Review

Mass Spectrometry Detects Sphingolipid Metabolites for Discovery of New Strategy for Cancer Therapy from the Aspect of Programmed Cell Death

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Abstract: Sphingolipids, a type of bioactive lipid, play crucial roles within cells, serving as integral components of membranes and exhibiting strong signaling properties that have potential therapeutic implications in anti-cancer treatments. However, due to the diverse group of lipids and intricate mechanisms, sphingolipids still face challenges in enhancing the efficacy of different therapy approaches. In recent decades, mass spectrometry has made significant advancements in uncovering sphingolipid biomarkers and elucidating their impact on cancer development, progression, and resistance. Primary sphingolipids, such as ceramide and sphingosine-1-phosphate, exhibit contrasting roles in regulating cancer cell death and survival. The evasion of cell death is a characteristic hallmark of cancer cells, leading to treatment failure and a poor prognosis. The escape initiates with long-established apoptosis and extends to other programmed cell death (PCD) forms when patients experience chemotherapy, radiotherapy, and/or immunotherapy. Gradually, supportive evidence has uncovered the fundamental molecular mechanisms underlying various forms of PCD leading to the development of innovative molecular, genetic, and pharmacological tools that specifically target sphingolipid signaling nodes. In this study, we provide a comprehensive overview of the sphingolipid biomarkers revealed through mass spectrometry in recent decades, as well as an in-depth analysis of the six main forms of PCD (apoptosis, autophagy, pyroptosis, necroptosis, ferroptosis, and cuproptosis) in aspects of tumorigenesis, metastasis, and tumor response to treatments. We review the corresponding small-molecule compounds associated with these processes and their potential implications in cancer therapy.

Keywords: sphingolipids metabolism; mass spectrometry; programmed cell death; novel anti-cancer therapy; small-molecule compounds

1. Introduction

Sphingolipids, present in the cell membrane of eukaryotic organisms, are a group of lipids characterized by their amino-alcohol backbones consisting of eighteen carbon atoms. The synthesis of sphingolipids primarily occurs in the endoplasmic reticulum [1].

They can also be formed from different precursors in the plasma membrane and Golgi apparatus [2,3]. In brief, the formation of ceramides, which serve as the fundamental structural units of all sphingolipids, involves the conjugation of sphingosine, derived from palmitoyl-CoA and serine, with very long-chain fatty acids. The enzymatic processes primarily catalyze sphingolipids to form sphingomyelin, cerebroside, and ganglioside (Figure 1). Sphingomyelins, which lack a glycerol backbone unlike other phospholipids, are synthesized by the transfer of phosphorylcholine from phosphatidylcholine to ceramide, resulting in the release of diacylglycerol. The cis and medial Golgi apparatus account for approximately 90% of the de novo synthesis of sphingomyelin. Although a certain amount of sphingomyelin synthesis takes place in the plasma membrane, particularly contributing to ceramide recycling, it is noteworthy that this process is the major source of sphingomyelin in oligodendrocytes and myelin membranes [4]. Cerebrosides are formed by linking either glucose or galactose to the C1 of ceramide. Mass spectrometry (MS) analysis has revealed a reduction in cerebroside levels and observed variations in fractions in numerous types of cancer [5,6]. Reliable investigation has indicated the potential significance of cerebroside variant proportions in understanding the complex biochemical and structural changes occurring during the neoplastic process. Due to structural abnormality and differentiation of various types of cells, the tumoral tissue exhibits a depleted capacity for the control of different processes concerning the synthesis of this group of lipids. Gangliosides, the most complex sphingolipids, are synthesized from galactocerebrosides and contain a huge polar head composed of several glycols, including at least one N-acetylneuraminic acid (sialic acid). The cell surface membrane is comprised of approximately 100 structurally distinct gangliosides, primarily concentrated in the brain, where they serve as crucial components [7]. Gangliosides act as differentiating markers during embryogenesis, oncogenesis and neoplastic transformation, and lymphoid differentiation. The complex sugars present on the outer surface of the membrane enable gangliosides to act as receptors for specific pituitary glycoprotein hormones that regulate essential physiological functions. Also, gangliosides can be receptors for bacterial protein toxins, such as cholera toxins. Hence, sphingolipids hold significant physiological and medicinal implications in the context of carcinogenesis. However, the metabolism of sphingolipids is subject to regulation at multiple levels and exhibits variations across different cell types. Even within a specific cell type, the regulation of sphingolipid metabolism is a dynamic process that responds to variable environmental conditions and adapts to different developmental stages. The complexity of this regulation makes it challenging to fully uncover and depict the complete story.
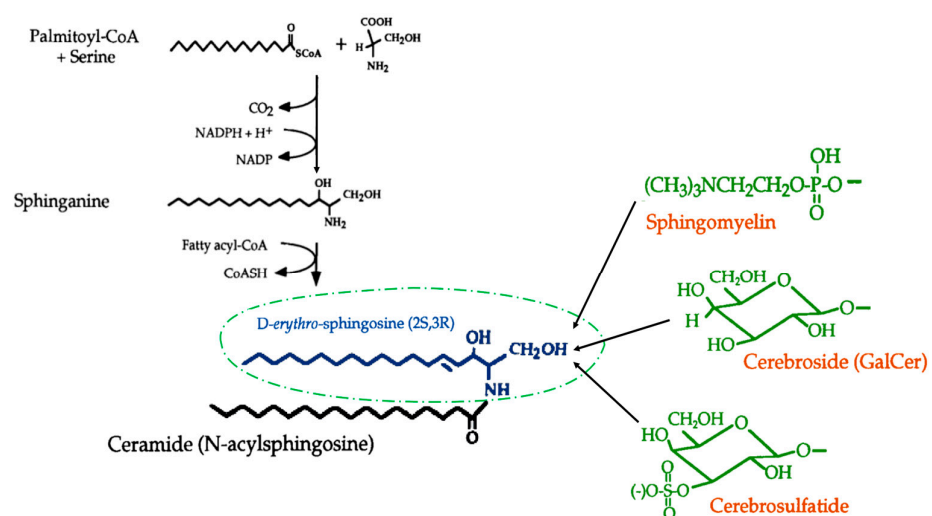
Figure 1. De novo biosynthesis of ceramide and primary components of sphingolipids.

Progress in MS analysis has facilitated the investigation of lipid metabolism, including sphingolipids, in almost all types of tumors over recent decades. These studies have revealed that the diverse sphingolipids and enzymes catabolizing sphingolipids involved in sphingolipid catabolism possess bioactive properties, playing a crucial role in regulating cellular functions and cell death. Meanwhile, programmed cell death (PCD) mechanisms explaining how tumor cells evade death are increasingly understood. Programmed cell death, a genetically regulated process known as apoptosis, is integral to the development, homeostasis, and integrity of multicellular organisms. Programmed cell death can refer to apoptosis, autophagy, pyroptosis, necroptosis, ferroptosis, and cuproptosis [8,9]. Numerous clinical trials focusing on targeting signaling pathways involved in PCD have emerged, evaluating strategies to modulate PCD pathways in different types of tumors. This review will discuss potential therapeutic targets derived from sphingolipid biomarkers detected through MS and their incorporation into PCD signaling pathways.

2. Mass Spectrometry Detected Sphingolipid Biomarkers for Carcinoma

Sphingolipids, a class of endogenous biologically active molecules with diverse structures, exhibit associations with various diseases due to metabolic disorders within this lipid category [10–17]. The comparative analysis of sphingolipid metabolic networks under different physiological conditions is crucial in identifying key differential metabolites involved in regulating sphingolipid metabolism. This method is important for interpreting the underlying mechanisms of sphingolipid metabolism in various diseases and tumors [18]. The measurement of these distinct sphingolipids with different fatty acyl chain lengths (as well as distinct long-chain base sphingosine backbones) in cancer cells, tumor tissues, or plasma is performed by quantitative sphingolipidomics using mass spectrometry-based assays [19]. Mass spectrometry (MS) offers enhanced sensitivity and enables the comprehensive analysis of a wide range of cell sphingolipid metabolites, making it an invaluable tool for studying sphingolipid metabolism [20]. Researchers currently have three strategies for studying sphingolipids: untargeted, targeted, and quasi-targeted metabolomics [21–23]. Untargeted metabolomics refers to conducting a comprehensive unbiased analysis of all measurable sphingolipids present in the sample without understanding the composition of the sample. It aims to extract new molecular information about sphingolipids by mining extensive data, maximizing the overall understanding of the sample's sphingolipid metabolic characteristics [24]. Targeted metabolomics mainly aims to verify whether specific sphingolipids exist in the sample and involves a selective analysis of the target sphingolipid molecules. This approach is highly focused, facilitating easier operation and analysis [25]. The combined use of these methods significantly improves information coverage and quantitative accuracy [26,27]. Below, we have summarized the specific methods and usage of MS in current sphingolipids research (Table 1).

2.1. Electron Ionization Mass Spectrometry

An electron ionization (EI) source in mass spectrometry employs electrons of specific energy to directly interact with sample molecules, resulting in their efficient ionization [28]. The advantages of EI include its ease of implementation, the reproducibility of mass spectra, and the provision of more fragment ion information in the obtained results. More importantly, EI can distinguish the isomers of sphingolipids [29,30], thereby facilitating speculations regarding the structure of unknown substances. In early studies of sphingolipids, the structures of ceramide and glucosylceramide were mainly determined through EI sources [31]. However, the excessive amount of cleaved fragment ions produced by this method posed challenges in effectively analyzing the constituents of complex mixtures of sphingolipids, necessitating prior chromatographic separation to enhance analytical capabilities [32].

2.2. Secondary Ion Mass Spectrometry

Secondary mass spectrometry is a soft ionization method that involves bombarding the surface with high-energy primary particles, followed by the analysis of secondary ions generated for mass spectrometry analysis [33,34]. This method is divided into fast atom bombardment mass spectrometry (FAB-MS) and liquid secondary ion mass spectrometry (LSI-MS). FAB-MS utilizes atomic beams as high-energy primary particles, whereas LSI-MS employs ion beams for the same purpose [35]. The generated excimer ions can be directly used to analyze complex sphingolipids such as ceramide, hexosylceramides, and sphingomyelin through the direct ionization of sphingolipids via high-energy atom or ion collisions. This approach yields crucial data for the structural analysis of sphingolipids [36]. This represents a significant advancement in sphingolipid metabolism research facilitated by mass spectrometry techniques.

2.3. Electrospray Ionization Mass Spectrometry

Electrospray ionization (ESI) uses a strong electrostatic field to produce highly charged droplets after repeated solvent volatilization and droplet splitting. A single multi-charged ion is formed through multiple cycles of solvent volatilization and droplet fragmentation. During the ionization progress, multiple protonated ions are generated [37]. Due to the possibility that sphingolipid molecules can be directly ionized in ESI mass spectrometry [38], ESI-MS can detect and analyze a wide variety of sphingolipid metabolites, including ceramide, sphingomyelin, cerebroside, phosphatidylserine, phosphatidylinositol, and dozens of others [39].

2.4. Liquid Chromatography and Electrospray Ionization Mass Spectrometry

The combination of liquid chromatography (LC) and mass spectrometry yields many advantages. LC efficiently purifies sphingolipid metabolites, isolating them from complex mixtures while reducing isomer interference and thereby improving the accuracy and sensitivity of subsequent mass spectrometry analysis. By utilizing this combination, one can accurately identify various sphingolipids such as ceramide, hexosylceramides, lacto-sylceramide, sphingomyelin, sphingolipid phosphorylation products, and other complex sphingolipids [40–44]. LC can adopt two separation modes: normal-phase chromatography and reverse-phase chromatography. Normal-phase chromatography separates compounds, such as sphingomyelin and glycosphingolipids, based on the polarity of their heads. On the other hand, reverse-phase chromatography separates these compounds by considering the carbon content and unsaturation of the main chain of sphingosine and the side chains of fatty acids [45–47].

2.5. Gas Chromatography Mass Spectrometry

Gas chromatography (GC) is a highly efficient chromatographic technique extensively employed for analyzing volatile lipid compounds. It serves as a prevalent method for analyzing fatty acids and cholesterol [48,49]. It is necessary to perform silanization or derivatization through esterification to analyze non-volatile compounds using GC. However, this requirement restricts the application of GC in lipidomics. The GC-MS method is a widely employed technique for detecting the composition of long-chain bases and fatty acids in sphingolipid samples and conducting structural analysis. While the GC-MS method is known for its accuracy and user-friendliness, one drawback is the requirement for sample pre-treatment, which can compromise structural integrity [49].

2.6. Fourier Transform Ion Cyclotron Resonance Mass Spectrometry

Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS) is a high-resolution mass spectrometry technique known for its exceptional mass resolution and accuracy. It enables the identification of small mass errors in ion peaks [50]. Therefore, Fourier transform ion cyclotron resonance mass spectrometry can effectively remove the interference of isomers and isotope lipids in sphingolipids analysis. Consequently, it enables a

more comprehensive component analysis of sphingolipid metabolites, facilitating the identification and structural determination of complex glycosyl sphingolipids [51], Additionally, FTICR-MS allows for the differentiation of various types of phosphate esters [52].

2.7. Matrix-Assisted Laser Desorption Ionization Mass Spectrometry

In matrix-assisted laser desorption ionization (MALDI), the sample is introduced into the ion source, where the matrix generates numerous molecules upon exposure to a UV laser beam. Subsequently, the analyte undergoes ionization [11]. MALDI-MS boasts fast analysis, ease of operation, and the advantage of requiring small sample sizes. The technique has proven successful in analyzing several sphingolipids, including ceramide, hex ceramide, and sphingomyelin [11,53]. MALDI-MS offers both spatial resolution and molecular specificity, making it a powerful technique for label-free tracking of endogenous and exogenous compounds. It is particularly useful for visualizing the spatial location of lipids in biological tissues [54,55].

With the development of mass spectrometry technology, it has provided a powerful tool for analyzing and identifying aspects of sphingolipid metabolism. Currently, mass spectrometry technology enables the identification of a wide range of essential compounds within sphingolipid metabolism networks and cell signal transduction. These compounds include the fatty acids-coenzyme, ceramide, sphingosine, sphingomyelin, galactosylceramide, among others [56]. Due to the occurrence of identical ion pairs or isotope peaks in mass spectrometry analysis among certain sphingolipid compound skeletons, it is essential to perform liquid-phase separation on the sample. This step is crucial in reducing false positive interference and enhancing detection sensitivity [57,58].

3. Sphingolipid Metabolism and Programmed Cell Death

Programmed cell death, an active and orderly form of cell death, is ubiquitous in the cell biological process and plays a crucial role in the process of tumor cell escape. Specifically, PCD refers to the suicidal protective measures initiated by gene programming and molecular mechanisms when organisms encounter internal and external stimuli in which non-essential cells undergo specialization and removal. Malignant tumor cells must surmount diverse types of cell death mechanisms to facilitate metastasis and recurrence. Relying on interactions with membrane proteins in the plasma membrane, sphingolipids form membrane microdomains with regulatory functions, thereby engaging in intricate interactions with nearly all PCD pathways (Table 2). There is no doubt that the association of sphingolipids and PCD pathways will provide new insights into cancer treatment. Here, we mainly discuss the relevance between sphingolipids and cell apoptosis, autophagy, pyroptosis, necroptosis, and ferroptosis, as well as the newly discovered cuproptosis.

3.1. Apoptosis

Specific cell death signaling pathways activate apoptosis. These pathways involve cytoplasmic shrinkage, budding of the plasma membrane, eversion of phosphatidylserine (PS), chromatin condensation, and DNA breakage [59,60], which gives rise to cell clearance [61]. During the whole process of apoptosis, the cell membrane remains intact, preventing the release of cellular endocytes and the onset of inflammatory responses [62]. There are two main pathways of apoptosis: the extrinsic receptor-mediated pathway and the intrinsic mitochondrial pathway. Extrinsic receptor-mediated pathways refer to death ligands such as tumor necrosis factor (TNF), Fas, and members of the tumor necrosis factor receptor family and their related ligands [63]. These ligands trigger the formation of a death-induced signaling complex (DISC) that recruits multiple effectors to facilitate cellular apoptosis. Sphingolipids play a crucial role as a regulator in the extrinsic receptor-mediated pathway of apoptosis. Sphingomyelinase hydrolyzes raft sphingomyelin to ceramide, and ceramide molecules spontaneously associate to form microdomains with the enrichment of ceramide. These ceramide-enriched microdomains can reorganize receptors and signaling molecules present both inside the cells and on the cell membranes, thereby facilitating and

amplifying specific receptor-mediated signaling transduction [64]. An example of such modulation is observed in the acid sphingomyelinase (aSMase)-mediated aggregation of CD95 within ceramide-rich membrane platforms. This aggregation event is crucial for DISC formation [65–67]. In addition, the binding of ligands with receptors, such as the p55 TNF receptor, interleukin-1 receptor, and Fas receptor, activates SMase, resulting in the generation of ceramide. Ceramide then functions as a second messenger, exerting regulatory control over apoptotic signaling pathways [68–71]. Multiple signaling pathways are transduced and aggregated to mitochondria, leading to the induction of mitochondrial outer membrane permeability (MOMP). This phenomenon is recognized as intrinsic mitochondrial-mediated apoptosis. The release of intercellular space proteins, including cytochrome c and Apaf1, triggers the activation of caspases and DNases within the cytoplasmic matrix. These proteins play a crucial role in regulating cell death processes [72]. The anti-apoptotic and pro-apoptotic members of the Bcl-2 family are highly homologous in one or more specific domains and regulate the process of MOMP. The overexpression of anti-apoptotic Bcl-2 members protects against apoptosis induced by various stimulus signals [73]. Various key enzymes in regulating sphingolipid metabolism exist in mitochondria, such as ceramide synthase, ceramidase, sphingosine kinase, and neuraminidase 4 [74–76]. Studies have provided evidence of elevated ceramide levels preceding the mitochondrial phase of apoptosis [77–79]. Moreover, increased ceramide levels are observed in response to various apoptotic stimuli [80,81]. Interestingly, as the metabolic content of ceramide decreases, cells can also avoid apoptosis. For example, glucosylceramide synthase (GCS) converts ceramide metabolism into glucosylceramide. Downregulating ceramide levels through this process provides cellular protection against ceramide-induced apoptosis and enables the cells to develop resistance against apoptosis, promoting anti-apoptotic responses [82]. As another ceramide metabolite, sphingosine-1-phosphate (S1P) and its metabolites exert the opposite effect on apoptosis: on the one hand, activating extracellular regulated protein kinases (ERK) and other anti-apoptotic signaling pathways inhibits apoptosis induced by ceramide [83–85]; on the other hand, S1P and its metabolite, acetaldehyde, can also promote apoptosis by activating the pro-apoptotic Bcl-2 proteins Bak and Bax [86,87].

Ceramide protects cells from apoptosis in certain cancer cells [88]. C16 ceramide protects head and neck squamous cell carcinoma (HNSCC) cells from the endoplasmic reticulum (ER) stress-mediated apoptosis [89]. Conversely, C24 ceramide protects HeLa cells against apoptosis induced by ionizing radiation (IR). However, in the case of cancer cells, C16 ceramide promotes apoptosis mediated by IR [90]. One possible explanation could be that the regulation of apoptosis by ceramide is affected by both the subcellular localization of ceramide and the type of stress stimulation (Figure 2). These factors contribute to the contrasting outcomes, either promoting or inhibiting apoptosis.

3.2. Autophagy

Autophagy is a highly conserved cellular degradation process in eukaryotes, responding to various stimuli to maintain the balance of cells, tissues, and organisms [91]. Autophagy can be divided into three types: macro-autophagy, micro-autophagy, and chaperone-mediated autophagy (CMA) [91–93]. The whole process of autophagy includes the initiation of autophagosomes, the nucleation of membranes, the expansion and extension of the autophagosome membrane, the closure of the autophagosome membrane, its fusion with lysosomes, and ultimately, the degradation of the autophagic contents [94,95]. When the body is under appropriate stimulation, the phosphorylation of the core complex ULK1 in the autophagy process is prevented. This cessation of ULK1-complex inhibition triggers the initiation of autophagosome formation [96,97]. The ULK1 complex activates another complex, Beclin-1, and enhances the activity of VPS34, which is located in PI3K. This activation leads to the formation of autophagosome membrane PI3P and the recruitment of crucial factors for autophagy induction, such as double FYVE-containing protein 1 (DFCP1) [98,99]. When the autophagosome matures, it undergoes fusion with the outer

membranes of lysosomes, resulting in the degradation of the cargo transported by lysosomes. The involvement of sphingolipids in autophagy is evident across various stages of the autophagic process. In the early stage of autophagy, Beclin-1, an important complex, binds to Bcl-2, resulting in the inhibition of Beclin-1 [100], while ceramide can induce the disassociation of Beclin-1 from the complex and the release of Beclin-1. This release of Beclin-1 plays a vital role in initiating autophagy [101]. In addition, the change in sphingolipid levels in the plasma membrane regulates the levels and activity of nutrient transporters [102,103]. These transporters play a critical role in controlling the cellular fuel supply. Conversely, ceramides downregulate the expression of amino acids and nutrient transporters, leading to cellular starvation. This, in turn, triggers the induction of survival autophagy through the reduction in mTOR signaling transduction or activation of AMPK signaling [104,105]. During autophagy, autophagosomes engulf various cytoplasmic components, as well as mitochondria, the endoplasmic reticulum, and peroxisomes [106]. In mitochondrial autophagy, ceramides can diminish the mitochondrial membrane potential, resulting in mitochondrial dysfunction. Furthermore, this process promotes the expression of the mitochondrial-associated cell death protein BNIP3 [107]. The ectopic expression of Cers1 leads to the formation of autophagosomes that directly bind to ceramides on the mitochondrial membrane [108]. Autophagosomes and lysosomes associate to form autolysosomes that degrade the substance regulated by sphingolipids [109]. More recently, it has been found that ceramide-1-phosphate, derived from sphingomyelin by sphingomyelinase and ceramide kinase, promotes calcium-dependent liposome fusion and regulates the fusion of the autophagosome and lysosome [110].
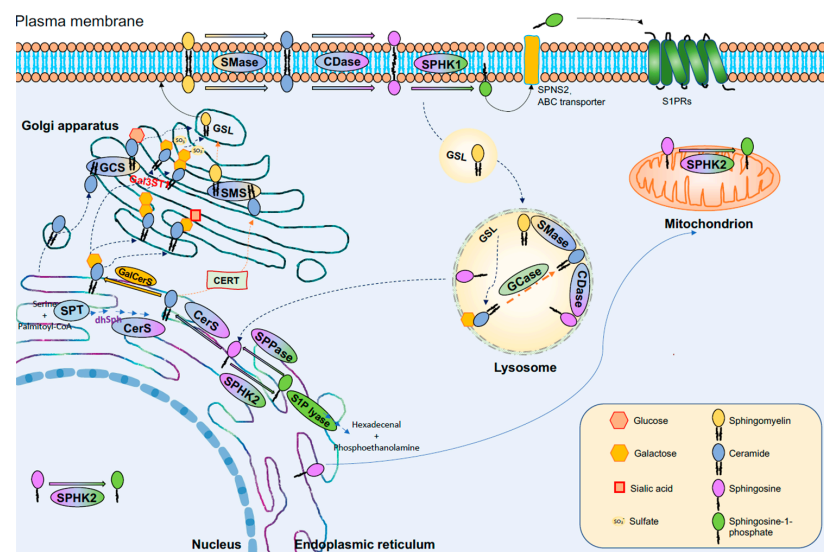
Figure 2. Subcellular compartmentalization of sphingolipid metabolism.

3.3. Necroptosis

Necrotic apoptosis, also known as necrotic apoptosis, represents an alternative form of programmed cell death. During necroptosis, apoptotic cells undergo rapid swelling, accompanied by concurrent organelle swelling [111]. Necroptosis involves key players such as RIPK3 and its substrate MLKL, whose interaction with lipids is a key step in the implementation of necroptosis [112,113]. Ceramide can target MLKL to activate necroptosis independent of the RIPK1/RIPK3 regulatory pathway in ovarian cancer [114]. Furthermore, during necroptosis, MLKL translocates to lipid rafts in the plasma membrane, and ceramide facilitates the relocation of MLKL to these lipid rafts [115]. Ceramide can form a complex with receptor-interacting Ser/Thr kinase 1 (RIPK1) and form a ceramide-enriched membrane pore channel mediated by non-muscle myosin IIA. In this process, ceramide binds to specific residues, such as ASP147 and ASN169 of RIPK1, while the interaction with myosin IIA involves the residues Arg258 and Leu293 [116].

3.4. Pyroptosis

Pyrosis is an inflammatory cell death in that various pathological factors, including related signals can trigger and activates Caspase-1 through classical inflammatory corpuscle signaling pathways or activates Caspase-4, -5, -11 through non-classical pathways to exert various downstream effects [117]. Ceramide is mainly located in the endoplasmic reticulum membrane and produces sphingosine through ceramidase. On the one hand, sphingosine translocates from the endoplasmic reticulum (ER) membrane to the cell membrane, where it undergoes conversion into sphingosine-1-phosphate (S1P) mediated by sphingosine kinase 1 (SPHK1). The buildup of sphingosine within the ER can trigger the activation of NLRP3 and oligomeric RLRP3 inflammasomes, leading to the homogeneous activation of Caspase-1 and subsequent production of IL-1/IL-18 [118]. In addition, when lysosomes are destroyed, Cathepsin B diffuses into the cytoplasmic matrix and cleaves SPHK1 [119]. Therefore, sphingosine-induced lysosomal membrane rupture leads to Cathepsin B translocation from the lysosome to the cytoplasm, which results in the cleaved SPHK1 and the inhibition of sphingosine conversion to S1P. As a result, the accumulated sphingosine activates the assembly of NLRP3 inflammasomes, ultimately inducing pyroptosis [118].

3.5. Ferroptosis and Cuproptosis

Ferroptosis is a form of cell death that relies on ferric ions to generate reactive oxygen species (ROS). These ROS initiate alterations in metabolic processes and the accumulation of lipid peroxides, leading to structural damage to cell membranes and ultimately resulting in cell death [120]. GXP4 is a defense inhibitor against LPO and iron apoptosis [121]. In cases of GSH deficiency, the activity of glutathione peroxidase 4 (GPX4) decreases, leading to an increase in intracellular lipid peroxide (LPO) levels. This imbalance in cellular redox status inclines cells towards undergoing ferroptosis [122]. Ceramide accumulation is observed in the early stages of iron-induced apoptosis, coinciding with glutathione depletion and ROS production. Ceramide can be activated by various stressors, such as genotoxic damage, inflammatory mediators, heat shock, oxidative stress, and anti-cancer drugs, forming membrane platforms enriched with ceramide [123]. aSMase-mediated ceramide has been proven to induce ROS production in cells. Additionally, ceramide is known to form membrane platforms enriched with ceramide. Concurrently, NADPH oxidase aggregates and becomes activated within these membrane platforms, producing ROS. As the ROS levels increase, the Cys-629 residue located in the aSMase undergoes oxidation. This oxidation event promotes the dimerization and activation of aSMase, facilitating the generation of more ceramide. This ceramide undergoes repeated circulation within the system [124,125]. Furthermore, aSMase also facilitates GPX4-dependent autophagy, an essential process associated with ferroptosis. Previous studies have shown that ferroptosis is accompanied by autophagy activation, while aSMase-mediated autophagy plays a key role in ferroptosis [124,126,127].

Cuproptosis is a recently identified form of cell death specifically triggered by copper. The mechanism underlying cuproptosis distinguishes it from all other known cell death pathways. Cuproptosis occurs due to the interaction between copper and the fatty acylation components within the tricarboxylic acid (TCA) cycle. This association leads to the buildup of fatty acylated proteins and the depletion of iron–sulfur cluster proteins. Consequently, protein toxic stress ensues, ultimately culminating in cell death [128]. At present, limited research exists on cuproptosis, and the relationship between sphingolipid metabolism and cuproptosis remains largely unknown. Further investigations are needed to elucidate this relationship. Experimental evidence has confirmed that copper can induce apoptosis by aSMase and subsequently trigger the release of ceramide [129,130]. Cu^{2+} initiates hepatocyte apoptosis by the activation of aSMase and subsequent release of ceramide. Additionally, Cu^{2+} induces the secretion of activated aSMase in leukocytes, leading to ceramide release and phosphatidylserine exposure in erythrocytes. This exposure facilitates the rapid clearance of affected erythrocytes within the bloodstream of mice [131].

Table 1. Sphingolipids detected by MS methods.

Mass Spectrometry	Advantages, Disadvantages, and Characteristics	Sphingolipids	Ref.
LC-MS, HPLC-ESI-MS	Detect and analyze a wide variety of sphingolipids metabolites	Ceramide	[40,56,132,133]
FTICR-MS, HPLC-ESI-MS	Detect and analyze a wide variety of sphingolipids metabolites	Sphingosine	[40,56]
FTICR-MS, LC-MS	Remove the interference of isomers and isotope lipids in sphingolipid analysis	Sphingomyelin	[56]
FTICR-MS with LC-MS	Reduce false positive interference and improve detection sensitivity	Dihydroceramide	[134,135]
LC-MS	Reduce the interference of isomers, improve the accuracy and sensitivity	Glucosylceramide	[136,137]
LC-MS/MS	Reduce the interference of isomers, improve the accuracy and sensitivity	Sphingosine-1-phosphate	[138]
LC-MS	Reduce the interference of isomers, improve the accuracy and sensitivity	Phosphatidylinositol	[139]

Table 2. Sphingolipids interplay with PCD pathways.

Cell Death Pathway	Sphingolipid	Morphological Feature	Function	Ref.
[64–67] [140] Apoptosis [83–85]	Ceramide, sphingomyelinase phosphate	Induces the extrinsic pathways of the apoptosis pathway	Adjusted, amplified the signal	[64–67]
	Ceramide	Induces the mitochondrial intrinsic apoptosis pathway	Induced	[140]
	Dihydroceramide	Interferes with the formation of ceramide channels in mitochondria, significantly reduces the permeability of the outer mitochondrial membrane, and inhibits ceramide-induced apoptosis	Inhibited	[141]
	Ceramide synthase 6 (CerS6), C16-Ceramide	Regulates activation of ER stress response	Inhibited	[89]
	Glucocerebroside; glycosphingolipid, (GSL); Glucosylceramide Synthase (GCS)	Induces mitochondrial intrinsic apoptosis pathway	Anti- Apoptosis	[82]
	S1P	Activates ERK and other signaling pathways	Anti- Apoptosis	[83–85]
	S1P	Activates pro-apoptotic Bcl-2 proteins Bak and Bax	Pro- apoptosis	[86]

Table 2. *Cont.*

Cell Death Pathway	Sphingolipid	Morphological Feature	Function	Ref.
Autophagy	Ceramide	Downregulates nutrient transporters	Promoted	[102,104,105]
		Induces the release of Beclin-1 by dissociation of the Beclin-1/Bcl-2 complex	Induced, promoted	[101]
		Mediates autophagosomes directly anchor to mitochondria	Promoted	[108]
		Reduces mitochondrial membrane potential and activates BNIP3 transcription	Induced	[107]
	Dihydroceramide desaturase 1, DES1	ATP synthesis damage activates Ampk, activating unc-51-like kinases to cause autophagosome formation	Promoted	[142]
	Sphingomyelin, sphingomyelinase, ceramide kinase, ceramide-1-phosphate	Calcium-dependent liposome fusion, which regulates the fusion of autophagosomes and lysosomes	Promoted	[110]
	Sphingosine-1-phosphate phosphohydrolase-1 (SPP1)	Deletion of SPP1 increases the expression of transcriptional regulators C/EBP homologous protein and Grp78/BiP, as well as phosphorylation of eukaryotic translation initiation factor-2 α (eIF2α), induces ER stress.	Induced	[143]
Necroptosis	Ceramide	Mediates MLKL repositioning into lipid rafts	Induced	[115]
		Ceramide–RIPK1 complex formation leads to disruption of lipid bilayer integrity	Induced	[116]
Pyroptosis	Sphingosine, SPHK1	Activates NLRP3 and oligomeric RLRP3 inflammasomes	Induced	[118]
Ferroptosis	Ceramide; acid sphingomyelinase, aSMase	Ceramide enrichment membrane plateau formation induces GPX4 autophagy degradation	Induced, promoted	[124]

Table 3. Inhibitors focusing on sphingolipid-motivated PCD in preclinical or clinical phase.

Inhibitor/Drug Name	Target Sphingolipids	Related PCD	Cancer Relevance	Trial Phase	Ref.
Myriocin	De novo ceramide synthesis	Apoptosis	Increased activity in response to chemotherapy and radiotherapy in breast cancer cells		[144–146]
FB1; HDAC1 or HDAC2	Synthesis of C18 (dihydro)ceramide	Autophagy	Induces mitophagy in head and neck and AML cell lines, mouse xenograft models, and patient-derived AML cells	Preclinical	[147–149]
FB1	Synthesis of C16 (dihydro)ceramide	Apoptosis—mitochondria	Induces caspase activation and cell death in lung cancer cells; preserves ER and Golgi integrity in head and neck cancer cells; elevated in breast tumour tissues; protects from GVHD in a mouse model of leukaemia	Preclinical	[89,150–156]
Fenretinide; ABC294640; C8-CPC	Ceramide synthesis	Apoptosis	Induces cell cycle arrest in neuroblastoma cells	Preclinical	[157]
Tri-cyclic anti-depressants	Ceramide generation	Apoptosis	Induces apoptosis in lymphoblasts; promotes haematogenous tumour metastasis in mouse models		[158–160]
GW4869	Ceramide generation	Apoptosis	Mediates cell cycle arrest in breast cancer cells; exosome release		[161–163]
THI	S1P breakdown	Apoptosis	Induces ceramide accumulation and colon cancer cell death		[164,165]
CHC	Ceramide transport from ER to Golgi	Autophagy	Inhibits pro-apoptotic ceramide signalling in breast cancer cells and tumours in mouse models	Preclinical	[166–168]
NVP-231	C1P generation	Apoptosis	Induces breast cancer cell survival in culture and mouse models	Preclinical	[169–171]
PPMP; PDMP	GlcCer synthesis	Apoptosis—mitochondria	Mediates drug resistance in patients with oral cancer and in breast cancer cells and xenografts		[172–176]
LCL-521	Ceramide cleavage	Apoptosis—mitochondria	Mediates resistance to cell death in prostate cancer cells and xenografts and elevated in tumours from patients	Preclinical	[177–180]
PF543	S1P generation	Apoptosis	Mediates pro-survival signalling and metastasis in bladder cancer, lung cancer, and melanoma cells in culture and in mouse models	Preclinical	[181–183]

4. Conclusions and Prospects

Advancements in genetic and molecular techniques have led to significant discoveries unveiling the involvement of sphingolipids in cancer progression and treatment over the past several decades. In particular, mass spectrometry is an analytic technology innovated and widely used to quantitate and distinguish specific sphingolipid metabolites relevant to cancer cells, cancer patients' peripheral blood, tumor tissues, and adjacent tissues or cells near tumor cell clusters. With the deep lipidomic excavation using MS, sphingolipid metabolites have been identified as crucial players in diverse signaling pathways implicated in cancer development, evasion of stem cells, drug resistance, and even modifications in drug response. The distinctive physiological functions of sphingolipids offer a novel avenue for studying cellular pathology and disease mechanisms, presenting exciting opportunities for further research. Mechanistically, most enzymes involved in sphingolipid metabolism can generate genetic models to study their roles in cancer signaling pathways. Additionally, these models contribute to developing and designing structure–function-based anti-cancer drugs, opening new avenues for therapeutic advancements. The synthesis and accumulation of sphingolipids can mediate cancer cell death through various mechanisms, including induction of apoptosis, necroptosis, autophagy, ferroptosis, and cellular stress like ER stress [88]. Several inhibitors specifically targeting sphingolipid-driven PCD have been developed and extensively investigated (Table 3). Many cancer treatments targeting the sphingolipid metabolism pathway have achieved clinical results. Fenretinide can target DES enzymes to reduce the de novo synthesis of ceramides and regulating the increase in dihydroceramide levels. But, there are some side effects after medication, including diarrhea, allergic reactions, and others [184]. One Phase III clinical trial of fenretinide showed no significant difference between treatment groups, accompanied by certain visual problems and toxic side effects of musculoskeletal disorders [185]. In addition, fenretinide showed insufficient antitumor activity, such as against breast cancer [186] and kidney cancer [187]. ABC294640 is an orally administered inhibitor of SPHK2 and DES1 that inhibits the synthesis of S1P, which is an anti-apoptotic sphingolipid metabolite, and increases the content of intracellular ceramides [188,189]. The current ABC294640 Phase I clinical trial results for solid tumors have shown a good tolerability; ABC294640 administration can cause S1P levels to decline in the first 12 h and return to baseline within 24 h with side effects include nausea, vomiting, and fatigue [190].

In addition, the different subcellular localizations cause sphingolipids to differentially mediate cell death pathways, varying depending on the cell and tissue type. This characteristic renders sphingolipids an appealing therapeutic target for anti-tumor interventions (Figure 2). MALDI visualizes and images the tissue localization of these distinct sphingolipids. Based on these techniques, ceramide and S1P are defined by anti-cancer and pro-oncogenic functions depending on the microenvironment and cell type [191–193]. The intracellular bioactive sphingolipid network is extremely complex and involved in many kinds of life activities. Cytoplasmic sphingolipids located regionally can lead to functional differences; thus, in the context of the entire biological organization, the study of sphingolipids is very difficult [194]. Nevertheless, the emergence of new technologies has provided various contributions to clarify the mechanism of sphingolipids in various life activities [88]. It is currently clear that different downstream targets of ceramide and S1P produce different results and, even in the same cell, sphingolipids in different subcellular structures changing will lead to different cell fate trajectories; this is why ceramide, S1P, or other kinds sphingolipid can participate in different types of PCD at the same time. In addition, sphingolipids represent components of the plasma membrane involved in regulating biofilm fluidity, forming sphingolipid-enrichment regions to form special functional platforms [124,195], and regulating cell growth and proliferation through signaling. A mitophagy-promoted cancer cell death induced by a mitochondrial accumulation of pyridinium ceramide has been discovered [196]. The intricate regulatory mechanisms of sphingolipids within different cells or organelles under varying microenvironments give

rise to diverse downstream targets. These targets ultimately influence cellular fate and outcomes. For example, SPHK2-generated S1P-protein binding regulates other S1P targets by allosterically mimicking protein phosphorylation. Notably, this interaction impacts various proteins, including PPARγ, HDAC1/2, and PHB2 [197–199]. This process plays a critical role in controlling drug-induced senescence and cancer-associated PCD. Revealing the roles of sphingolipids in signal transduction and cross-talk between tumors and host cells (primary stromal cells, endothelial cells, osteoclasts, or platelets) can help to develop novel therapeutic strategies to inhibit cancer growth and metastasis.

However, numerous preclinical and clinical drugs have been developed and subjected to trials, capitalizing on the aforementioned advantages (Table 3). Most trials have failed or been stopped due to our limited understanding of the intricate signal transduction processes involved in cancer. The central key of cancer biology is the type and condition of cells transformed to form tumors. Therefore, effective treatment strategies should be rooted in a deep understanding of carcinoma and the overall metabolic profiles of individual patients. The cornerstone of cancer biology lies in comprehending the characteristics and status of cells that transform to give rise to tumors. Therefore, effective treatment strategies should be rooted in a deep understanding of carcinoma and the overall metabolic profiles of individual patients. Tracing and defining the roles of sphingolipids in regulating PCD versus survival is a challenge. This challenge arises from the rapid in vivo metabolic interconversions, intricate trafficking, and multifaceted signaling roles that sphingolipids assume within cell and organelle membranes. Moreover, further studies are needed to ascertain the precise mechanisms by which sphingolipids function in immunocyte-mediated tumor killing and how they influence the effectiveness of immunotherapy in combating cancer. For example, immunotherapy with ch14.18, GM-CSF, and interleukin-2 was associated with a significantly improved outcome as compared with standard therapy in patients with high-risk neuroblastoma. Rapid advancements in MS technology have paved the way for significant breakthroughs in unraveling the molecular interactions and signaling networks underlying the immune metabolism of sphingolipids. This progress holds promising prospects for the future development of improved treatments for solid tumors.

References

1. Breslow, D.K. Sphingolipid homeostasis in the endoplasmic reticulum and beyond. *Cold Spring Harb. Perspect. Biol.* **2013**, *5*, a013326. [CrossRef] [PubMed]
2. Zahumensky, J.; Mota Fernandes, C.; Vesela, P.; Del Poeta, M.; Konopka, J.B.; Malinsky, J. Microdomain Protein Nce102 Is a Local Sensor of Plasma Membrane Sphingolipid Balance. *Microbiol. Spectr.* **2022**, *10*, e0196122. [CrossRef]
3. Gault, C.R.; Obeid, L.M.; Hannun, Y.A. An overview of sphingolipid metabolism: From synthesis to breakdown. *Adv. Exp. Med. Biol.* **2010**, *688*, 1–23.
4. Merrill, A.H., Jr.; Liotta, D.C.; Riley, R.T. Fumonisins: Fungal toxins that shed light on sphingolipid function. *Trends Cell Biol.* **1996**, *6*, 218–223. [CrossRef]
5. Jiang, Y.; DiVittore, N.A.; Young, M.M.; Jia, Z.; Xie, K.; Ritty, T.M.; Kester, M.; Fox, T.E. Altered sphingolipid metabolism in patients with metastatic pancreatic cancer. *Biomolecules* **2013**, *3*, 435–448. [CrossRef]
6. Faedo, R.R.; da Silva, G.; da Silva, R.M.; Ushida, T.R.; da Silva, R.R.; Lacchini, R.; Matos, L.L.; Kowalski, L.P.; Lopes, N.P.; Leopoldino, A.M. Sphingolipids signature in plasma and tissue as diagnostic and prognostic tools in oral squamous cell carcinoma. *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* **2022**, *1867*, 159057. [CrossRef]

7. Kumar, S.; Sharma, B.; Bhadwal, P.; Sharma, P.; Agnihotri, N. *Chapter 3—Lipids as nutraceuticals: A shift in paradigm In Therapeutic Foods*; Academic Press: Cambridge, MA, USA, 2018; p. 51. [CrossRef]

8. Gao, W.; Wang, X.; Zhou, Y.; Wang, X.; Yu, Y. Autophagy, ferroptosis, pyroptosis, and necroptosis in tumor immunotherapy. *Signal Transduct. Target. Ther.* **2022**, *7*, 196. [CrossRef] [PubMed]

9. Tong, X.; Tang, R.; Xiao, M.; Xu, J.; Wang, W.; Zhang, B.; Liu, J.; Yu, X.; Shi, S. Targeting cell death pathways for cancer therapy: Recent developments in necroptosis, pyroptosis, ferroptosis, and cuproptosis research. *J. Hematol. Oncol.* **2022**, *15*, 174. [CrossRef] [PubMed]

10. Sui, J.; He, M.; Wang, Y.; Zhao, X.; He, Y.; Shi, B. Sphingolipid metabolism in type 2 diabetes and associated cardiovascular complications. *Exp. Ther. Med.* **2019**, *18*, 3603–3614. [CrossRef]

11. Suzuki, Y.; Suzuki, M.; Ito, E.; Goto-Inoue, N.; Miseki, K.; Iida, J.; Yamazaki, Y.; Yamada, M.; Suzuki, A. Convenient structural analysis of glycosphingolipids using MALDI-QIT-TOF mass spectrometry with increased laser power and cooling gas flow. *J. Biochem.* **2006**, *139*, 771–777. [CrossRef]

12. Chaurasia, B.; Ying, L.; Talbot, C.L.; Maschek, J.A.; Cox, J.; Schuchman, E.H.; Hirabayashi, Y.; Holland, W.L.; Summers, S.A. Ceramides are necessary and sufficient for diet-induced impairment of thermogenic adipocytes. *Mol. Metab.* **2021**, *45*, 101145. [CrossRef] [PubMed]

13. Fenger, M.; Linneberg, A.; Jeppesen, J. Network-based analysis of the sphingolipid metabolism in hypertension. *Front. Genet.* **2015**, *6*, 84. [CrossRef] [PubMed]

14. Suh, J.H.; Saba, J.D. Sphingosine-1-phosphate in inflammatory bowel disease and colitis-associated colon cancer: The fat's in the fire. *Transl. Cancer Res.* **2015**, *4*, 469–483. [CrossRef]

15. De Wit, N.M.; Mol, K.; Rodriguez-Lorenzo, S.; de Vries, H.E.; Kooij, G. The Role of Sphingolipids and Specialized Pro-Resolving Mediators in Alzheimer's Disease. *Front. Immunol.* **2020**, *11*, 620348. [CrossRef]

16. Mingo-Casas, P.; Sanchez-Cespedes, J.; Blazquez, A.B.; Casas, J.; Balsera-Manzanero, M.; Herrero, L.; Vazquez, A.; Pachon, J.; Aguilar-Guisado, M.; Cisneros, J.M.; et al. Lipid signatures of West Nile virus infection unveil alterations of sphingolipid metabolism providing novel biomarkers. *Emerg. Microbes Infect.* **2023**, *12*, 2231556. [CrossRef] [PubMed]

17. Hua, F.; Wang, H.R.; Bai, Y.F.; Sun, J.P.; Wang, W.S.; Xu, Y.; Zhang, M.S.; Liu, J. Substance P promotes epidural fibrosis via induction of type 2 macrophages. *Neural Regen. Res.* **2023**, *18*, 2252–2259. [CrossRef]

18. Ejsing, C.S.; Moehring, T.; Bahr, U.; Duchoslav, E.; Karas, M.; Simons, K.; Shevchenko, A. Collision-induced dissociation pathways of yeast sphingolipids and their molecular profiling in total lipid extracts: A study by quadrupole TOF and linear ion trap-orbitrap mass spectrometry. *J. Mass. Spectrom.* **2006**, *41*, 372–389. [CrossRef]

19. Haynes, C.A.; Allegood, J.C.; Park, H.; Sullards, M.C. Sphingolipidomics: Methods for the comprehensive analysis of sphingolipids. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* **2009**, *877*, 2696–2708. [CrossRef]

20. Capolupo, L.; Khven, I.; Lederer, A.R.; Mazzeo, L.; Glousker, G.; Ho, S.; Russo, F.; Montoya, J.P.; Bhandari, D.R.; Bowman, A.P.; et al. Sphingolipids control dermal fibroblast heterogeneity. *Science* **2022**, *376*, eabh1623. [CrossRef]

21. Poisson, L.M.; Suhail, H.; Singh, J.; Datta, I.; Denic, A.; Labuzek, K.; Hoda, M.N.; Shankar, A.; Kumar, A.; Cerghet, M.; et al. Untargeted Plasma Metabolomics Identifies Endogenous Metabolite with Drug-like Properties in Chronic Animal Model of Multiple Sclerosis. *J. Biol. Chem.* **2015**, *290*, 30697–30712. [CrossRef]

22. Xu, J.; Zhang, Y.; Qi, D.; Huo, H.; Dong, X.; Tian, L.; Liu, C.; Cao, Y. Metabolomic and transcriptomic analyses highlight the influence of lipid changes on the post-harvest softening of Pyrus ussurian Max. 'Zaoshu Shanli'. *Genomics* **2021**, *113*, 919–926. [CrossRef] [PubMed]

23. Li, Y.; Ruan, Q.; Li, Y.; Ye, G.; Lu, X.; Lin, X.; Xu, G. A novel approach to transforming a non-targeted metabolic profiling method to a pseudo-targeted method using the retention time locking gas chromatography/mass spectrometry-selected ions monitoring. *J. Chromatogr. A* **2012**, *1255*, 228–236. [CrossRef]

24. Zahoor, I.; Suhail, H.; Datta, I.; Ahmed, M.E.; Poisson, L.M.; Waters, J.; Rashid, F.; Bin, R.; Singh, J.; Cerghet, M.; et al. Blood-based untargeted metabolomics in relapsing-remitting multiple sclerosis revealed the testable therapeutic target. *Proc. Natl. Acad. Sci. USA* **2022**, *119*, e2123265119. [CrossRef]

25. Petit, C.S.; Lee, J.J.; Boland, S.; Swarup, S.; Christiano, R.; Lai, Z.W.; Mejhert, N.; Elliott, S.D.; McFall, D.; Haque, S.; et al. Inhibition of sphingolipid synthesis improves outcomes and survival in GARP mutant wobbler mice, a model of motor neuron degeneration. *Proc. Natl. Acad. Sci. USA* **2020**, *117*, 10565–10574. [CrossRef]

26. Chen, S.; Kong, H.; Lu, X.; Li, Y.; Yin, P.; Zeng, Z.; Xu, G. Pseudotargeted metabolomics method and its application in serum biomarker discovery for hepatocellular carcinoma based on ultra high-performance liquid chromatography/triple quadrupole mass spectrometry. *Anal. Chem.* **2013**, *85*, 8326–8333. [CrossRef]

27. Lv, W.; Wang, L.; Xuan, Q.; Zhao, X.; Liu, X.; Shi, X.; Xu, G. Pseudotargeted Method Based on Parallel Column Two-Dimensional Liquid Chromatography-Mass Spectrometry for Broad Coverage of Metabolome and Lipidome. *Anal. Chem.* **2020**, *92*, 6043–6050. [CrossRef]

28. Vargas Medina, D.A.; Burato, J.; Borsatto, J.V.B.; Lancas, F.M. Porous layer open tubular nano liquid chromatography directly coupled to electron ionization mass spectrometry. *J. Chromatogr. A* **2022**, *1674*, 463143. [CrossRef] [PubMed]

29. Hammarstrom, S.; Samuelsson, B. On the biosynthesis of cerebrosides from 2-hydroxy acid ceramides: Use of deuterium labeled substrate and multiple ion detector. *Biochem. Biophys. Res. Commun.* **1970**, *41*, 1027–1035. [CrossRef] [PubMed]

30. Samuelsson, K.; Sameulsson, B. Gas chromatographic and mass spectrometric studies of synthetic and naturally occurring ceramides. *Chem. Phys. Lipids* **1970**, *5*, 44–79. [CrossRef]

31. Samuelsson, B.; Samuelsson, K. Gas--liquid chromatography-mass spectrometry of synthetic ceramides. *J. Lipid Res.* **1969**, *10*, 41–46. [CrossRef]

32. Tsugawa, H.; Ikeda, K.; Tanaka, W.; Senoo, Y.; Arita, M.; Arita, M. Comprehensive identification of sphingolipid species by in silico retention time and tandem mass spectral library. *J. Cheminform.* **2017**, *9*, 19. [CrossRef] [PubMed]

33. Verkhoturov, D.S.; Crulhas, B.P.; Eller, M.J.; Han, Y.D.; Verkhoturov, S.V.; Bisrat, Y.; Revzin, A.; Schweikert, E.A. Nanoprojectile Secondary Ion Mass Spectrometry for Analysis of Extracellular Vesicles. *Anal. Chem.* **2021**, *93*, 7481–7490. [CrossRef] [PubMed]

34. Aldossari, S.; McMahon, G.; Lockyer, N.P.; Moore, K.L. Microdistribution and quantification of the boron neutron capture therapy drug BPA in primary cell cultures of human glioblastoma tumour by NanoSIMS. *Analyst* **2019**, *144*, 6214–6224. [CrossRef] [PubMed]

35. Wilson, I.D. The state of the art in thin-layer chromatography-mass spectrometry: A critical appraisal. *J. Chromatogr. A* **1999**, *856*, 429–442. [CrossRef]

36. Cahoon, R.E.; Solis, A.G.; Markham, J.E.; Cahoon, E.B. Mass Spectrometry-Based Profiling of Plant Sphingolipids from Typical and Aberrant Metabolism. *Methods Mol. Biol.* **2021**, *2295*, 157–177. [CrossRef]

37. Yang, Y.; Fu, H.; Qian, C.; Li, H.; Chen, D.D.Y. Characterization of interaction between Bcl-2 oncogene promoter I-Motif DNA and flavonoids using electrospray ionization mass spectrometry and pressure-assisted capillary electrophoresis frontal analysis. *Talanta* **2020**, *215*, 120885. [CrossRef]

38. Han, X.; Yang, J.; Cheng, H.; Ye, H.; Gross, R.W. Toward fingerprinting cellular lipidomes directly from biological samples by two-dimensional electrospray ionization mass spectrometry. *Anal. Biochem.* **2004**, *330*, 317–331. [CrossRef]

39. Han, X.; Gross, R.W. Global analyses of cellular lipidomes directly from crude extracts of biological samples by ESI mass spectrometry: A bridge to lipidomics. *J. Lipid Res.* **2003**, *44*, 1071–1079. [CrossRef]

40. Sullards, M.C. Analysis of sphingomyelin, glucosylceramide, ceramide, sphingosine, and sphingosine 1-phosphate by tandem mass spectrometry. *Methods Enzymol.* **2000**, *312*, 32–45. [CrossRef]

41. Sullards, M.C.; Merrill, A.H., Jr. Analysis of sphingosine 1-phosphate, ceramides, and other bioactive sphingolipids by high-performance liquid chromatography-tandem mass spectrometry. *Sci. STKE* **2001**, *2001*, pl1. [CrossRef]

42. Sakai, E.; Kurano, M.; Morita, Y.; Aoki, J.; Yatomi, Y. Establishment of a Measurement System for Sphingolipids in the Cerebrospinal Fluid Based on Liquid Chromatography-Tandem Mass Spectrometry, and Its Application in the Diagnosis of Carcinomatous Meningitis. *J. Appl. Lab. Med.* **2020**, *5*, 656–670. [CrossRef]

43. Chao, Y.; Chen, X.; Shi, X.; Li, N.; Gao, S.; Yang, J.; Dong, X. Quantification of alpha-hydroxy ceramides in mice serum by LC-MS/MS: Application to sepsis study. *J. Chromatogr. B Analyt Technol. Biomed. Life Sci.* **2023**, *1225*, 123764. [CrossRef] [PubMed]

44. Ge, K.; Zheng, D.; Wang, J.; Jia, W.; Zhao, A. A method for quantifying hepatic and intestinal ceramides on mice by UPLC-MS/MS. *Anal. Biochem.* **2023**, *661*, 114982. [CrossRef] [PubMed]

45. Pacetti, D.; Boselli, E.; Hulan, H.W.; Frega, N.G. High performance liquid chromatography-tandem mass spectrometry of phospholipid molecular species in eggs from hens fed diets enriched in seal blubber oil. *J. Chromatogr. A* **2005**, *1097*, 66–73. [CrossRef] [PubMed]

46. Kaga, N.; Kazuno, S.; Taka, H.; Iwabuchi, K.; Murayama, K. Isolation and mass spectrometry characterization of molecular species of lactosylceramides using liquid chromatography-electrospray ion trap mass spectrometry. *Anal. Biochem.* **2005**, *337*, 316–324. [CrossRef]

47. Lee, M.H.; Lee, G.H.; Yoo, J.S. Analysis of ceramides in cosmetics by reversed-phase liquid chromatography/electrospray ionization mass spectrometry with collision-induced dissociation. *Rapid Commun. Mass. Spectrom.* **2003**, *17*, 64–75. [CrossRef]

48. Zanfini, A.; Dreassi, E.; Berardi, A.; Piomboni, P.; Costantino-Ceccarini, E.; Luddi, A. GC-EI-MS analysis of fatty acid composition in brain and serum of twitcher mouse. *Lipids* **2014**, *49*, 1115–1125. [CrossRef]

49. Son, H.H.; Moon, J.Y.; Seo, H.S.; Kim, H.H.; Chung, B.C.; Choi, M.H. High-temperature GC-MS-based serum cholesterol signatures may reveal sex differences in vasospastic angina. *J. Lipid Res.* **2014**, *55*, 155–162. [CrossRef] [PubMed]

50. Marshall, A.G.; Hendrickson, C.L. High-resolution mass spectrometers. *Annu. Rev. Anal. Chem.* **2008**, *1*, 579–599. [CrossRef]

51. McFarland, M.A.; Marshall, A.G.; Hendrickson, C.L.; Nilsson, C.L.; Fredman, P.; Mansson, J.E. Structural characterization of the GM1 ganglioside by infrared multiphoton dissociation, electron capture dissociation, and electron detachment dissociation electrospray ionization FT-ICR MS/MS. *J. Am. Soc. Mass. Spectrom.* **2005**, *16*, 752–762. [CrossRef]

52. Jones, J.J.; Stump, M.J.; Fleming, R.C.; Lay, J.O., Jr.; Wilkins, C.L. Strategies and data analysis techniques for lipid and phospholipid chemistry elucidation by intact cell MALDI-FTMS. *J. Am. Soc. Mass. Spectrom.* **2004**, *15*, 1665–1674. [CrossRef] [PubMed]

53. Tran, A.; Wan, L.; Xu, Z.; Haro, J.M.; Li, B.; Jones, J.W. Lithium Hydroxide Hydrolysis Combined with MALDI TOF Mass Spectrometry for Rapid Sphingolipid Detection. *J. Am. Soc. Mass. Spectrom.* **2021**, *32*, 289–300. [CrossRef]

54. Zhao, C.; Xie, P.; Yong, T.; Wang, H.; Chung, A.C.K.; Cai, Z. MALDI-MS Imaging Reveals Asymmetric Spatial Distribution of Lipid Metabolites from Bisphenol S-Induced Nephrotoxicity. *Anal. Chem.* **2018**, *90*, 3196–3204. [CrossRef] [PubMed]

55. Bowman, A.P.; Bogie, J.F.J.; Hendriks, J.J.A.; Haidar, M.; Belov, M.; Heeren, R.M.A.; Ellis, S.R. Evaluation of lipid coverage and high spatial resolution MALDI-imaging capabilities of oversampling combined with laser post-ionisation. *Anal. Bioanal. Chem.* **2020**, *412*, 2277–2289. [CrossRef]

56. Haynes, C.A.; Allegood, J.C.; Sims, K.; Wang, E.W.; Sullards, M.C.; Merrill, A.H., Jr. Quantitation of fatty acyl-coenzyme As in mammalian cells by liquid chromatography-electrospray ionization tandem mass spectrometry. *J. Lipid Res.* **2008**, *49*, 1113–1125. [CrossRef] [PubMed]

57. Ramstedt, B.; Slotte, J.P. Separation and purification of sphingomyelin diastereomers by high-performance liquid chromatography. *Anal. Biochem.* **2000**, *282*, 245–249. [CrossRef]

58. Muthing, J.; Unland, F. Improved separation of isomeric gangliosides by anion-exchange high-performance liquid chromatography. *J. Chromatogr. B Biomed. Appl.* **1994**, *658*, 39–45. [CrossRef]

59. Hacker, G. The morphology of apoptosis. *Cell Tissue Res.* **2000**, *301*, 5–17. [CrossRef]

60. Saraste, A.; Pulkki, K. Morphologic and biochemical hallmarks of apoptosis. *Cardiovasc. Res.* **2000**, *45*, 528–537. [CrossRef]

61. Fuchs, Y.; Steller, H. Programmed cell death in animal development and disease. *Cell* **2011**, *147*, 742–758. [CrossRef]

62. Hengartner, M.O. Apoptosis: Corralling the corpses. *Cell* **2001**, *104*, 325–328. [CrossRef] [PubMed]

63. Ashkenazi, A.; Dixit, V.M. Death receptors: Signaling and modulation. *Science* **1998**, *281*, 1305–1308. [CrossRef] [PubMed]

64. Bollinger, C.R.; Teichgraber, V.; Gulbins, E. Ceramide-enriched membrane domains. *Biochim. Biophys. Acta* **2005**, *1746*, 284–294. [CrossRef] [PubMed]

65. Grassme, H.; Cremesti, A.; Kolesnick, R.; Gulbins, E. Ceramide-mediated clustering is required for CD95-DISC formation. *Oncogene* **2003**, *22*, 5457–5470. [CrossRef] [PubMed]

66. Grassme, H.; Jekle, A.; Riehle, A.; Schwarz, H.; Berger, J.; Sandhoff, K.; Kolesnick, R.; Gulbins, E. CD95 signaling via ceramide-rich membrane rafts. *J. Biol. Chem.* **2001**, *276*, 20589–20596. [CrossRef] [PubMed]

67. Gulbins, E.; Kolesnick, R. Raft ceramide in molecular medicine. *Oncogene* **2003**, *22*, 7070–7077. [CrossRef]

68. Schutze, S.; Potthoff, K.; Machleidt, T.; Berkovic, D.; Wiegmann, K.; Kronke, M. TNF activates NF-kappa B by phosphatidylcholine-specific phospholipase C-induced "acidic" sphingomyelin breakdown. *Cell* **1992**, *71*, 765–776. [CrossRef]

69. Wiegmann, K.; Schutze, S.; Machleidt, T.; Witte, D.; Kronke, M. Functional dichotomy of neutral and acidic sphingomyelinases in tumor necrosis factor signaling. *Cell* **1994**, *78*, 1005–1015. [CrossRef]

70. Liu, P.; Anderson, R.G. Compartmentalized production of ceramide at the cell surface. *J. Biol. Chem.* **1995**, *270*, 27179–27185. [CrossRef]

71. Cifone, M.G.; De Maria, R.; Roncaioli, P.; Rippo, M.R.; Azuma, M.; Lanier, L.L.; Santoni, A.; Testi, R. Apoptotic signaling through CD95 (Fas/Apo-1) activates an acidic sphingomyelinase. *J. Exp. Med.* **1994**, *180*, 1547–1552. [CrossRef]

72. Patwardhan, G.A.; Beverly, L.J.; Siskind, L.J. Sphingolipids and mitochondrial apoptosis. *J. Bioenerg. Biomembr.* **2016**, *48*, 153–168. [CrossRef] [PubMed]

73. Chipuk, J.E.; Moldoveanu, T.; Llambi, F.; Parsons, M.J.; Green, D.R. The BCL-2 family reunion. *Mol. Cell* **2010**, *37*, 299–310. [CrossRef] [PubMed]

74. Bigi, A.; Morosi, L.; Pozzi, C.; Forcella, M.; Tettamanti, G.; Venerando, B.; Monti, E.; Fusi, P. Human sialidase NEU4 long and short are extrinsic proteins bound to outer mitochondrial membrane and the endoplasmic reticulum, respectively. *Glycobiology* **2010**, *20*, 148–157. [CrossRef] [PubMed]

75. Bionda, C.; Portoukalian, J.; Schmitt, D.; Rodriguez-Lafrasse, C.; Ardail, D. Subcellular compartmentalization of ceramide metabolism: MAM (mitochondria-associated membrane) and/or mitochondria? *Biochem. J.* **2004**, *382*, 527–533. [CrossRef] [PubMed]

76. Yamaguchi, K.; Hata, K.; Koseki, K.; Shiozaki, K.; Akita, H.; Wada, T.; Moriya, S.; Miyagi, T. Evidence for mitochondrial localization of a novel human sialidase (NEU4). *Biochem. J.* **2005**, *390*, 85–93. [CrossRef] [PubMed]

77. Thomas, R.L., Jr.; Matsko, C.M.; Lotze, M.T.; Amoscato, A.A. Mass spectrometric identification of increased C16 ceramide levels during apoptosis. *J. Biol. Chem.* **1999**, *274*, 30580–30588. [CrossRef]

78. Kroesen, B.J.; Pettus, B.; Luberto, C.; Busman, M.; Sietsma, H.; de Leij, L.; Hannun, Y.A. Induction of apoptosis through B-cell receptor cross-linking occurs via de novo generated C16-ceramide and involves mitochondria. *J. Biol. Chem.* **2001**, *276*, 13606–13614. [CrossRef]

79. Rodriguez-Lafrasse, C.; Alphonse, G.; Broquet, P.; Aloy, M.T.; Louisot, P.; Rousson, R. Temporal relationships between ceramide production, caspase activation and mitochondrial dysfunction in cell lines with varying sensitivity to anti-Fas-induced apoptosis. *Biochem. J.* **2001**, *357*, 407–416. [CrossRef]

80. Garcia-Ruiz, C.; Colell, A.; Mari, M.; Morales, A.; Fernandez-Checa, J.C. Direct effect of ceramide on the mitochondrial electron transport chain leads to generation of reactive oxygen species. Role of mitochondrial glutathione. *J. Biol. Chem.* **1997**, *272*, 11369–11377. [CrossRef]

81. Geilen, C.C.; Bektas, M.; Wieder, T.; Kodelja, V.; Goerdt, S.; Orfanos, C.E. 1alpha,25-dihydroxyvitamin D3 induces sphingomyelin hydrolysis in HaCaT cells via tumor necrosis factor alpha. *J. Biol. Chem.* **1997**, *272*, 8997–9001. [CrossRef]

82. Liu, Y.Y.; Yu, J.Y.; Yin, D.; Patwardhan, G.A.; Gupta, V.; Hirabayashi, Y.; Holleran, W.M.; Giuliano, A.E.; Jazwinski, S.M.; Gouaze-Andersson, V.; et al. A role for ceramide in driving cancer cell resistance to doxorubicin. *FASEB J.* **2008**, *22*, 2541–2551. [CrossRef] [PubMed]

83. Shida, D.; Takabe, K.; Kapitonov, D.; Milstien, S.; Spiegel, S. Targeting SphK1 as a new strategy against cancer. *Curr. Drug Targets* **2008**, *9*, 662–673. [CrossRef] [PubMed]

84. Stevenson, C.E.; Takabe, K.; Nagahashi, M.; Milstien, S.; Spiegel, S. Targeting sphingosine-1-phosphate in hematologic malignancies. *Anticancer. Agents Med. Chem.* **2011**, *11*, 794–798. [CrossRef]

85.   Takabe, K.; Spiegel, S. Export of sphingosine-1-phosphate and cancer progression. *J. Lipid Res.* **2014**, *55*, 1839–1846. [CrossRef]
86.   Chipuk, J.E.; McStay, G.P.; Bharti, A.; Kuwana, T.; Clarke, C.J.; Siskind, L.J.; Obeid, L.M.; Green, D.R. Sphingolipid metabolism cooperates with BAK and BAX to promote the mitochondrial pathway of apoptosis. *Cell* **2012**, *148*, 988–1000. [CrossRef]
87.   Wang, P.; Yuan, Y.; Lin, W.; Zhong, H.; Xu, K.; Qi, X. Roles of sphingosine-1-phosphate signaling in cancer. *Cancer Cell Int.* **2019**, *19*, 295. [CrossRef] [PubMed]
88.   Ogretmen, B. Sphingolipid metabolism in cancer signalling and therapy. *Nat. Rev. Cancer* **2018**, *18*, 33–50. [CrossRef]
89.   Senkal, C.E.; Ponnusamy, S.; Manevich, Y.; Meyers-Needham, M.; Saddoughi, S.A.; Mukhopadyay, A.; Dent, P.; Bielawski, J.; Ogretmen, B. Alteration of ceramide synthase 6/C16-ceramide induces activating transcription factor 6-mediated endoplasmic reticulum (ER) stress and apoptosis via perturbation of cellular Ca2+ and ER/Golgi membrane network. *J. Biol. Chem.* **2011**, *286*, 42446–42458. [CrossRef] [PubMed]
90.   Mesicek, J.; Lee, H.; Feldman, T.; Jiang, X.; Skobeleva, A.; Berdyshev, E.V.; Haimovitz-Friedman, A.; Fuks, Z.; Kolesnick, R. Ceramide synthases 2, 5, and 6 confer distinct roles in radiation-induced apoptosis in HeLa cells. *Cell Signal.* **2010**, *22*, 1300–1307. [CrossRef] [PubMed]
91.   Spiess, C.; Meyer, A.S.; Reissmann, S.; Frydman, J. Mechanism of the eukaryotic chaperonin: Protein folding in the chamber of secrets. *Trends Cell Biol.* **2004**, *14*, 598–604. [CrossRef]
92.   He, C.; Klionsky, D.J. Regulation mechanisms and signaling pathways of autophagy. *Annu. Rev. Genet.* **2009**, *43*, 67–93. [CrossRef] [PubMed]
93.   Mizushima, N.; Levine, B.; Cuervo, A.M.; Klionsky, D.J. Autophagy fights disease through cellular self-digestion. *Nature* **2008**, *451*, 1069–1075. [CrossRef] [PubMed]
94.   Xie, Z.; Klionsky, D.J. Autophagosome formation: Core machinery and adaptations. *Nat. Cell Biol.* **2007**, *9*, 1102–1109. [CrossRef] [PubMed]
95.   Levy, J.M.M.; Towers, C.G.; Thorburn, A. Targeting autophagy in cancer. *Nat. Rev. Cancer* **2017**, *17*, 528–542. [CrossRef]
96.   Jung, C.H.; Jun, C.B.; Ro, S.H.; Kim, Y.M.; Otto, N.M.; Cao, J.; Kundu, M.; Kim, D.H. ULK-Atg13-FIP200 complexes mediate mTOR signaling to the autophagy machinery. *Mol. Biol. Cell* **2009**, *20*, 1992–2003. [CrossRef]
97.   Mizushima, N. The role of the Atg1/ULK1 complex in autophagy regulation. *Curr. Opin. Cell Biol.* **2010**, *22*, 132–139. [CrossRef]
98.   Russell, R.C.; Tian, Y.; Yuan, H.; Park, H.W.; Chang, Y.Y.; Kim, J.; Kim, H.; Neufeld, T.P.; Dillin, A.; Guan, K.L. ULK1 induces autophagy by phosphorylating Beclin-1 and activating VPS34 lipid kinase. *Nat. Cell Biol.* **2013**, *15*, 741–750. [CrossRef]
99.   Obara, K.; Ohsumi, Y. PtdIns 3-Kinase Orchestrates Autophagosome Formation in Yeast. *J. Lipids* **2011**, *2011*, 498768. [CrossRef]
100. Pattingre, S.; Tassa, A.; Qu, X.; Garuti, R.; Liang, X.H.; Mizushima, N.; Packer, M.; Schneider, M.D.; Levine, B. Bcl-2 antiapoptotic proteins inhibit Beclin 1-dependent autophagy. *Cell* **2005**, *122*, 927–939. [CrossRef]
101. Pattingre, S.; Espert, L.; Biard-Piechaczyk, M.; Codogno, P. Regulation of macroautophagy by mTOR and Beclin 1 complexes. *Biochimie* **2008**, *90*, 313–323. [CrossRef]
102. Guenther, G.G.; Peralta, E.R.; Rosales, K.R.; Wong, S.Y.; Siskind, L.J.; Edinger, A.L. Ceramide starves cells to death by downregulating nutrient transporter proteins. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 17402–17407. [CrossRef]
103. Romero Rosales, K.; Singh, G.; Wu, K.; Chen, J.; Janes, M.R.; Lilly, M.B.; Peralta, E.R.; Siskind, L.J.; Bennett, M.J.; Fruman, D.A.; et al. Sphingolipid-based drugs selectively kill cancer cells by down-regulating nutrient transporter proteins. *Biochem. J.* **2011**, *439*, 299–311. [CrossRef]
104. Peralta, E.R.; Edinger, A.L. Ceramide-induced starvation triggers homeostatic autophagy. *Autophagy* **2009**, *5*, 407–409. [CrossRef] [PubMed]
105. Edinger, A.L. Starvation in the midst of plenty: Making sense of ceramide-induced autophagy by analysing nutrient transporter expression. *Biochem. Soc. Trans.* **2009**, *37*, 253–258. [CrossRef] [PubMed]
106. Gomes, L.C.; Scorrano, L. Mitochondrial morphology in mitophagy and macroautophagy. *Biochim. Biophys. Acta* **2013**, *1833*, 205–212. [CrossRef] [PubMed]
107. 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. [CrossRef]
108. Sentelle, R.D.; Senkal, C.E.; Jiang, W.; Ponnusamy, S.; Gencer, S.; Selvam, S.P.; Ramshesh, V.K.; Peterson, Y.K.; Lemasters, J.J.; Szulc, Z.M.; et al. Ceramide targets autophagosomes to mitochondria and induces lethal mitophagy. *Nat. Chem. Biol.* **2012**, *8*, 831–838. [CrossRef]
109. Russo, S.B.; Baicu, C.F.; Van Laer, A.; Geng, T.; Kasiganesan, H.; Zile, M.R.; Cowart, L.A. Ceramide synthase 5 mediates lipid-induced autophagy and hypertrophy in cardiomyocytes. *J. Clin. Investig.* **2012**, *122*, 3919–3930. [CrossRef]
110. Hinkovska-Galcheva, V.T.; Boxer, L.A.; Mansfield, P.J.; Harsh, D.; Blackwood, A.; Shayman, J.A. The formation of ceramide-1-phosphate during neutrophil phagocytosis and its role in liposome fusion. *J. Biol. Chem.* **1998**, *273*, 33203–33209. [CrossRef]
111. Chen, J.; Kos, R.; Garssen, J.; Redegeld, F. Molecular Insights into the Mechanism of Necroptosis: The Necrosome As a Potential Therapeutic Target. *Cells* **2019**, *8*, 1486. [CrossRef]
112. Dondelinger, Y.; Declercq, W.; Montessuit, S.; Roelandt, R.; Goncalves, A.; Bruggeman, I.; Hulpiau, P.; Weber, K.; Sehon, C.A.; Marquis, R.W.; et al. MLKL compromises plasma membrane integrity by binding to phosphatidylinositol phosphates. *Cell Rep.* **2014**, *7*, 971–981. [CrossRef]

113. Quarato, G.; Guy, C.S.; Grace, C.R.; Llambi, F.; Nourse, A.; Rodriguez, D.A.; Wakefield, R.; Frase, S.; Moldoveanu, T.; Green, D.R. Sequential Engagement of Distinct MLKL Phosphatidylinositol-Binding Sites Executes Necroptosis. *Mol. Cell* **2016**, *61*, 589–601. [CrossRef] [PubMed]

114. Zhang, X.; Matsuda, M.; Yaegashi, N.; Nabe, T.; Kitatani, K. Regulation of Necroptosis by Phospholipids and Sphingolipids. *Cells* **2020**, *9*, 627. [CrossRef]

115. Zhang, X.; Kitatani, K.; Toyoshima, M.; Ishibashi, M.; Usui, T.; Minato, J.; Egiz, M.; Shigeta, S.; Fox, T.; Deering, T.; et al. Ceramide Nanoliposomes as a MLKL-Dependent, Necroptosis-Inducing, Chemotherapeutic Reagent in Ovarian Cancer. *Mol. Cancer Ther.* **2018**, *17*, 50–59. [CrossRef] [PubMed]

116. Nganga, R.; Oleinik, N.; Kim, J.; Selvam, S.P.; De Palma, R.; Johnson, K.A.; Parikh, R.Y.; Gangaraju, V.; Peterson, Y.; Dany, M.; et al. Receptor-interacting Ser/Thr kinase 1 (RIPK1) and myosin IIA-dependent ceramidosomes form membrane pores that mediate blebbing and necroptosis. *J. Biol. Chem.* **2019**, *294*, 502–519. [CrossRef]

117. Rathinam, V.A.; Vanaja, S.K.; Fitzgerald, K.A. Regulation of inflammasome signaling. *Nat. Immunol.* **2012**, *13*, 333–342. [CrossRef] [PubMed]

118. Wang, X.; Yang, Y.; Cai, W.Q.; Lu, Y. The Relationship of Sphingosine Kinase 1 With Pyroptosis Provides a New Strategy for Tumor Therapy. *Front. Immunol.* **2020**, *11*, 574990. [CrossRef]

119. Taha, T.A.; El-Alwani, M.; Hannun, Y.A.; Obeid, L.M. Sphingosine kinase-1 is cleaved by cathepsin B in vitro: Identification of the initial cleavage sites for the protease. *FEBS Lett.* **2006**, *580*, 6047–6054. [CrossRef] [PubMed]

120. Stockwell, B.R.; Friedmann Angeli, J.P.; Bayir, H.; Bush, A.I.; Conrad, M.; Dixon, S.J.; Fulda, S.; Gascon, S.; Hatzios, S.K.; Kagan, V.E.; et al. Ferroptosis: A Regulated Cell Death Nexus Linking Metabolism, Redox Biology, and Disease. *Cell* **2017**, *171*, 273–285. [CrossRef]

121. Yang, W.S.; SriRamaratnam, R.; Welsch, M.E.; Shimada, K.; Skouta, R.; Viswanathan, V.S.; Cheah, J.H.; Clemons, P.A.; Shamji, A.F.; Clish, C.B.; et al. Regulation of ferroptotic cancer cell death by GPX4. *Cell* **2014**, *156*, 317–331. [CrossRef]

122. Lei, P.; Bai, T.; Sun, Y. Mechanisms of Ferroptosis and Relations With Regulated Cell Death: A Review. *Front. Physiol.* **2019**, *10*, 139. [CrossRef]

123. Galadari, S.; Rahman, A.; Pallichankandy, S.; Thayyullathil, F. Tumor suppressive functions of ceramide: Evidence and mechanisms. *Apoptosis* **2015**, *20*, 689–711. [CrossRef] [PubMed]

124. Thayyullathil, F.; Cheratta, A.R.; Alakkal, A.; Subburayan, K.; Pallichankandy, S.; Hannun, Y.A.; Galadari, S. Acid sphingomyelinase-dependent autophagic degradation of GPX4 is critical for the execution of ferroptosis. *Cell Death Dis.* **2021**, *12*, 26. [CrossRef] [PubMed]

125. Qiu, H.; Edmunds, T.; Baker-Malcolm, J.; Karey, K.P.; Estes, S.; Schwarz, C.; Hughes, H.; Van Patten, S.M. Activation of human acid sphingomyelinase through modification or deletion of C-terminal cysteine. *J. Biol. Chem.* **2003**, *278*, 32744–32752. [CrossRef]

126. Wu, Z.; Geng, Y.; Lu, X.; Shi, Y.; Wu, G.; Zhang, M.; Shan, B.; Pan, H.; Yuan, J. Chaperone-mediated autophagy is involved in the execution of ferroptosis. *Proc. Natl. Acad. Sci. USA* **2019**, *116*, 2996–3005. [CrossRef] [PubMed]

127. Gao, M.; Monian, P.; Pan, Q.; Zhang, W.; Xiang, J.; Jiang, X. Ferroptosis is an autophagic cell death process. *Cell Res.* **2016**, *26*, 1021–1032. [CrossRef]

128. Tsvetkov, P.; Coy, S.; Petrova, B.; Dreishpoon, M.; Verma, A.; Abdusamad, M.; Rossen, J.; Joesch-Cohen, L.; Humeidi, R.; Spangler, R.D.; et al. Copper induces cell death by targeting lipoylated TCA cycle proteins. *Science* **2022**, *375*, 1254–1261. [CrossRef]

129. Gaetke, L.M.; Chow-Johnson, H.S.; Chow, C.K. Copper: Toxicological relevance and mechanisms. *Arch. Toxicol.* **2014**, *88*, 1929–1938. [CrossRef]

130. Rana, S.V. Metals and apoptosis: Recent developments. *J. Trace Elem. Med. Biol.* **2008**, *22*, 262–284. [CrossRef]

131. Lang, F.; Ullrich, S.; Gulbins, E. Ceramide formation as a target in beta-cell survival and function. *Expert. Opin. Ther. Targets* **2011**, *15*, 1061–1071. [CrossRef]

132. Triebl, A.; Weissengruber, S.; Trotzmuller, M.; Lankmayr, E.; Kofeler, H. Quantitative analysis of N-acylphosphatidylethanolamine molecular species in rat brain using solid-phase extraction combined with reversed-phase chromatography and tandem mass spectrometry. *J. Sep. Sci.* **2016**, *39*, 2474–2480. [CrossRef] [PubMed]

133. Kataria, H.; Alizadeh, A.; Karimi-Abdolrezaee, S. Neuregulin-1/ErbB network: An emerging modulator of nervous system injury and repair. *Prog. Neurobiol.* **2019**, *180*, 101643. [CrossRef]

134. Ivanova, P.T.; Cerda, B.A.; Horn, D.M.; Cohen, J.S.; McLafferty, F.W.; Brown, H.A. Electrospray ionization mass spectrometry analysis of changes in phospholipids in RBL-2H3 mastocytoma cells during degranulation. *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 7152–7157. [CrossRef]

135. Schuhmann, K.; Almeida, R.; Baumert, M.; Herzog, R.; Bornstein, S.R.; Shevchenko, A. Shotgun lipidomics on a LTQ Orbitrap mass spectrometer by successive switching between acquisition polarity modes. *J. Mass. Spectrom.* **2012**, *47*, 96–104. [CrossRef]

136. Yang, K.; Dilthey, B.G.; Gross, R.W. Identification and quantitation of fatty acid double bond positional isomers: A shotgun lipidomics approach using charge-switch derivatization. *Anal. Chem.* **2013**, *85*, 9742–9750. [CrossRef] [PubMed]

137. Abdul Rashid, K.; Ibrahim, K.; Wong, J.H.D.; Mohd Ramli, N. Lipid Alterations in Glioma: A Systematic Review. *Metabolites* **2022**, *12*, 1280. [CrossRef]

138. Rosen, H.; Stevens, R.C.; Hanson, M.; Roberts, E.; Oldstone, M.B. Sphingosine-1-phosphate and its receptors: Structure, signaling, and influence. *Annu. Rev. Biochem.* **2013**, *82*, 637–662. [CrossRef] [PubMed]

139. Kilaru, A.; Isaac, G.; Tamura, P.; Baxter, D.; Duncan, S.R.; Venables, B.J.; Welti, R.; Koulen, P.; Chapman, K.D. Lipid profiling reveals tissue-specific differences for ethanolamide lipids in mice lacking fatty acid amide hydrolase. *Lipids* **2010**, *45*, 863–875. [CrossRef]

140. Obeid, L.M.; Linardic, C.M.; Karolak, L.A.; Hannun, Y.A. Programmed cell death induced by ceramide. *Science* **1993**, *259*, 1769–1771. [CrossRef]

141. Stiban, J.; Fistere, D.; Colombini, M. Dihydroceramide hinders ceramide channel formation: Implications on apoptosis. *Apoptosis* **2006**, *11*, 773–780. [CrossRef]

142. Siddique, M.M.; Li, Y.; Wang, L.; Ching, J.; Mal, M.; Ilkayeva, O.; Wu, Y.J.; Bay, B.H.; Summers, S.A. Ablation of dihydroceramide desaturase 1, a therapeutic target for the treatment of metabolic diseases, simultaneously stimulates anabolic and catabolic signaling. *Mol. Cell Biol.* **2013**, *33*, 2353–2369. [CrossRef]

143. Lepine, S.; Allegood, J.C.; Park, M.; Dent, P.; Milstien, S.; Spiegel, S. Sphingosine-1-phosphate phosphohydrolase-1 regulates ER stress-induced autophagy. *Cell Death Differ.* **2011**, *18*, 350–361. [CrossRef]

144. Han, G.; Gupta, S.D.; Gable, K.; Niranjanakumari, S.; Moitra, P.; Eichler, F.; Brown, R.H., Jr.; Harmon, J.M.; Dunn, T.M. Identification of small subunits of mammalian serine palmitoyltransferase that confer distinct acyl-CoA substrate specificities. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 8186–8191. [CrossRef] [PubMed]

145. Bode, H.; Bourquin, F.; Suriyanarayanan, S.; Wei, Y.; Alecu, I.; Othman, A.; Von Eckardstein, A.; Hornemann, T. HSAN1 mutations in serine palmitoyltransferase reveal a close structure-function-phenotype relationship. *Hum. Mol. Genet.* **2016**, *25*, 853–865. [CrossRef] [PubMed]

146. Kramer, R.; Bielawski, J.; Kistner-Griffin, E.; Othman, A.; Alecu, I.; Ernst, D.; Kornhauser, D.; Hornemann, T.; Spassieva, S. Neurotoxic 1-deoxysphingolipids and paclitaxel-induced peripheral neuropathy. *FASEB J.* **2015**, *29*, 4461–4472. [CrossRef] [PubMed]

147. Meyers-Needham, M.; Ponnusamy, S.; Gencer, S.; Jiang, W.; Thomas, R.J.; Senkal, C.E.; Ogretmen, B. Concerted functions of HDAC1 and microRNA-574-5p repress alternatively spliced ceramide synthase 1 expression in human cancer cells. *EMBO Mol. Med.* **2012**, *4*, 78–92. [CrossRef]

148. 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.; 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. [CrossRef]

149. Thomas, R.J.; Oleinik, N.; Panneer Selvam, S.; Vaena, S.G.; Dany, M.; Nganga, R.N.; Depalma, R.; Baron, K.D.; Kim, J.; Szulc, Z.M.; et al. HPV/E7 induces chemotherapy-mediated tumor suppression by ceramide-dependent mitophagy. *EMBO Mol. Med.* **2017**, *9*, 1030–1051. [CrossRef]

150. Fekry, B.; Jeffries, K.A.; Esmaeilniakooshkghazi, A.; Ogretmen, B.; Krupenko, S.A.; Krupenko, N.I. CerS6 Is a Novel Transcriptional Target of p53 Protein Activated by Non-genotoxic Stress. *J. Biol. Chem.* **2016**, *291*, 16586–16596. [CrossRef]

151. White-Gilbertson, S.; Mullen, T.; Senkal, C.; Lu, P.; Ogretmen, B.; Obeid, L.; Voelkel-Johnson, C. Ceramide synthase 6 modulates TRAIL sensitivity and nuclear translocation of active caspase-3 in colon cancer cells. *Oncogene* **2009**, *28*, 1132–1141. [CrossRef]

152. Lee, H.; Rotolo, J.A.; Mesicek, J.; Penate-Medina, T.; Rimner, A.; Liao, W.C.; Yin, X.; Ragupathi, G.; Ehleiter, D.; Gulbins, E.; et al. Mitochondrial ceramide-rich macrodomains functionalize Bax upon irradiation. *PLoS ONE* **2011**, *6*, e19783. [CrossRef]

153. Jensen, S.A.; Calvert, A.E.; Volpert, G.; Kouri, F.M.; Hurley, L.A.; Luciano, J.P.; Wu, Y.; Chalastanis, A.; Futerman, A.H.; Stegh, A.H. Bcl2L13 is a ceramide synthase inhibitor in glioblastoma. *Proc. Natl. Acad. Sci. USA* **2014**, *111*, 5682–5687. [CrossRef]

154. Senkal, C.E.; Ponnusamy, S.; Bielawski, J.; Hannun, Y.A.; Ogretmen, B. Antiapoptotic roles of ceramide-synthase-6-generated C16-ceramide via selective regulation of the ATF6/CHOP arm of ER-stress-response pathways. *FASEB J.* **2010**, *24*, 296–308. [CrossRef]

155. Schiffmann, S.; Sandner, J.; Birod, K.; Wobst, I.; Angioni, C.; Ruckhaberle, E.; Kaufmann, M.; Ackermann, H.; Lotsch, J.; Schmidt, H.; et al. Ceramide synthases and ceramide levels are increased in breast cancer tissue. *Carcinogenesis* **2009**, *30*, 745–752. [CrossRef] [PubMed]

156. Sofi, M.H.; Heinrichs, J.; Dany, M.; Nguyen, H.; Dai, M.; Bastian, D.; Schutt, S.; Wu, Y.; Daenthanasanmak, A.; Gencer, S.; et al. Ceramide synthesis regulates T cell activity and GVHD development. *JCI Insight* **2017**, *2*, e91701. [CrossRef] [PubMed]

157. Rahmaniyan, M.; Curley, R.W., Jr.; Obeid, L.M.; Hannun, Y.A.; Kraveka, J.M. Identification of dihydroceramide desaturase as a direct in vitro target for fenretinide. *J. Biol. Chem.* **2011**, *286*, 24754–24764. [CrossRef] [PubMed]

158. Gorelik, A.; Illes, K.; Heinz, L.X.; Superti-Furga, G.; Nagar, B. Crystal structure of mammalian acid sphingomyelinase. *Nat. Commun.* **2016**, *7*, 12196. [CrossRef]

159. Santana, P.; Pena, L.A.; Haimovitz-Friedman, A.; Martin, S.; Green, D.; McLoughlin, M.; Cordon-Cardo, C.; Schuchman, E.H.; Fuks, Z.; Kolesnick, R. Acid sphingomyelinase-deficient human lymphoblasts and mice are defective in radiation-induced apoptosis. *Cell* **1996**, *86*, 189–199. [CrossRef]

160. Carpinteiro, A.; Becker, K.A.; Japtok, L.; Hessler, G.; Keitsch, S.; Pozgajova, M.; Schmid, K.W.; Adams, C.; Muller, S.; Kleuser, B.; et al. Regulation of hematogenous tumor metastasis by acid sphingomyelinase. *EMBO Mol. Med.* **2015**, *7*, 714–734. [CrossRef]

161. Airola, M.V.; Shanbhogue, P.; Shamseddine, A.A.; Guja, K.E.; Senkal, C.E.; Maini, R.; Bartke, N.; Wu, B.X.; Obeid, L.M.; Garcia-Diaz, M.; et al. Structure of human nSMase2 reveals an interdomain allosteric activation mechanism for ceramide generation. *Proc. Natl. Acad. Sci. USA* **2017**, *114*, E5549–E5558. [CrossRef]

162. Shamseddine, A.A.; Clarke, C.J.; Carroll, B.; Airola, M.V.; Mohammed, S.; Rella, A.; Obeid, L.M.; Hannun, Y.A. P53-dependent upregulation of neutral sphingomyelinase-2: Role in doxorubicin-induced growth arrest. *Cell Death Dis.* **2015**, *6*, e1947. [CrossRef] [PubMed]

163. Trajkovic, K.; Hsu, C.; Chiantia, S.; Rajendran, L.; Wenzel, D.; Wieland, F.; Schwille, P.; Brugger, B.; Simons, M. Ceramide triggers budding of exosome vesicles into multivesicular endosomes. *Science* **2008**, *319*, 1244–1247. [CrossRef] [PubMed]

164. Degagne, E.; Pandurangan, A.; Bandhuvula, P.; Kumar, A.; Eltanawy, A.; Zhang, M.; Yoshinaga, Y.; Nefedov, M.; de Jong, P.J.; Fong, L.G.; et al. Sphingosine-1-phosphate lyase downregulation promotes colon carcinogenesis through STAT3-activated microRNAs. *J. Clin. Investig.* **2014**, *124*, 5368–5384. [CrossRef] [PubMed]

165. Oskouian, B.; Sooriyakumaran, P.; Borowsky, A.D.; Crans, A.; Dillard-Telm, L.; Tam, Y.Y.; Bandhuvula, P.; Saba, J.D. Sphingosine-1-phosphate lyase potentiates apoptosis via p53- and p38-dependent pathways and is down-regulated in colon cancer. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 17384–17389. [CrossRef]

166. Heering, J.; Weis, N.; Holeiter, M.; Neugart, F.; Staebler, A.; Fehm, T.N.; Bischoff, A.; Schiller, J.; Duss, S.; Schmid, S.; et al. Loss of the ceramide transfer protein augments EGF receptor signaling in breast cancer. *Cancer Res.* **2012**, *72*, 2855–2866. [CrossRef]

167. Lee, A.J.; Roylance, R.; Sander, J.; Gorman, P.; Endesfelder, D.; Kschischo, M.; Jones, N.P.; East, P.; Nicke, B.; Spassieva, S.; et al. CERT depletion predicts chemotherapy benefit and mediates cytotoxic and polyploid-specific cancer cell death through autophagy induction. *J. Pathol.* **2012**, *226*, 482–494. [CrossRef]

168. Hullin-Matsuda, F.; Tomishige, N.; Sakai, S.; Ishitsuka, R.; Ishii, K.; Makino, A.; Greimel, P.; Abe, M.; Laviad, E.L.; Lagarde, M.; et al. Limonoid compounds inhibit sphingomyelin biosynthesis by preventing CERT protein-dependent extraction of ceramides from the endoplasmic reticulum. *J. Biol. Chem.* **2012**, *287*, 24397–24411. [CrossRef]

169. Wijesinghe, D.S.; Brentnall, M.; Mietla, J.A.; Hoeferlin, L.A.; Diegelmann, R.F.; Boise, L.H.; Chalfant, C.E. Ceramide kinase is required for a normal eicosanoid response and the subsequent orderly migration of fibroblasts. *J. Lipid Res.* **2014**, *55*, 1298–1309. [CrossRef]

170. Payne, A.W.; Pant, D.K.; Pan, T.C.; Chodosh, L.A. Ceramide kinase promotes tumor cell survival and mammary tumor recurrence. *Cancer Res.* **2014**, *74*, 6352–6363. [CrossRef]

171. Pastukhov, O.; Schwalm, S.; Zangemeister-Wittke, U.; Fabbro, D.; Bornancin, F.; Japtok, L.; Kleuser, B.; Pfeilschifter, J.; Huwiler, A. The ceramide kinase inhibitor NVP-231 inhibits breast and lung cancer cell proliferation by inducing M phase arrest and subsequent cell death. *Br. J. Pharmacol.* **2014**, *171*, 5829–5844. [CrossRef]

172. Kim, J.W.; Park, Y.; Roh, J.L.; Cho, K.J.; Choi, S.H.; Nam, S.Y.; Kim, S.Y. Prognostic value of glucosylceramide synthase and P-glycoprotein expression in oral cavity cancer. *Int. J. Clin. Oncol.* **2016**, *21*, 883–889. [CrossRef] [PubMed]

173. Roh, J.L.; Kim, E.H.; Park, J.Y.; Kim, J.W. Inhibition of Glucosylceramide Synthase Sensitizes Head and Neck Cancer to Cisplatin. *Mol. Cancer Ther.* **2015**, *14*, 1907–1915. [CrossRef] [PubMed]

174. Stefanovic, M.; Tutusaus, A.; Martinez-Nieto, G.A.; Barcena, C.; de Gregorio, E.; Moutinho, C.; Barbero-Camps, E.; Villanueva, A.; Colell, A.; Mari, M.; et al. Targeting glucosylceramide synthase upregulation reverts sorafenib resistance in experimental hepatocellular carcinoma. *Oncotarget* **2016**, *7*, 8253–8267. [CrossRef] [PubMed]

175. Liu, Y.Y.; Patwardhan, G.A.; Bhinge, K.; Gupta, V.; Gu, X.; Jazwinski, S.M. Suppression of glucosylceramide synthase restores p53-dependent apoptosis in mutant p53 cancer cells. *Cancer Res.* **2011**, *71*, 2276–2285. [CrossRef]

176. Gupta, V.; Bhinge, K.N.; Hosain, S.B.; Xiong, K.; Gu, X.; Shi, R.; Ho, M.Y.; Khoo, K.H.; Li, S.C.; Li, Y.T.; et al. Ceramide glycosylation by glucosylceramide synthase selectively maintains the properties of breast cancer stem cells. *J. Biol. Chem.* **2012**, *287*, 37195–37205. [CrossRef]

177. Eliyahu, E.; Park, J.H.; Shtraizent, N.; He, X.; Schuchman, E.H. Acid ceramidase is a novel factor required for early embryo survival. *FASEB J.* **2007**, *21*, 1403–1409. [CrossRef]

178. Cheng, J.C.; Bai, A.; Beckham, T.H.; Marrison, S.T.; Yount, C.L.; Young, K.; Lu, P.; Bartlett, A.M.; Wu, B.X.; Keane, B.J.; et al. Radiation-induced acid ceramidase confers prostate cancer resistance and tumor relapse. *J. Clin. Investig.* **2013**, *123*, 4344–4358. [CrossRef]

179. Beckham, T.H.; Cheng, J.C.; Lu, P.; Shao, Y.; Troyer, D.; Lance, R.; Marrison, S.T.; Norris, J.S.; Liu, X. Acid ceramidase induces sphingosine kinase 1/S1P receptor 2-mediated activation of oncogenic Akt signaling. *Oncogenesis* **2013**, *2*, e49. [CrossRef]

180. Tirodkar, T.S.; Lu, P.; Bai, A.; Scheffel, M.J.; Gencer, S.; Garrett-Mayer, E.; Bielawska, A.; Ogretmen, B.; Voelkel-Johnson, C. Expression of Ceramide Synthase 6 Transcriptionally Activates Acid Ceramidase in a c-Jun N-terminal Kinase (JNK)-dependent Manner. *J. Biol. Chem.* **2015**, *290*, 13157–13167. [CrossRef]

181. Wang, Z.; Min, X.; Xiao, S.H.; Johnstone, S.; Romanow, W.; Meininger, D.; Xu, H.; Liu, J.; Dai, J.; An, S.; et al. Molecular basis of sphingosine kinase 1 substrate recognition and catalysis. *Structure* **2013**, *21*, 798–809. [CrossRef]

182. Kawamori, T.; Kaneshiro, T.; Okumura, M.; Maalouf, S.; Uflacker, A.; Bielawski, J.; Hannun, Y.A.; Obeid, L.M. Role for sphingosine kinase 1 in colon carcinogenesis. *FASEB J.* **2009**, *23*, 405–414. [CrossRef]

183. Zhang, Y.; Wang, Y.; Wan, Z.; Liu, S.; Cao, Y.; Zeng, Z. Sphingosine kinase 1 and cancer: A systematic review and meta-analysis. *PLoS ONE* **2014**, *9*, e90362. [CrossRef] [PubMed]

184. Kummar, S.; Gutierrez, M.E.; Maurer, B.J.; Reynolds, C.P.; Kang, M.; Singh, H.; Crandon, S.; Murgo, A.J.; Doroshow, J.H. Phase I trial of fenretinide lym-x-sorb oral powder in adults with solid tumors and lymphomas. *Anticancer. Res.* **2011**, *31*, 961–966. [PubMed]

185. Rao, R.D.; Cobleigh, M.A.; Gray, R.; Graham, M.L., 2nd; Norton, L.; Martino, S.; Budd, G.T.; Ingle, J.N.; Wood, W.C. Phase III double-blind, placebo-controlled, prospective randomized trial of adjuvant tamoxifen vs. tamoxifen and fenretinide in postmenopausal women with positive receptors (EB193): An intergroup trial coordinated by the Eastern Cooperative Oncology Group. *Med. Oncol.* **2011**, *28* (Suppl. S1), S39–S47. [CrossRef] [PubMed]

186. Veronesi, U.; Mariani, L.; Decensi, A.; Formelli, F.; Camerini, T.; Miceli, R.; Di Mauro, M.G.; Costa, A.; Marubini, E.; Sporn, M.B.; et al. Fifteen-year results of a randomized phase III trial of fenretinide to prevent second breast cancer. *Ann. Oncol.* **2006**, *17*, 1065–1071. [CrossRef]

187. Vaishampayan, U.; Heilbrun, L.K.; Parchment, R.E.; Jain, V.; Zwiebel, J.; Boinpally, R.R.; LoRusso, P.; Hussain, M. Phase II trial of fenretinide in advanced renal carcinoma. *Investig. New Drugs* **2005**, *23*, 179–185. [CrossRef]

188. French, K.J.; Zhuang, Y.; Maines, L.W.; Gao, P.; Wang, W.; Beljanski, V.; Upson, J.J.; Green, C.L.; Keller, S.N.; Smith, C.D. Pharmacology and antitumor activity of ABC294640, a selective inhibitor of sphingosine kinase-2. *J. Pharmacol. Exp. Ther.* **2010**, *333*, 129–139. [CrossRef] [PubMed]

189. Zhou, J.; Chen, J.; Yu, H. Targeting sphingosine kinase 2 by ABC294640 inhibits human skin squamous cell carcinoma cell growth. *Biochem. Biophys. Res. Commun.* **2018**, *497*, 535–542. [CrossRef] [PubMed]

190. Shaw, J.; Costa-Pinheiro, P.; Patterson, L.; Drews, K.; Spiegel, S.; Kester, M. Novel Sphingolipid-Based Cancer Therapeutics in the Personalized Medicine Era. *Adv. Cancer Res.* **2018**, *140*, 327–366. [CrossRef]

191. Choi, R.H.; Tatum, S.M.; Symons, J.D.; Summers, S.A.; Holland, W.L. Ceramides and other sphingolipids as drivers of cardiovascular disease. *Nat. Rev. Cardiol.* **2021**, *18*, 701–711. [CrossRef]

192. Hait, N.C.; Maiti, A. The Role of Sphingosine-1-Phosphate and Ceramide-1-Phosphate in Inflammation and Cancer. *Mediators Inflamm.* **2017**, *2017*, 4806541. [CrossRef] [PubMed]

193. Tabasinezhad, M.; Samadi, N.; Ghanbari, P.; Mohseni, M.; Saei, A.A.; Sharifi, S.; Saeedi, N.; Pourhassan, A. Sphingosin 1-phosphate contributes in tumor progression. *J. Cancer Res. Ther.* **2013**, *9*, 556–563. [CrossRef] [PubMed]

194. Hannun, Y.A.; Obeid, L.M. Sphingolipids and their metabolism in physiology and disease. *Nat. Rev. Mol. Cell Biol.* **2018**, *19*, 175–191. [CrossRef] [PubMed]

195. Becker, K.A.; Gellhaus, A.; Winterhager, E.; Gulbins, E. Ceramide-enriched membrane domains in infectious biology and development. *Subcell. Biochem.* **2008**, *49*, 523–538. [CrossRef]

196. Dany, M.; Gencer, S.; Nganga, R.; Thomas, R.J.; Oleinik, N.; Baron, K.D.; Szulc, Z.M.; Ruvolo, P.; Kornblau, S.; Andreeff, M.; et al. Targeting FLT3-ITD signaling mediates ceramide-dependent mitophagy and attenuates drug resistance in AML. *Blood* **2016**, *128*, 1944–1958. [CrossRef]

197. Parham, K.A.; Zebol, J.R.; Tooley, K.L.; Sun, W.Y.; Moldenhauer, L.M.; Cockshell, M.P.; Gliddon, B.L.; Moretti, P.A.; Tigyi, G.; Pitson, S.M.; et al. Sphingosine 1-phosphate is a ligand for peroxisome proliferator-activated receptor-gamma that regulates neoangiogenesis. *FASEB J.* **2015**, *29*, 3638–3653. [CrossRef]

198. Hait, N.C.; Allegood, J.; Maceyka, M.; Strub, G.M.; Harikumar, K.B.; Singh, S.K.; Luo, C.; Marmorstein, R.; Kordula, T.; Milstien, S.; et al. Regulation of histone acetylation in the nucleus by sphingosine-1-phosphate. *Science* **2009**, *325*, 1254–1257. [CrossRef]

199. Strub, G.M.; Paillard, M.; Liang, J.; Gomez, L.; Allegood, J.C.; Hait, N.C.; Maceyka, M.; Price, M.M.; Chen, Q.; Simpson, D.C.; et al. Sphingosine-1-phosphate produced by sphingosine kinase 2 in mitochondria interacts with prohibitin 2 to regulate complex IV assembly and respiration. *FASEB J.* **2011**, *25*, 600–612. [CrossRef]