Manifold Roles of Ceramide Metabolism in Non-Alcoholic Fatty Liver Disease and Liver Cancer

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Abstract

Non-alcoholic fatty liver disease (NAFLD) is a metabolic disorder manifested in hepatic fat accumulation (hepatic steatosis) in the absence of heavy alcohol use. NAFLD consists of four major stages ranging from simple steatosis or non-alcoholic fatty liver (NAFL) to more advanced stages, non-alcoholic steatohepatitis (NASH), fibrosis, and cirrhosis. NFLAD may further advance to hepatocellular carcinoma (HCC). Primary causes of NAFLD are obesity and obesity-associated insulin resistance (IR). As a result of the obesity pandemic, NAFLD has become one of the most common liver disorders worldwide and both the incidence and mortality rate of HCC that develops from NAFLD are increasing steadily. As treatment options are not available for advanced NAFLD, a better understanding of the molecular mechanisms for NAFLD development and progression is urgently needed. Emerging evidence suggests that dysregulation of the metabolism of sphingolipids contributes to development and progression of NAFLD and NAFLD-associated HCC. The present chapter summarizes roles of bioactive sphingolipids, ceramides, sphingosine, and sphingosine-1-phosphate (S1P) and their metabolizing enzymes in NAFLD and HCC.

Keywords

Ceramide · Non-alcoholic fatty liver disease (NAFLD) · Non-alcoholic steatohepatitis (NASH) · Hepatocellular carcinoma (HCC) · Insulin resistance

11.1 Overview of NAFLD and HCC

Non-alcoholic fatty liver disease (NAFLD) is a metabolic disorder manifested in hepatic fat accumulation (hepatic steatosis) in the absence of heavy alcohol consumption in past medical history. NAFLD exhibits a spectrum of conditions ranging from simple steatosis or non-alcoholic fatty liver (NAFLD) to non-alcoholic steatohepatitis (NASH) or cirrhosis, which may further advance to hepatocellular carcinoma (HCC) [1]. Primary causes of NAFLD are obesity and obesity-associated insulin resistance (IR). As a result of the obesity pandemic, NAFLD has become one of the most common liver disorders worldwide and currently affects around 25% of

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the general population of Western countries [2, 3]. NASH is expected to become the leading cause of liver transplant [3]. However, NASH currently has no effective treatment apart from lifestyle interventions [4]. Therefore, there are unmet medical needs for understanding the molecular and cellular mechanisms for the progression of NAFLD to NASH and cirrhosis and developing novel approaches to treating advanced NAFLD based on an understanding of the pathogenesis of this disease.

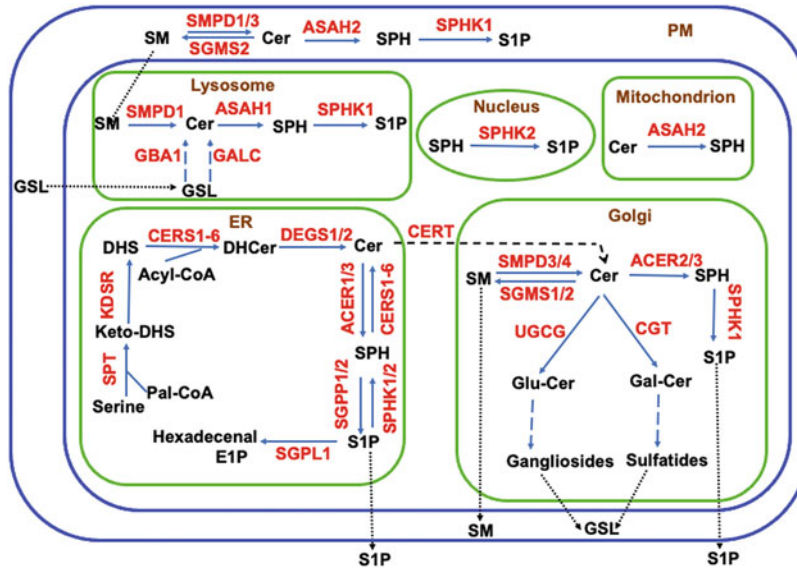
Obesity [2] and IR [5] have been suggested to be major risk factors for NAFLD. Obesity and IR result in accumulation of free fatty acids (FFAs) in the liver [2]. Hepatic FFAs are metabolized into many lipotoxic species that cause various forms of hepatocellular stress, including oxidative stress, endoplasmic reticulum (ER) stress, and cell death [1], thus leading to liver damage, inflammation, and fibrosis, which are the hallmark comorbidities of NASH [6]. The ER plays central roles in calcium ion storage, lipid biosynthesis, and protein sorting and processing [7]. Accumulation of unfolded proteins in the ER lumen or impairment of the ER membrane integrity results in ER stress which in turn activates a series of signaling processes called the unfolded protein response (UPR) [8]. Transient activation of the UPR restores the homeostasis of the ER while chronic UPR activation due to persistence of ER stress results in cell death and cellular injury. Chronic UPR activation has been observed in liver and/or adipose tissue of dietary and genetic murine models of obesity, and in human obesity and NAFLD [8]. The chronic responses to cell death and cellular injury lead to chronic hepatocyte turnover, the recruitment of immune cells, and activation of hepatic stellate cells (HSCs), thus contributing to the development of liver fibrosis and cirrhosis [9–11]. It has become evident that, besides apoptosis, necroptosis is a highly relevant form of programmed cell death (PCD) in the liver [9, 12, 13]. In addition to PCD, increasing studies have implicated autophagy in the pathogenesis of NAFLD [14]. Autophagy is a lysosomal degradative pathway that promotes cell survival by supplying energy under the stress of energy crisis or by removing damaged organelles and proteins after cellular injury [15]. An initial study suggests that autophagy mediates the breakdown of lipids in hepatocytes [16]. Subsequent studies have implicated autophagy in regulating several pathological effects of NAFLD, such as insulin sensitivity, hepatocellular injury, innate immunity, fibrosis, and liver carcinogenesis [14].

However, lipotoxic species that mediate ER stress, cell death, and autophagy in the context of NAFLD have not been completely identified. Increasing studies have implicated bioactive sphingolipids, such as ceramides, sphingosine, and sphingosine-1-phosphate (S1P), in both initiation of NAFLD and its progression to HCC.

11.2 Metabolism of Ceramides in NAFLD

11.2.1 Dysregulation of Metabolism of Sphingolipids in the Liver of Patients with NAFLD

Increasing studies have demonstrated that ceramides, a subclass of sphingolipids, are potential lipotoxic mediators of saturated FFAs in driving NAFLD onset and progression [17, 18] . Palmitic acid, the most abundant hepatic FFA in NAFLD patients, is an essential precursor of ceramides and other sphingolipids [19, 20] (Scheme 11.1). Palmitic acid is activated to form palmitoyl-CoA, which is condensed with serine into keto-dihydrosphingosine by the action of serine palmitoyltransferase (SPT) [19]. Keto-dihydrosphingosine is reduced to form dihydrosphingosine [20], which is acylated by various fatty acids to form dihydroceramides with various acyl-chains by the action of 6 (dihydro)ceramides synthases (CERS1–6) [21, 22]. Dihydroceramides are then converted to ceramides by the action of dihydroceramide desaturase. These enzymatic steps occur in the ER and belong to so-called the de novo pathway for ceramide formation. Once generated in the ER, ceramides are transported from the ER to the Golgi complex where they are then incorporated into more complex sphingolipids



Scheme 11.1 Metabolism of sphingolipids. *Cer* ceramide, *CGT* galactosylceramide synthase, *Dhcer* dihydroceramide, *DHS* dihydrosphingosine, *E1P* phosphoethanolamine, *Gal-Cer* galactosylceramide, *GLS* glycosphingolipid, *Glu-cer* glucosylceramide, *KDSR* ketodihydrosphingosine reductase, *Pal-CoA* palmitoyl-CoA, *SPH* sphingosine, *S1P* sphingosine-1-phosphate, *SM* sphingomyelin

such as sphingomyelins and glycosphingolipids. Sphingomyelins on the plasma membrane can be hydrolyzed to form ceramides by the action of neutral sphingomyelinase. This metabolic pathway is termed the sphingomyelin-hydrolysis pathway for ceramide formation. Complex sphingolipids can also be transported back to the lysosomes where they are converted back to ceramides, which are hydrolyzed into sphingosine by the action of ceramidases [23, 24]. Sphingosine can be converted back to ceramides by the action of CERS, which is termed the salvage pathway for ceramide formation. Therefore, there are three major metabolic pathways that lead to the formation of ceramides in cells, including the de novo, sphingomyelin-hydrolysis, and salvage pathways. In vitro cellular studies demonstrated that oversupply of palmitate markedly increases the levels of ceramides in hepatocytes [25]. Several clinical studies demonstrated that the hepatic content of ceramides is elevated in patients with NAFLD. Apostolopoulou et al. [26] found that the hepatic levels of ceramides were increased in NASH patients compared to NAFL patients or healthy individuals. Luukkonen et al. [27] demonstrated that the hepatic levels of ceramides and dihydroceramides were elevated in patients with steatosis plus IR compared to patients with steatosis. However, Vvedenskaya et al. [28] showed that the hepatic levels of ceramides were similar between healthy individuals and patients with NAFL or NASH. Several other studies demonstrated that the hepatic levels of ceramides were also increased in murine models of NASH [29–32]. Accumulated ceramides are derived from both de novo pathway and sphingomyelin-hydrolysis pathway. Enzymes in both ceramide-generating pathways have been shown to be upregulated in the liver of mice with NAFLD, including SPT, CERS1, CERS2, CERS4, CERS6, and SMPD1 [33–37]. These results indicate that NAFLD is indeed associated with increased hepatic levels of ceramides.

As mentioned earlier, ceramides can be hydrolyzed into sphingosine by the action of 5 different ceramidases including acid ceramidase (ASAH1) [38], neutral ceramidase (ASAH2) [39], alkaline ceramidase 1 (ACER1) [40], alkaline ceramidase 2 (ACER2) [41], and alkaline ceramidase 3 (ACER3) [42]. As such, the hepatic content of sphingosine may be increased with increasing FFAs and ceramides in NAFLD patients and animal models of NAFLD. Indeed, the content of sphingosine was also increased in human hepatocytes treated with palmitate, a cellular model of steatosis [43, 44]. Hepatic sphingosine has been shown to be elevated in several

mouse models of NAFLD [26, 29, 30, 45] although it remains unclear whether this is also the case in patients with NAFLD.

As sphingosine can be phosphorylated to form S1P by the action of SPHK1 and/or SPHK2 in liver cells, several studies have shown that hepatic levels of S1P are increased in patients with NAFLD [30] and murine models of NAFLD [46–48].

11.2.2 Role of Ceramide and Sphingosine in Hepatocyte Injury

In addition to structural components of membranes of mammalian cells, ceramides are bioactive lipids implicated in regulating oxidative stress [49], ER stress [50], and PCD [51, 52], which are key drivers of hepatic injury in NASH. This indicates that increased hepatic ceramides may contribute to the pathogenesis of NAFLD. In line with this notion, several animal studies demonstrated that inhibiting the generation of ceramides protected mice from diet-induced NASH. Treatment with myriocin, a specific and potent SPT inhibitor that inhibits the biosynthesis of ceramides, reduced liver damage and fibrosis, and expression of the inflammation markers, IL-1β, MCP-1, and TNFα [53]. Myriocin has also been found to be effective in suppressing high-fat diet (HFD)-induced steatosis, inflammation, fibrosis, and apoptosis in *LDLr* $^{-/-}$ mice, a genetic mouse model of NASH [54]. Moreover, myriocin was also shown to reduce the severity of NAFLD in rats fed diet enriched in fat and cholesterol [55]. In contrast to myriocin, treatment of ceramide per se was found to induce hepatic steatosis by enhancing hepatic lipogenesis [56]. These results suggest that ceramides or their metabolites accumulated in the liver may drive NASH onset and progression.

Indeed, several recent studies revealed the distinct roles of specific ceramide species in the pathogenesis of NAFLD. We showed that knocking down ACER3 increased the levels of $C_{18:1}$-ceramide and alleviated oxidative stress and cell death in human hepatocytes oversupplied with palmitic acid [57]. We also demonstrated that knocking out the mouse *Acer3* protected mice from hepatic oxidative stress and death of hepatocytes in a mouse model of NAFLD likely by increasing hepatic levels of $C_{18:1}$-ceramide [57]. These results suggest a protective role of the unsaturated long-chain ceramide in NAFLD. *CerS2* haploinsufficiency decreased very-long-chain ceramides while inducing a compensatory increase of C_{16}-ceramide in mice, resulting in inhibition of beta-oxidation and advanced steatohepatitis [58], suggesting either a protective role of very-long-chain ceramides or a pathological role of C_{16}-ceramide in NAFLD. A series of studies demonstrated that increased C_{16}-ceramide due to *Cers6* deficiency promoted lipogenesis and impaired mitochondrial respiration in NAFLD with obesity background [58–60]. Additionally, C_{16}-ceramide treatment per se induced steatosis in mice by upregulating lipogenesis [56]. Interestingly, *Cers5* knockout decreased the levels of C_{16}-ceramide in various tissues including the liver in a feeding-independent manner and protected mice from weight gain, white adipose tissue inflammation, and hyperglycemia after HFD challenge [61]. These results suggest that C_{16}-ceramide produced by the action of different CERS isozymes has distinct roles in regulating NAFLD.

Similar to ceramides, previous studies indicate that sphingosine also functions as a bioactive lipid to mediate PCD in human cells in response to different stressful insults, such as the pro-death cytokine TNF-α [62], glucocorticoid [63], serum deprivation [64], or oxidative stress [65]. However, which ceramidase is responsible for generating the pro-death sphingosine remained unclear until our recent studies have firmly established that ACER2 is a major ceramidase responsible for production of sphingosine that mediates cell death in response to a variety of stress stimuli. We demonstrated that genotoxic cancer chemotherapeutic agents [66] or ionizing radiation increased the levels of sphingosine in human tumor cells in a p53-dependent manner by upregulating ACER2 [67]. Knocking out ACER2 inhibited not only the generation of sphingosine but also PCD in response to DNA-damaging

agents, whereas overexpression of ACER2 was sufficient to induce both sphingosine generation and PCD in cells [66, 67]. Furthermore, we identified ACER2 as a novel transcriptional target of p53, explaining why DNA-damaging agents and ionizing radiation upregulate both ACER2 and sphingosine in cells in a p53-dependent manner [67]. Many previous studies suggest that ceramides act as bioactive lipids to mediate PCD in tumor cells in response to different forms of stress, including DNA damage [68–70]. However, we demonstrated that an increase in the levels of ceramides due to ACER2 knockout or knockdown in cells failed to induce cell death [66], suggesting that ceramides that serve as endogenous substrates of ACER2 do not directly mediate PCD. These results suggest that sphingosine converted from ceramides by the action of ACER2 may also mediate the pathogenesis of NAFLD by inducing PCD of hepatocytes. Gulibositan et al. recently reported that hepatocyte-specific knockout of *Sphk2*, which phosphorylates sphingosine into S1P, significantly impaired insulin sensitivity and glucose tolerance in HFD-fed mice [71]. Intriguingly, the mechanistic study by the same group found that hepatocyte-specific ablation of *Sphk2* increased the hepatic levels of sphingosine without affecting those of S1P, suggesting that sphingosine may be a bona fide mediator of HFD-induced insulin resistance and glucose intolerance [71].

11.2.3 Role of S1P in Steatosis and Inflammation in NAFLD

Emerging evidence suggests that S1P plays roles in inflammation and fibrosis in the context of NAFLD. In vitro study reported that SPHK1 expression protected hepatocytes from lipotoxicity by inhibiting IRE1α activation and JNK phosphorylation and thereby ER-stress-associated apoptosis of hepatocytes [72]. However, in vivo studies found that knocking out *Sphk1* alleviated steatosis and hepatic inflammation in a mouse model of NAFLD, suggesting a role of S1P in NAFLD progression [48, 73]. In contrast, *Sphk2* knockout mice were found to be more susceptible to NAFLD induction likely due to the compensatory upregulation of *Sphk1* [47], further confirming the pathological role of S1P in NAFLD.

11.3 Sphingolipid Metabolism in HCC

11.3.1 Dysregulation of Ceramide Metabolism in HCC

Several studies demonstrated that ceramide metabolism is dysregulated in HCC in humans and animals. Krautbauer et al. found that most ceramide species (C_{16}-$C_{24:1}$) were decreased in human HCC tissues compared to their matched nontumor hepatic tissues [74]. In line with this study, Ismail et al. [75] and Li et al. [76] showed that the hepatic levels of ceramides were significantly decreased in human HCC tumors compared to paired nontumor hepatic tissues. In contrast to the above studies, Miura et al. found that the hepatic levels of several subclass sphingolipids, including ceramides, were significantly increased in human HCC tumors compared with their matched nontumor hepatic tissues [77]. The discrepancies among these studies might be attributed to variations in the etiology of HCC patients. Preclinical studies also indicate that hepatic levels of ceramides are altered in chemically induced liver tumors compared to nontumor liver tissues in mouse models of HCC. Haberl et al. [78] demonstrated that the levels of ceramides were higher in liver tumors than in nontumor hepatic tissues in mice treated with the carcinogen N-nitrosodiethylamine (DEN).

11.3.2 Role of Ceramides in HCC

Ceramides derived from either the de novo or sphingomyelin-hydrolysis pathway have been implicated in regulating HCC development and progression. Targeting specific CERS with a genetical approach has revealed that ceramides

with different acyl-chains may have distinct roles in regulating HCC in mice. It has been shown that *Cers2* deficiency decreased the hepatic levels of very-long-chain ($C_{22–24}$) ceramides while increasing those of C_{16}-ceramide and promoted sporadic liver tumor formation and chemical-induced hepatocarcinogenesis in mice [79, 80]. These results suggest that very-long-chain ceramides have a tumor suppressor role in HCC or that long-chain ceramide has an oncogenic role in HCC. Silencing CERS6, which synthesizes C_{16}-ceramide, was found to mediate the cytotoxicity of antifolate methotrexate in HCC cell, suggesting that C_{16}-ceramide might be a mediator of chemotherapeutic agents in HCC [81]. CERS4 was found to be upregulated in HCC tissues and its upregulation promoted the proliferation and survival of HCC cells [82]. These data suggest that ceramides with different chain lengths and saturation degrees, which are produced by specific CERSs, may function distinctly in HCC.

The sphingomyelin-hydrolysis pathway is also involved in the pathogenesis of HCC. Neutral sphingomyelinase 1 encoded by the gene *SMPD2* was found to be downregulated in HCC tissues and its downregulation negatively correlates with poor long-term survival of patients with HCC [83]. Neutral sphingomyelinase 2 encoded by the gene *SMPD3* was identified as a tumor suppressor-like gene that negatively correlates with early recurrence of human HCC after curative surgery [84]. *Smpd3* deficiency in mice promoted survival and proliferation of cancer stem-like cells, resulting in spontaneous HCC. Unexpectedly, *Smpd3* deficiency increased both sphingomyelin and C_{16}-ceramide levels in mouse HCC tissues, especially in cancer stem-like cells. The increased sphingomyelin and C_{16}-ceramide were attributed to the compensatory upregulation of *Cers5* [85]. These results suggest that sphingomyelinases play an anti-cancer role in HCC.

On the other hand, inhibition of glucosylceramide synthesis was also found to suppress HCC. Jennemann et al. found that glucosylceramide synthase encoded by the *UGCG* gene was significantly overexpressed in human HCC tissues as compared to nontumor liver tissues and that knockout of the mouse *Ucgc* specifically in the hepatocyte inhibited HCC initiation and progression in a chemically induced HCC model [86]. Su et al. found that ganglioside synthesis was increased in the livers of an animal model of activation and expansion of liver cancer cells, and pharmacological inhibition of ganglioside synthesis suppressed proliferation and sphere growth of liver cancer cells [87]. Guri et al. [88] demonstrated that activating the mTOR signaling pathway specifically in the liver through the liver-specific knockout of both *Tsc1* and *Pten* increased synthesis of fatty acids and sphingolipids including glucosylceramides in the liver, resulting in hepatosteatosis and HCC, which occurred sequentially. Inhibiting sphingolipid biosynthesis with myriocin, which specifically inhibits SPT, or knocking down hepatic Ugcg by RNA interference markedly reduced liver tumor numbers in *Tsc1* and *Pten* double knockout mice. This study provides compelling evidence that increasing glucosylceramides or more complex glycosphingolipids may promote NAFLD development and progression to HCC.

The breakdown of ceramide catalyzed by ceramidases plays important role in regulating cancer-related pathobiology [24]. Pharmacological and siRNA inhibition of acid ceramidase was found to inhibit growth of liver tumor xenografts of HepG2 cells and enhanced the cytotoxicity of daunorubicin on HCC cells by upregulating oxidative stress and apoptosis [89]. The expression of ACER3 was found to be upregulated in different liver cancer cell lines compared to normal liver cells and its increased expression inversely correlates with the overall and disease-free survival of HCC patients [90]. Knockdown of ACER3 inhibited cell growth and promoted apoptosis in HCC cells but had no influence on growth or apoptosis in normal hepatocyte cells. Similar to ACER3, Liu et al. found that ACER2 was also upregulated in HCC and its upregulation promoted HCC cell survival and migration [91]. These results suggest that increased alkaline ceramidases promotes liver tumorigenesis likely by increasing hydrolysis of ceramides.

11.3.3 Role of S1P in HCC

SPHK-derived S1P has been shown to function as an oncogenic lipid of HCC by promoting survival, migration, and proliferation in HCC cells. SPHK1 was found to be upregulated in human HCC tissues compared to adjacent non-tumorous liver tissues, and the overexpression of SPHK1 was associated with advanced malignancy and poor prognosis of HCC [92–95]. Bao et al. found that the SphK1-induced migration and invasion of HCC cells was mediated by the S1P receptor S1PR1 [92]. Mu et al. demonstrated that SPHK1 could mediate the migration of hepatoma cells induced by hepatocyte growth factor (HGF) [96]. SPHK1-mediated invasion and metastasis were also attributed to induction of the mesenchymal transition (EMT) in HCC cells as increased SPHK1 accelerates lysosomal degradation of the cell-cell adhesion molecule E-cadherin (CDH1) [97]. Notably, this study found that SPHK1-produced S1P bound to TRAF2 and stimulated lysine 63-linked ubiquitination and beclin1 activation, resulting in autophagic degradation of E-cadherin and thereby EMT [97]. SPHK1 has been shown to mediate HCC proliferation by activating the Ras/ERK, MEK1/2, FAK/MLC-2, Wnt5A/b-catenin, Akt/GSK3β, and Akt/NF-kb signaling pathways [98–100]. In addition to regulating the malignancy of HCC, *Sphk1* deletion in mice was found to suppress carcinogenesis in a chemically induced HCC model, supporting a role of SPHK1 in promoting hepatocarcinogenesis [101]. SPHK2 has a similar role in HCC. SPHK2 mRNA levels were found to be increased in HCC tissues and positively correlated with intra- and extra-hepatic recurrence [95]. Shi et al. reported that SPHK2 overexpression was associated with regorafenib resistance in HCC by activating NF-κB and STAT3 [102]. Beljanski et al. also reported that inhibition of SPHK2 in combination with sorafenib suppressed cell growth through the MAPK pathway in HCC [103]. Interestingly, S1P lyase was also increased in HCC tissues, and higher S1P lyase mRNA levels in HCC were associated with increased proliferation and poorer differentiation of HCC [95]. These results suggest that S1P may have an important role in liver tumorigenesis and that targeting the S1P pathway may improve the therapy of HCC.

11.3.4 Therapeutic Role of Exogenous Ceramide in HCC

Exogenous treatment of ceramide and sphingosine have been demonstrated to exert cytotoxic effects in HCC. Short-chain ceramide, including C_2- and C_6-ceramide, are well-studied antitumor lipids that induce growth arrest and cell death in various types of cancer [104]. Since Obeid et al. demonstrated for the first time that C_2-ceramide induces PCD [105], short-chain ceramides have come into the spotlight of cancer research. C_2-ceramide was later shown to induce cell death in HCC cell, including apoptosis and necrosis, possibly by downregulating Bcl-2 and inhibiting respiratory chain to produce reactive oxygen species (ROS), exhausting ATP, and impairing mitochondria function [106, 107]. C_6-ceramide was shown to induce apoptosis in HCC cells concurrent with release of cytochrome c and activation of caspase-3 without affecting mitochondrial respiratory chain [107]. Co-administration of C_6-ceramide was also found to enhance cytotoxic and pro-apoptotic effects of mTOR complex 1/2 (mTORC1/2) dual inhibitor AZD-8055 in a panel of HCC cell lines and primary cultured human HCC cells, with no adverse effect on growth and survival of normal human hepatocytes [108]. Nanoliposomal C_6-ceramide, which can be administered intravenously and has improved bioavailability and solubility [109], was then applied in several preclinical studies to evaluate its therapeutic efficiency in HCC. Tagaram et al. reported that nanoliposomal C_6-ceramide administration suppressed HCC growth in mice engrafted with HCC cells by reducing tumor vascularization and proliferation, inducing tumor cell apoptosis, and inhibiting phosphorylation of AKT [110]. More recently, in addition to tumor growth suppression, nanoliposomal C_6-ceramide was found to improve antitumor immune response [111]. Nanoliposomal C_6-ceramide injection

could reduce numbers of tumor-associated macrophages and their production of ROS while inducing differentiation of tumor-associated macrophages into M1 phenotype of macrophages, which reduce immune suppression and increase activity of anti-cancer $CD8^+$ T cell [111]. Similar to liposomal C_6-ceramide, liposomal C_8-ceramide was also found to induce apoptosis in HCC by activating caspase pathways and apoptosis signal-regulating kinase 1 (ASK1)-Jun N-terminal protein kinase (JNK) signaling, and injection of liposomal C_8-ceramide inhibited HepG2 xenograft growth in severe combined immuno-deficient mice and improved their survival [112]. The liposomal short-chain ceramides were also found to enhance the effect of several chemotherapeutic drugs. Co-treatment with nanoliposomal C_6-ceramide and vinblastine synergistically inhibited growth in HCC cells, probably by inhibiting autophagy flux and increasing apoptosis [113]. Similarly, liposomal C_6-ceramide augmented the growth inhibitory effects of mTOR complex 1/2 inhibitor AZD-8055 in HCC mice xenografts with HCC cells [108]. Notably, co-loading C_6–ceramide with sorafenib into liposomes synergistically increased antitumor activity in HCC cells with reduced systemic toxicity of sorafenib [114]. Similar to ceramides, sphingosine treatment was found to induce apoptosis by suppressing the activation of AKT kinase and upregulating caspase pathways in HCC cells [115–117]. All the data highlight the anti-cancer activities of ceramides and sphingosine mainly by inducing cell death in HCC, which warrants further clinical studies to evaluate the potential of ceramide and sphingosine treatment for HCC.

11.4 Conclusions

Recent findings strongly suggest that specific ceramide species, sphingosine, sphingosine-1-phosphate, and other sphingolipids play prominent roles in NAFLD and liver tumorigenesis. Targeting the metabolism of these bioactive sphingolipids may represent a novel approach to halting NAFLD development and/or its progression to HCC. Therefore, an important area of future study in the sphingolipid field is to develop novel drugs targeting ceramide metabolism to enhance therapeutic response and improve survival outcome in HCC patients.

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