaks to the importance of sphingolipids in head and neck cancer.

Novel approaches to sphingolipid-based cancer therapies include the exploitation of immune cell activation by galactosylceramide. In 2008, Uchida and colleagues reported the results of a phase I study in Japan evaluating the safety and feasibility of administering antigen presenting cells (APCs) pulsed with α -galactosylceramide (α -GalCer) into the nasal submucosa [84]. This study was preceded in 2005 by a phase I trial with similar objectives in the settings of advanced and recurrent non-small cell lung cancer [85]. In the head and neck cancer trial, nine patients with unresectable lesions or recurrent disease received two injections of α -GalCer-pulsed autologous APCs into the anterior portion of the bilateral inferior turbinate. No serious adverse events were observed. Moreover, analysis of peripheral blood mononuclear cells demonstrated an increased number of natural killer T (NKT) cells in four patients and an enhancement of natural killer activity, as assessed by interferon- γ (IFN- γ) production, in eight patients [84]. These results not only implicate a significant role of the regional immune system in the upper respiratory and digestive organs in modulating both local and systemic immunologic functions, but also confirm the applicability of α -GalCer as a potent immune-modulatory compound.

4.2. Sphingolipid-based clinical trials in other cancers: Potentials for head and neck cancer therapy

While there are few clinical trials targeting sphingolipid metabolism specifically in head and neck cancer, there are several sphingolipid-based approaches in other cancers that have progressed to clinical investigation. As previously mentioned, the SK1/PKC inhibitor safinol was used in a Phase I trial in combination with cisplatin chemotherapy in patients with advanced solid tumors. This study was completed in early 2009, and though the results have not yet been published, preliminary results were presented in 2006 at the annual meeting of the American Society of Clinical Oncology [51]. The early report indicated safety in dose escalation of safinol with steady doses of cisplatin. While the potential of a synthetic sphingosine isomer as a clinical agent is encouraging, it is important to remember the mechanism of this drug is not specific to SK1, and it is not fully known to what extent the inhibition of PKC is responsible for promising preclinical and clinical results. Regardless, a systemic therapy utilizing a sphingolipid analog in clinical trials is promising, and publication of the results is eagerly awaited.

Taking an approach that circumvents the traditional problems of lipid-based therapies, the Sabbadini group from San Diego State University has taken the targeting of sphingolipid metabolism in an entirely new direction. They have developed an anti-S1P monoclonal antibody [86] which they report has a remarkably high affinity for S1P, even higher than the affinity of S1P for its own receptors [87]. This addition to the increasingly popular therapeutic modality of monoclonal antibodies brings to light a new method of targeting sphingolipids and may provide a direct means of reducing levels of tumor-promoting S1P in human cancers while avoiding the problems of hydrophobicity with analogs and the common toxicity of small molecules. Encouragingly, a recently completed Phase I trial did not reveal dose-limiting toxicity, and a Phase II trial is currently being planned.

Numerous clinical trials have been completed and more still are underway on the promising immunosuppressive agent FTY720, known as fingolimod. FTY720 is phosphorylated by sphingosine kinase 2, resulting in formation of the bioactive S1P structural analog phospho-FTY720. While the exact mechanism of FTY720 in solid tumors is not known, it has been suggested that its action may be through downregulation of S1P receptors [88], interference with sphingolipid biosynthesis [89,90], or a combination thereof. Most of the investigation of FTY720 thus far has focused on its ability to suppress lymphoproliferation, thus it is of interest as an immunosuppressive for organ transplantation as well as autoimmune conditions like multiple sclerosis. There are presently 23 completed or active clinical trials in multiple sclerosis and organ transplant rejection, with multiple sclerosis trials having progressed as far as phase III trials. Interestingly, there are promising preclinical results that suggest this drug may be of use as an anti-cancer agent. Following its profound effect on lymphoproliferation, it was investigated as an anti-leukemia agent, with promising results in chronic lymphocytic leukemia [88], chronic myelogenous leukemia [91], and multiple myeloma [92]. It has since gone on to receive attention as an agent against multiple solid tumors, exhibiting effectiveness against lung tumor development [93], pancreatic [94,95], prostate [96], breast [97] and colon cancers [98]. Clearly there is much excitement that FTY720 may be used clinically as an anti-cancer therapy, and given its activity against numerous solid tumors there is reason to believe its broad action may also apply to head and neck cancer.

These trials in non-head and neck cancers offer promise for future implementation of therapies targeting sphingolipids in head and neck cancer. There is reason to be hopeful that these and other therapies in earlier stages of development will take advantage of the knowledge that has been generated about sphingolipid metabolism and push forward new therapeutic options for patients with head and neck cancers.

5. Expert Opinion and Conclusion

Our understanding of how sphingolipid metabolism impacts cancer development and progression has expanded dramatically over the past two decades. This new knowledge not only constitutes a new level of understanding of head and neck cancer development but also provides ample evidence that ceramide metabolism is a promising new avenue for treatment of head and neck cancers. However, overcoming the hydrophobicity and low cell permeability of sphingolipid analogs, developing non-lipid inhibitors, and developing enzyme-specific inhibitors are critical challenges that must be met for the full potential of sphingolipid-based therapy to be realized. Fortunately, the research community is already developing new technologies that are making sphingolipid-based treatments increasingly feasible. Unlocking the potential of these new therapeutic targets is as important in the treatment of head and neck cancer as in any other cancer. The sheer anatomic density of the region often makes even skillfully executed surgeries disfiguring and debilitating, and the limitations of radiotherapy leave patients and practitioners wishing for chemotherapeutic improvements. The validity of sphingolipid metabolism as a target for head and neck cancer has been shown on the bench, and with new discoveries and further improvements in therapeutic implementation, sphingolipid-based therapies will soon improve patient outcomes at the bedside.

6. Article Highlights Box

- Accumulation of the sphingolipid ceramide is a key event in the initiation of apoptosis in response to stressors including chemotherapy and radiation, and dysregulation of ceramide metabolism commonly confers tumor resistance to therapy.
- Aberrations in sphingolipid metabolism are observed in many forms of head and neck cancer, a group of diseases for which there are currently few chemotherapeutic options. These alterations represent new therapeutic targets in the treatment of head and neck cancer.
- Sphingolipid-based therapies are under development in clinical and preclinical trials and are showing promise in many cancers, including head and neck cancer.
- Ceramide is important for the induction of apoptosis in many cancer therapies and targeting its metabolism is a promising avenue for improving therapeutic options and outcomes for people with head and neck cancer.

Abbreviations

C1P	Ceramide-1-phosphate
GCS	Glucosylceramide synthase
HNSCC	Head and neck squamous cell carcinoma
SCC	Squamous cell carcinoma
S1P	Sphingosine-1-phosphate
SK1	Sphingosine kinase 1

Acknowledgments

Declaration of interest

This paper has been sponsored by a NIH/NCI grant number P01 CA97132.

X. Liu is Director of preclinical and translational research at Sphingogene, Inc.

S.J. Norris is Chairman of the Board at Sphingogene, Inc.

References

1. Cancer Facts and Figures 2009. American Cancer Society; Atlanta: 2009.
2. Day TA, et al. Oral cancer treatment. *Curr Treat Options Oncol* 2003;4:27–41. [PubMed: 12525277]
3. Futerman AH, Hannun YA. The complex life of simple sphingolipids. *EMBO Rep* 2004;5:777–782. [PubMed: 15289826]
4. Ogretmen B, Hannun YA. Biologically active sphingolipids in cancer pathogenesis and treatment. *Nat Rev Cancer* 2004;4:604–616. [PubMed: 15286740]
5. Hannun YA. Functions of ceramide in coordinating cellular responses to stress. *Science* 1996;274:1855–1859. [PubMed: 8943189]
6. Hannun YA, Luberto C. Ceramide in the eukaryotic stress response. *Trends Cell Biol* 2000;10:73–80. [PubMed: 10652518]
7. Hannun YA, Obeid LM. Ceramide: an intracellular signal for apoptosis. *Trends in Biochemical Sciences* 1996;20:73–77. [PubMed: 7701566]
8. Mathias S, Pena LA, Kolesnick RN. Signal transduction of stress via ceramide. *Biochem J* 1998;335 (Pt 3):465–480. [PubMed: 9794783]
9. Yao B, Zhang Y, Delikat S, Mathias S, Basu S, Kolesnick R. Phosphorylation of Raf by ceramide-activated protein kinase. *Nature* 1995;378:307–310. [PubMed: 7477354]
10. Chalfant CE, et al. 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* 2002;277:12587–12595. [PubMed: 11801602]
11. Dobrowsky RT, Hannun YA. Ceramide stimulates a cytosolic protein phosphatase. *J Biol Chem* 1992;267:5048–5051. [PubMed: 1312082]
12. Kashiwagi K, Shirai Y, Kuriyama M, Sakai N, Saito N. Importance of C1B domain for lipid messenger-induced targeting of protein kinase C. *J Biol Chem* 2002;277:18037–18045. [PubMed: 11877428]
13. Deiss LP, Galinka H, Berissi H, Cohen O, Kimchi A. Cathepsin D protease mediates programmed cell death induced by interferon-gamma, Fas/APO-1 and TNF-alpha. *EMBO J* 1996;15:3861–3870. [PubMed: 8670891]
14. Pattingre S, Bauvy C, Carpentier S, Levade T, Levine B, Codogno P. Role of JNK1-dependent Bcl-2 phosphorylation in ceramide-induced macroautophagy. *J Biol Chem* 2009;284:2719–2728. [PubMed: 19029119]
15. Scarlatti F, et al. Ceramide-mediated macroautophagy involves inhibition of protein kinase B and up-regulation of beclin 1. *J Biol Chem* 2004;279:18384–18391. [PubMed: 14970205]
16. Daido S, Kanzawa T, Yamamoto A, Takeuchi H, Kondo Y, Kondo S. Pivotal role of the cell death factor BNIP3 in ceramide-induced autophagic cell death in malignant glioma cells. *Cancer Res* 2004;64:4286–4293. [PubMed: 15205343]
17. Xin M, Deng X. Protein phosphatase 2A enhances the proapoptotic function of Bax through dephosphorylation. *J Biol Chem* 2006;281:18859–18867. [PubMed: 16679323]
18. Lin SS, et al. PP2A regulates BCL-2 phosphorylation and proteasome-mediated degradation at the endoplasmic reticulum. *J Biol Chem* 2006;281:23003–23012. [PubMed: 16717086]
19. Reynolds CP, Maurer BJ, Kolesnick RN. Ceramide synthesis and metabolism as a target for cancer therapy. *Cancer Lett* 2004;206:169–180. [PubMed: 15013522]
20. Verheij M, van Blitterswijk WJ, Bartelink H. Radiation-induced apoptosis--the ceramide-SAPK signaling pathway and clinical aspects. *Acta Oncol* 1998;37:575–581. [PubMed: 9860316]
21. Nishina H, Wada T, Katada T. Physiological roles of SAPK/JNK signaling pathway. *J Biochem* 2004;136:123–126. [PubMed: 15496581]
22. Verheij M, et al. Requirement for ceramide-initiated SAPK/JNK signalling in stress-induced apoptosis. *Nature* 1996;380:75–79. [PubMed: 8598911]
23. Kolesnick R. The therapeutic potential of modulating the ceramide/sphingomyelin pathway. *J Clin Invest* 2002;110:3–8. [PubMed: 12093880]

24. Huwiler A, Zangemeister-Wittke U. Targeting the conversion of ceramide to sphingosine 1-phosphate as a novel strategy for cancer therapy. *Crit Rev Oncol Hematol* 2007;63:150–159. [PubMed: 17560117]
25. Radin NS. Killing cancer cells by poly-drug elevation of ceramide levels: a hypothesis whose time has come? *Eur J Biochem* 2001;268:193–204. [PubMed: 11168352]
26. Saddoughi SA, Song P, Ogretmen B. Roles of bioactive sphingolipids in cancer biology and therapeutics. *Subcell Biochem* 2008;49:413–440. [PubMed: 18751921]
27. Hanada K, Kumagai K, Tomishige N, Yamaji T. CERT-mediated trafficking of ceramide. *Biochim Biophys Acta* 2009;1791:684–691. [PubMed: 19416656]
28. Swanton C, et al. Regulators of mitotic arrest and ceramide metabolism are determinants of sensitivity to paclitaxel and other chemotherapeutic drugs. *Cancer Cell* 2007;11:498–512. [PubMed: 17560332]
29. Gouaze V, et al. Overexpression of glucosylceramide synthase and P-glycoprotein in cancer cells selected for resistance to natural product chemotherapy. *Mol Cancer Ther* 2004;3:633–639. [PubMed: 15141021]
30. Liu Y-Y, Han T-Y, Giuliano AE, Cabot MC. Expression of Glucosylceramide Synthase, Converting Ceramide to Glucosylceramide, Confers Adriamycin Resistance in Human Breast Cancer Cells. *J Biol Chem* 1999;274:1140–1146. [PubMed: 9873062]
31. Liu YY, Han TY, Giuliano AE, Cabot MC. Ceramide glycosylation potentiates cellular multidrug resistance. *FASEB J* 2001;15:719–730. [PubMed: 11259390]
32. Chalfant CE, Ogretmen B, Galadari S, Kroesen BJ, Pettus BJ, Hannun YA. FAS activation induces dephosphorylation of SR proteins; dependence on the de novo generation of ceramide and activation of protein phosphatase 1. *Journal of Biological Chemistry* 2001;276:44848–44855. [PubMed: 11502750]
33. Liu Y-Y, et al. Oligonucleotides blocking glucosylceramide synthase expression selectively reverse drug resistance in cancer cells. *J Lipid Res* 2004;45:933–940. [PubMed: 14967819]
34. Liu YY, et al. A role for ceramide in driving cancer cell resistance to doxorubicin. *FASEB J* 2008;22:2541–2551. [PubMed: 18245173]
35. Weiss M, Hettmer S, Smith P, Ladisch S. Inhibition of melanoma tumor growth by a novel inhibitor of glucosylceramide synthase. *Cancer Res* 2003;63:3654–3658. [PubMed: 12839955]
36. Chalfant CE, Spiegel S. Sphingosine 1-phosphate and ceramide 1-phosphate: expanding roles in cell signaling. *J Cell Sci* 2005;118:4605–4612. [PubMed: 16219683]
37. Gomez-Munoz A, et al. Ceramide-1-phosphate promotes cell survival through activation of the phosphatidylinositol 3-kinase/protein kinase B pathway. *FEBS Lett* 2005;579:3744–3750. [PubMed: 15978590]
38. Engelman JA. Targeting PI3K signalling in cancer: opportunities, challenges and limitations. *Nat Rev Cancer* 2009;9:550–562. [PubMed: 19629070]
39. Elojeimy S, et al. Role of Acid Ceramidase in Resistance to FasL: Therapeutic Approaches Based on Acid Ceramidase Inhibitors and FasL Gene Therapy. *Mol Ther* 2007;15:1259–1263. [PubMed: 17426710]
40. Kim WJ, et al. Mutations in the neutral sphingomyelinase gene SMPD3 implicate the ceramide pathway in human leukemias. *Blood* 2008;111:4716–4722. [PubMed: 18299447]
41. Ruckhaberle E, et al. Microarray analysis of altered sphingolipid metabolism reveals prognostic significance of sphingosine kinase 1 in breast cancer. *Breast Cancer Res Treat* 2008;112:41–52. [PubMed: 18058224]
42. Coward J, et al. Safingol (L-threo-sphinganine) induces autophagy in solid tumor cells through inhibition of PKC and the PI3-kinase pathway. *Autophagy* 2009;5:184–193. [PubMed: 19098447]
43. Noda T, Iwai S, Hamada M, Fujita Y, Yura Y. Induction of apoptosis of detached oral squamous cell carcinoma cells by safingol. Possible role of Bim, focal adhesion kinase and endonuclease G. *Apoptosis* 2009;14:287–297. [PubMed: 19199036]
44. Senkal CE, et al. Potent Antitumor Activity of a Novel Cationic Pyridinium-Ceramide Alone or in Combination with Gemcitabine against Human Head and Neck Squamous Cell Carcinomas in Vitro and in Vivo. *J Pharmacol Exp Ther* 2006;317:1188–1199. [PubMed: 16510697]
45. Mahdy AE, et al. Acid ceramidase upregulation in prostate cancer cells confers resistance to radiation: AC inhibition, a potential radiosensitizer. *Mol Ther* 2009;17:430–438. [PubMed: 19107118]

46. Grammatikos G, et al. Overexpression of acid sphingomyelinase sensitizes glioma cells to chemotherapy. *Antioxid Redox Signal* 2007;9:1449–1456. [PubMed: 17576160]
47. Holman D, et al. Lysosomotropic acid ceramidase inhibitor induces apoptosis in prostate cancer cells. *Cancer Chemotherapy and Pharmacology* 2008;61:231–242. [PubMed: 17429631]
48. Liu, X., et al. Implementation of acid ceramidase inhibitors as adjuncts to increase the efficacy of cancer therapy. In: Ochiai, T.; Shimada, H.; Tagawa, M., editors. *Gene Therapy* 2007. 1st ed. 21st Century's Center of Excellence Program of Japanese Ministry of Education and Science; Chiba: 2007. p. 58-65.
49. Saad AF, et al. The functional effects of acid ceramidase overexpression in prostate cancer progression and resistance to chemotherapy. *Cancer Biol Ther* 2007;6:1455–1460. [PubMed: 17881906]
50. French KJ, Upson JJ, Keller SN, Zhuang Y, Yun JK, Smith CD. Antitumor activity of sphingosine kinase inhibitors. *J Pharmacol Exp Ther* 2006;318:596–603. [PubMed: 16632640]
51. Carvajal RD, Merrill AH Jr, Dials HAB, Schwartz GK. A phase I clinical study of safinol followed by cisplatin: promising activity in refractory adrenocortical cancer with novel pharmacology. *Journal of Clinical Oncology* 2006;24:13044.
52. Yuan Y, Chi F, Wang S, Wang Z. Significance of ceramide and DNA ploidy in laryngeal carcinogenesis. *ORL J Otorhinolaryngol Relat Spec* 2007;69:283–288. [PubMed: 17565231]
53. Chi FL, Yuan YS, Wang SY, Wang ZM. Study on ceramide expression and DNA content in patients with healthy mucosa, leukoplakia, and carcinoma of the larynx. *Arch Otolaryngol Head Neck Surg* 2004;130:307–310. [PubMed: 15023837]
54. Young MR, Neville BW, Chi AC, Lathers DM, Gillespie MB, Day TA. Autocrine motility-stimulatory pathways of oral premalignant lesion cells. *Clin Exp Metastasis* 2007;24:131–139. [PubMed: 17370039]
55. Koybasi S, et al. 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* 2004;279:44311–44319. [PubMed: 15317812]
56. Karahatay S, et al. Clinical relevance of ceramide metabolism in the pathogenesis of human head and neck squamous cell carcinoma (HNSCC): attenuation of C(18)-ceramide in HNSCC tumors correlates with lymphovascular invasion and nodal metastasis. *Cancer Lett* 2007;256:101–111. [PubMed: 17619081]
57. Marques Filho MF, et al. Glycosphingolipid expression in squamous cell carcinoma of the upper aerodigestive tract. *Braz J Otorhinolaryngol* 2006;72:25–30. [PubMed: 16917549]
58. Bolot G, et al. Analysis of glycosphingolipids of human head and neck carcinomas with comparison to normal tissue. *Biochem Mol Biol Int* 1998;46:125–135. [PubMed: 9784847]
59. Fish RG. Role of gangliosides in tumour progression: a molecular target for cancer therapy? *Med Hypotheses* 1996;46:140–144. [PubMed: 8692038]
60. Miller AV, Alvarez SE, Spiegel S, Lebman DA. Sphingosine kinases and sphingosine-1-phosphate are critical for transforming growth factor beta-induced extracellular signal-regulated kinase 1 and 2 activation and promotion of migration and invasion of esophageal cancer cells. *Molecular and cellular biology* 2008;28:4142–4151. [PubMed: 18426913]
61. Bergelin N, et al. Sphingosine kinase as an oncogene: autocrine sphingosine 1-phosphate modulates ML-1 thyroid carcinoma cell migration by a mechanism dependent on protein kinase C-alpha and ERK1/2. *Endocrinology* 2009;150:2055–2063. [PubMed: 19116345]
62. Senchenkov A, Litvak DA, Cabot MC. Targeting Ceramide Metabolism--a Strategy for Overcoming Drug Resistance. *J Natl Cancer Inst* 2001;93:347–357. [PubMed: 11238696]
63. Dumitru CA, Weller M, Gulbins E. Ceramide metabolism determines glioma cell resistance to chemotherapy. *J Cell Physiol*. 2009
64. Rath G, et al. De novo ceramide synthesis is responsible for the anti-tumor properties of camptothecin and doxorubicin in follicular thyroid carcinoma. *Int J Biochem Cell Biol* 2009;41:1165–1172. [PubMed: 19027085]
65. Kolesnick R, Fuks Z. Radiation and ceramide-induced apoptosis. *Oncogene* 2003;22:5897–5906. [PubMed: 12947396]

66. Chmura SJ, Nodzenski E, Beckett MA, Kufe DW, Quintans J, Weichselbaum RR. Loss of ceramide production confers resistance to radiation-induced apoptosis. *Cancer Research* 1997;57:1270–1275. [PubMed: 9102212]
67. Chmura SJ, et al. Down-regulation of ceramide production abrogates ionizing radiation-induced cytochrome c release and apoptosis. *Mol Pharmacol* 2000;57:792–796. [PubMed: 10727527]
68. Alphonse G, Bionda C, Aloy MT, Ardail D, Rousson R, Rodriguez-Lafrasse C. Overcoming resistance to gamma-rays in squamous carcinoma cells by poly-drug elevation of ceramide levels. *Oncogene* 2004;23:2703–2715. [PubMed: 15048093]
69. Radin NS. Designing anticancer drugs via the achilles heel: ceramide, allylic ketones, and mitochondria. *Bioorg Med Chem* 2003;11:2123–2142. [PubMed: 12713822]
70. Stover TC, Sharma A, Robertson GP, Kester M. Systemic delivery of liposomal short-chain ceramide limits solid tumor growth in murine models of breast adenocarcinoma. *Clin Cancer Res* 2005;11:3465–3474. [PubMed: 15867249]
71. Shabbits JA, Mayer LD. Intracellular delivery of ceramide lipids via liposomes enhances apoptosis in vitro. *Biochim Biophys Acta* 2003;1612:98–106. [PubMed: 12729935]
72. Norris JS, et al. Combined therapeutic use of AdGFP_{FasL} and small molecule inhibitors of ceramide metabolism in prostate and head and neck cancers: a status report. *Cancer Gene Ther* 2006;13:1045–1051. [PubMed: 16763610]
73. Selzner M, et al. Induction of apoptotic cell death and prevention of tumor growth by ceramide analogues in metastatic human colon cancer. *Cancer Res* 2001;61:1233–1240. [PubMed: 11221856]
74. Szulc ZM, et al. Novel analogs of D-e-MAPP and B13. Part 1: Synthesis and evaluation as potential anticancer agents. *Bioorganic & Medicinal Chemistry* 2008;16:1015–1031. [PubMed: 17869115]
75. Liu X, et al. Modulation of ceramide metabolism enhances viral protein apoptin's cytotoxicity in prostate cancer. *Mol Ther* 2006;14:637–646. [PubMed: 16887394]
76. Litvak DA, Bilchik AJ, Cabot MC. Modulators of ceramide metabolism sensitize colorectal cancer cells to chemotherapy: a novel treatment strategy. *J Gastrointest Surg* 2003;7:140–148. discussion 148. [PubMed: 12559195]
77. Kawamori T, et al. Sphingosine kinase 1 is up-regulated in colon carcinogenesis. *FASEB Journal* 2006;20:386–388. [PubMed: 16319132]
78. Taha TA, Kitatani K, El-Alwani M, Bielawski J, Hannun YA, Obeid LM. Loss of sphingosine kinase-1 activates the intrinsic pathway of programmed cell death: modulation of sphingolipid levels and the induction of apoptosis. *FASEB J* 2006;20:482–484. [PubMed: 16507765]
79. Van, Brooklyn, JR; Jackson, CA.; Pearl, DK.; Kotur, MS.; Snyder, PJ.; Prior, TW. Sphingosine kinase-1 expression correlates with poor survival of patients with glioblastoma multiforme: roles of sphingosine kinase isoforms in growth of glioblastoma cell lines. *J Neuropathol Exp Neurol* 2005;64:695–705. [PubMed: 16106218]
80. Barry JM, Lisa M, Carolyn B, Myles CC, Reynolds CP. Synergistic cytotoxicity in solid tumor cell lines between N-(4-hydroxyphenyl)retinamide and modulators of ceramide metabolism. *Journal of the National Cancer Institute* 2000;92:1897. [PubMed: 11106681]
81. Hamada M, Sumi T, Iwai S, Nakazawa M, Yura Y. Induction of endonuclease G-mediated apoptosis in human oral squamous cell carcinoma cells by protein kinase C inhibitor safinol. *Apoptosis* 2006;11:47–56. [PubMed: 16374540]
82. French KJ, et al. Discovery and Evaluation of Inhibitors of Human Sphingosine Kinase. *Cancer Res* 2003;63:5962–5969. [PubMed: 14522923]
83. Senkal CE, et al. 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* 2007;6:712–722. [PubMed: 17308067]
84. Uchida T, et al. Phase I study of alpha-galactosylceramide-pulsed antigen presenting cells administration to the nasal submucosa in unresectable or recurrent head and neck cancer. *Cancer Immunol Immunother* 2008;57:337–345. [PubMed: 17690880]
85. Ishikawa A, et al. A phase I study of alpha-galactosylceramide (KRN7000)-pulsed dendritic cells in patients with advanced and recurrent non-small cell lung cancer. *Clin Cancer Res* 2005;11:1910–1917. [PubMed: 15756017]

86. Visentin B, et al. Validation of an anti-sphingosine-1-phosphate antibody as a potential therapeutic in reducing growth, invasion, and angiogenesis in multiple tumor lineages. *Cancer Cell* 2006;9:225–238. [PubMed: 16530706]
87. O'Brien N, et al. Production and characterization of monoclonal anti-sphingosine-1-phosphate antibodies. *J Lipid Res.* 2009
88. Liu Q, et al. FTY720 demonstrates promising preclinical activity for chronic lymphocytic leukemia and lymphoblastic leukemia/lymphoma. *BLOOD* 2008;111:275–284. [PubMed: 17761520]
89. Lahiri S, Park H, Laviad EL, Lu X, Bittman R, Futerman AH. Ceramide synthesis is modulated by the sphingosine analog FTY720 via a mixture of uncompetitive and noncompetitive inhibition in an Acyl-CoA chain length-dependent manner. *J Biol Chem* 2009;284:16090–16098. [PubMed: 19357080]
90. Berdyshev EV, et al. FTY720 inhibits ceramide synthases and up-regulates dihydrosphingosine 1-phosphate formation in human lung endothelial cells. *J Biol Chem* 2009;284:5467–5477. [PubMed: 19119142]
91. Neviani P, et al. FTY720, a new alternative for treating blast crisis chronic myelogenous leukemia and Philadelphia chromosome-positive acute lymphocytic leukemia. *J Clin Invest* 2007;117:2408–2421. [PubMed: 17717597]
92. Yasui H, et al. FTY720 induces apoptosis in multiple myeloma cells and overcomes drug resistance. *Cancer Res* 2005;65:7478–7484. [PubMed: 16103102]
93. Salinas NR, Oshima CT, Cury PM, Cordeiro JA, Bueno V. FTY720 and lung tumor development. *Int Immunopharmacol* 2009;9:689–693. [PubMed: 19146992]
94. Shen Y, et al. Antiproliferative and overadditive effects of rapamycin and FTY720 in pancreatic cancer cells in vitro. *Transplant Proc* 2008;40:1727–1733. [PubMed: 18589181]
95. Shen Y, et al. FTY720, a synthetic compound from *Isaria sinclairii*, inhibits proliferation and induces apoptosis in pancreatic cancer cells. *Cancer Lett* 2007;254:288–297. [PubMed: 17462818]
96. Zhou C, Ling MT, Kin-Wah Lee T, Man K, Wang X, Wong YC. FTY720, a fungus metabolite, inhibits invasion ability of androgen-independent prostate cancer cells through inactivation of RhoA-GTPase. *Cancer Lett* 2006;233:36–47. [PubMed: 16473668]
97. Azuma H, et al. Marked prevention of tumor growth and metastasis by a novel immunosuppressive agent, FTY720, in mouse breast cancer models. *Cancer Res* 2002;62:1410–1419. [PubMed: 11888913]
98. Nagaoka Y, Otsuki K, Fujita T, Uesato S. Effects of phosphorylation of immunomodulatory agent FTY720 (fingolimod) on antiproliferative activity against breast and colon cancer cells. *Biol Pharm Bull* 2008;31:1177–1181. [PubMed: 18520051]