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Review Article

Potential therapeutic targets for atherosclerosis in sphingolipid metabolism

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Sphingolipids, such as sphingomyelins, ceramides, glycosphingolipids, and sphingosine-1-phosphates (S1P) are a large group of structurally and functionally diverse molecules. Some specific species are found associated with atherogenesis and provide novel therapeutic targets. Herein, we briefly review how sphingolipids are implicated in the progression of atherosclerosis and related diseases, and then we discuss the potential therapy options by targetting several key enzymes in sphingolipid metabolism.

Sphingolipids metabolism

Overview of sphingolipid biosynthesis

Sphingolipids are based on a ceramide parent structure. Ceramides are composed of a hydrophobic sphingoid backbone and a fatty acyl chain, linked to the backbone via an amino bond [1]. Three metabolic pathways are involved in ceramide production (Figure 1). (1) *De novo* synthesis begins in the cytosolic layer of the endoplasmic reticulum (ER) with the condensation of the amino acid serine and palmitoyl-coenzyme A (CoA) via serine palmitoyltransferase (SPT), generating 3-ketosphinganine. 3-ketosphinganine is then reduced to sphinganine, the 18-carbon backbone, via 3-ketosphinganine reductase (KSR). Finally, N-acylation of sphinganine by ceramide synthases (CerS) generates dihydroceramide, which is subsequently converted into ceramide via dihydroceramide desaturase (DES) [2]. This *de novo* pathway is the major source of ceramide in cells, and all eukaryotic cells have the capacity to produce sphingolipids in this way. (2) A catabolic pathway occurs in lysosomes, including hydrolysis of sphingomyelin via sphingomyelinase (SMase) and catabolism of glycosphingolipids via glycosidases hydrolyzing glycosidic bonds [3]. (3) A salvage pathway generates ceramides by recycling sphingosine via CerS, as the sphingosine is produced by the hydrolysis of ceramide catalyzed by ceramidase (CDase) [4]. At least half of the sphingosine enters this reutilization pathway, playing an important role in sphingolipid homeostasis [3].

Ceramides have three main ways to be incorporated into various sphingolipids. (1) In the ER, ceramide can be deacetylated to form sphingosine, which in turn is phosphorylated to serine palmitoyltransferase (S1P) via sphingosine kinase (SphK) [5]. (2) In the Golgi, after being transported from the ER by ceramide transfer protein (CERT) in a non-vesicular pathway, ceramide acquires a phosphorylcholine moiety from phosphatidylcholine to form sphingomyelin and diacylglycerol via sphingomyelin synthase (SMS); when transported from the ER via transport vesicles, ceramide add a glucose/galactose to form glucosylceramide (GluCer)/galactosylceramide (GalCer) via glucosylceramide synthase (GCS, also named UDP-glucose ceramide glucosyltransferase) or galactosylceramide synthase (GalCerS, also named 2-hydroxyacylsphingosine 1-β-galactosyltransferase). Then, GluCer is transferred from the *cis*-Golgi to the *trans*-Golgi by vesicular transport or a carrier protein, four-phosphate adapter protein 2 (FAPP2), to generate lactosylceramide (LacCer) catalyzed by lactose ceramide synthase (LCS), also named glucosylceramide β1→4 galactosyltransferase (GalT-2) [6]. In the *trans*-Golgi, LacCer can further produce complex globosides and gangliosides [7]. (3) In the Golgi, ceramides can also be phosphorylated to form ceramide-1-phosphate (C1P) via ceramide kinase (CERK).

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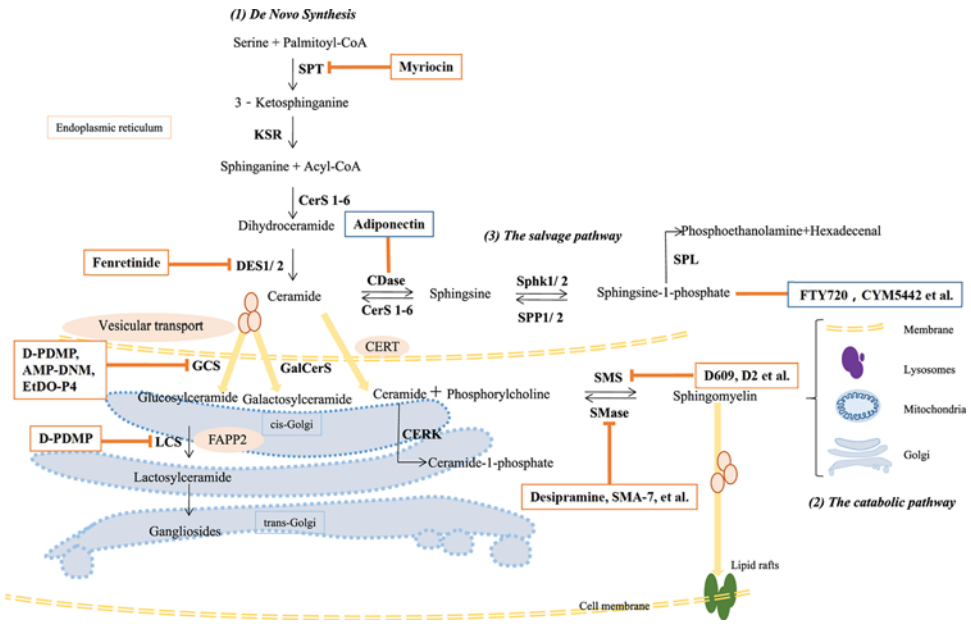

Figure 1. Sphingolipid biosynthesis and sphingolipid-centric theraputics

(1) *De novo* sphingolipid synthesis starts in the ER with the decarboxylation of a serine residue and condensation with a palmitoyl-CoA catalyzed by SPT. Sequential reactions lead to the production of ceramides, which are precursors for the biosynthesis of sphingomyelins and glycosphingolipids. In the ER, ceramides can be deacylated by CDase to form sphingosine. Sphingosine can be phosphorylated to form sphingosine-1-phosphate (S1P) by SphK1/2. In the Golgi, ceramides transferred by CERT are predestined to synthesize sphingomyelins by the addition of phosphocholine head group or be phosphorylated to form ceramide-1-phosphate. Ceramides transferred by vesicular transport can be glycosylated to form glucosylceramides or galactosylceramides. FAPP2 can transfer glucosylceramides from the *cis*-Golgi to the *trans*-Golgi, where they are converted into lactosylceramides. (2) Bidirectionally, in the plasma membrane, lysosome, mitochondria, and Golgi, sphingomyelins can be converted into ceramides by SMSases. Similarly, ceramide-1-phosphate and glycosphingolipids can be hydrolyzed to form ceramides (not shown). (3) Sphingosine can be recycled to generate ceramides by CerSs. Myriocin is a SPT inhibitor; Fenretinide plays a role in DES1 inhibition; Adiponectin exerts its metabolic improvement functions through CDase signaling; FTY720 and CYM5442 are S1P analogs; D609 and D2-series are SMS inhibitors; Desipramine and SMA-7 et al. are inhibitors of SMase. D-PDMP inhibits both GCS and LCS; AMP-DNM and EtDO-P4 are specific GCS inhibitors. Abbreviations: AMP-DNM, N-(5-adamantane-1-yl-methoxy)-pentyl-1-deoxynoijirimycin; CDase, ceramidase; CERK, ceramide kinase; CerS1-6, ceramide synthase1-6; CERT, ceramide transfer protein; DES1/2, dihydroceramide desaturase1/2; D-PDMP, D-threo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol; EtDO-P4, D-threo-1-ethylendioxyphenyl-2-palmitoylamino-3-pyrrolidino-propanol; FAPP2, four-phosphate adaptor protein 2; FTY720, 2-amino-2-[2-(4-octylphenyl)ethyl] propane-1, 3-diolhydrochloride; GalCerS, galactosylceramide synthase; GCS, glucosylceramide synthase; KSR, 3-ketosphinganine reductase; LCS, lactose CerS; SMS, sphingomyelin synthase; SphK1/2, sphingosine kinase1/2; SPP1/2, S1P phosphatase1/2; SPL, S1P lyase.

Regulation in sphingolipid metabolism

The abundance and species of sphingolipids can be regulated by the availability of different substrates and the activity of various enzymes. It was proven that feeding rodents and rabbits a diet enriched in saturated fats increased their levels of various sphingolipids. In humans, different diets also affect the serum levels of ceramides. Thus, the oversupply of substrate palmitate and serine may promote *de novo* ceramide biosynthesis [8].

In addition, many key enzymes not only influence the synthetic rate but also introduce variations into the basic structure. SPT, acting as a rate-limiting enzyme, can generate a multitude of sphingoid bases by altering the substrate specificity. More specifically, SPT can utilize alanine or glycine instead of serine and prefer myristate or stearate as a fatty acid substrate, instead of the canonical palmitate. The sphingoid bases can be further compounded by an additional double-bond via DES1 and an OH via DES2 [9]. The N-linked fatty acid chains also display wide variations with various chain lengths, unsaturation levels, and hydroxylation levels. Distinct CerS isoforms prefer specific fatty acyl-CoAs with different chain lengths, such as the CerS1 mainly involved in the synthesis of C18:0 ceramides [10].

Distribution and transport of sphingolipids

Plasma sphingolipids are very rare, mainly consisting of the most prevalent sphingomyelins (∼87%), complex glycosphingolipids (9-10%), and ceramides (∼3%) [7]. Insoluble lipids are associated with apolipoprotein (apo), forming lipoproteins for transport in circulation and metabolism. According to their flotation density, lipoproteins are classified as chylomicrons, very-low-density lipoproteins (VLDL), low-density lipoproteins (LDL), or high-density lipoproteins (HDL). Approximately, sphingomyelins are distributed into VLDL/LDL (63–75%) and HDL (25–35%); the most abundant glycosphingolipids, GluCer and LacCer, are present as VLDL (8–14%), LDL (46–60%), and HDL (28–44%), while ceramides are distributed equally as VLDL, LDL, and HDL [11]. How sphingolipids are incorporated into lipoprotein particles is not very clear. Recently, it was demonstrated that microsomal triglyceride transfer protein (MTP), by helping apoB lipoproteins with assembly, plays a crucial role in the plasma levels of sphingomyelin and ceramides, along with GluCer concentrations [12].

Intracellular sphingolipids have specific compartmentalizations and can be transported between different membranes via two routes, as mentioned above: vesicular transport and non-vesicle transporters. Apart from CERT for ceramide transport and FAPP2 for GluCer transport, there are other identified transfer proteins, such as protein spinster homolog 2 (SPNS2) for S1P, C1P transfer protein (CPTP) for C1P, and glycolipid transfer protein (GLTP) for LacCer [9].

Sphingolipids associated with metabolic disease

The metabolic syndrome, mainly driven by obesity, defines a multiplex risk factor for atherosclerotic vascular disease and type 2 diabetes [13]. It is a growing epidemic, composed of dyslipidemia, insulin resistance, hypertension, a pro-thrombotic state, and a pro-inflammatory state. Also, non-alcoholic fatty liver disease (NAFLD), which progresses from steatosis alone to ultimate cirrhosis, is a common metabolic disease. Countless studies have shown that subjects with the above metabolic disorders exhibit greater plasma or tissue levels of one or more of the sphingolipid species [14–16]. Some specific sphingolipids are even emerging as biomarkers and prognostic indicators, such as for cardiovascular disease [17]. Sphingolipid metabolism is strongly associated with the pathogenesis of a repertoire of metabolic diseases. Great efforts have been exerted in identifying the critical sphingolipids, modulating sphingolipid synthesis and catabolism, recognizing the biological functions, identifying the transporting mode, and locating the sphingolipid-dependent signal pathways in diverse pathologies. More importantly, disrupting sphingolipid metabolism has proven to provide novel therapeutic avenues for metabolic disorders, which is the ultimate goal.

The sphingolipidome is extremely diverse and complex, so in this brief review, we focus on relationships between specific sphingolipids and atherosclerosis, a leading cause of worldwide morbidity and mortality, and summarize how metabolic pathways are being regulated for anti-atherosclerosis effects.

Sphingomyelins and atherosclerosis

Human studies investigating the role of sphingomyelins

Employing a novel high-throughput enzymatic method for plasma lipid determination, Jiang et al. [18] systematically assessed plasma sphingomyelin for the first time. Higher plasma sphingomyelin level was found in coronary artery disease (CAD) patients, and proposed as an independent risk factor for CAD. Also, the arterial tissues obtained by coronary artery bypass grafting (CABG) surgery had a higher concentration than normal vascular tissues [19]. Subsequently, the relationships of plasma sphingomyelin with left ventricle systolic dysfunction and clinical cardiac events were investigated [20,21]. What is more, Nelson et al. [22] found the sphingomyelin level was positively correlated with earlier, subclinical atherosclerotic disease, such as carotid intimal–medial wall thickness.

The question as to whether higher plasma sphingomyelin concentration is risk factor for CAD and indicates worse prognosis remains controversial. Yeboah et al. [23] assessed the predictive value in a cohort study of participants free of clinical CAD at baseline. After 5 years of follow-up, the data showed no association between plasma sphingomyelin levels and incident CAD events (myocardial infarction, definite angina, coronary revascularization, resuscitated cardiac arrest, and cardiovascular death). Sigruener et al. [24] found long chain saturated sphingomyelins (23:0, 24:0) seemed to be associated with a protective effect on cardiovascular mortality. Therefore, more studies are needed to determine how sphingomyelins are implicated in cardiovascular diseases and to illuminate the mechanisms involved.

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SMSs as potential therapeutic targets for atherosclerosis

SMSs act on the last step of sphingomyelin synthesis; three homologs have been identified: SMS1, SMS2, and SMSr (SMS-related protein) [25]. SMS1 and SMS2 both possess SMS activity, albeit with distinguishable subcellular localization. SMS1 is mostly located in the Golgi apparatus and SMS2 is mainly found in plasma membranes. SMSr serves as monofunctional ceramide phosphoethanolamine (CPE, a sphingomyelin analog) synthase in the ER.

It was reported that overexpression of SMS1 and SMS2 would increase plasma sphingomyelin level and aggravate atherosclerosis in mice models [26,27]. Conversely, inhibiting SMS1 and SMS2 activity *in vitro* could lower sphingomyelin concentrations [28] and cause blunt NFκB activation responding to inflammatory stimuli [29]. Further *in vivo* experiments showed that atherosclerotic lesions were efficiently decreased and inflammatory responses were lowered in the SMS2 deficient ($SMS2^{-/-}$) mice models [30–32]. Besides, SMS2 deficiency protected mice against tissue and whole-body insulin resistance [33,34], which might be associated with less liver steatosis [35].

Although SMS1 deficiency in macrophages showed similar anti-atherosclerotic effects in a mice model [36], SMS1 is indeed not an appropriate therapeutic target. To date, it has been reported that $SMS1^{-/-}$ mice exhibited several severe abnormalities, including defective insulin secretion [37], progressive hearing loss at a low frequency range [38], $CD4^+$ T-cell dysfunction [39], adipocyte lipid storage dysfunction [40], male spermatogenesis defects [41], and mesenchymal transition of epithelial cells derived from the renal papillary collecting duct [42]. Disruption of SMSr in mice resulted in marginal changes in the plasma levels of sphingomyelin, ceramide, and S1P [43,44]. Thus, compared with SMS1 and SMSr, SMS2 is a more promising therapeutic target for atherosclerosis, but it is a challenge to develop highly specific SMS2 inhibitors without cross-reaction with SMS1.

Tricyclodecan-9-yl-xanthogenate

Tricyclodecan-9-yl-xanthogenate (D-609) was the first compound reported as an inhibitor against sphingomyelin synthesis from *Bacillus cereus* [45,46]. Some undesired properties of D609 and its prodrugs, e.g. instability, low specificity, and week potency hindered them from being practical drugs (Table 1) [47].

D2 group inhibitors

The D2 group inhibitors with more potent and better performance on inhibiting SMS2 than D-609 were found by applying structure-based virtual screening [48]. However, D2-series are still not applicable drugs as they possess a toxic α-aminonitrile group [25].

2-Quinolone SMS2 inhibitors

Recently, Adachi et al. [25] established a high throughput screening-compatible assay condition and identified a 2-quinolone derivative as an SMS2 selective inhibitor. There was no further specific experimental data to evaluate their safety and efficacy.

4-benzyloxybenzo[d]isoxazole-3-amine

Very recently, 4-benzyloxybenzo[d]isoxazole-3-amine derivatives were identified as potent SMS2 selective inhibitors [49]. What was more, one of the compounds were proved to significantly attenuate chronic inflammation in db/db mice after oral dosing for 6 weeks. Undoubtedly, the study provides robust evidence of developing selective SMS2 inhibitors to prevent inflammation-associated diseases, e.g. atherosclerosis.

SMases as potential therapeutic targets for atherosclerosis

There are three types of SMases depending on the optimum pH value: acid, neutral, and alkaline. The secretory form of acid SMase could convert sphingomyelins in lipoproteins into ceramide. The acid SMase was believed to be one potent inducer of subendothelial lipoprotein aggregation and foam cell formation [50]. It remains controversial whether the level of circulating acid SMase activity affects atherosclerosis development. In 2008, Devlin et al. [51] compared acid SMase gene-deficient ($Asm^{-/-}$) mice and non-deficient ($Asm^{+/+}$) mice on the $ApoE^{-/-}$ and $LDLr^{-/-}$ backgrounds, and found the absence of acid SMase strikingly contributed to reductions in lipoprotein retention within early lesions. But in 2011, Leger et al. [52] found no expected accelerated or exacerbated lesions in $ApoE^{-/-}$ mice which concurrently overexpressed acid SMase by injecting recombinant adeno-associated virus. Several compounds have been tested as acid SMase inhibitors, such as the tricyclic antidepressants (desipramine, imipramine, and amitryptiline), SMA-7, and siramesine [53,54]. To date, no references were found to demonstrate that any kind of SMase inhibitors has definite effects on anti-atherosclerosis.

Table 1 Human studies investigating the role of diverse sphingolipid species in atherosclerosis related diseases

Study population	*n*	Correlating sphingolipids	Clinical end points	Reference
Human studies investigating the role of sphingomyelins				
African American and whites with CAD	279 cases and 277 controls	Higher plasma sphingomyelins	CAD	Jiang et al. (2000) [18]
CABG patients	32 CABG patients	Higher concentration of sphingomyelins in arterial tissues	Coronary arteries obtained on CABG surgery	Kummerow et al. (2001) [19]
CAD patients	1102 CAD patients and 444 controls	Higher plasma sphingomyelins	6-year, a predictor for myocardial infarction (MI) and cardiovascular death	Schlitt et al. (2006) [20]
Asymptomatic adults, MESA study	6814 adults	Higher plasma sphingomyelins	Subclinical atherosclerosis (carotid intimal–medial wall thickness, ankle-arm blood pressure, and Agatston coronary artery calcium score)	Nelson et al. (2006) [22]
Adults free of clinical CAD in MESA	6809 adults	Plasma sphingomyelins, not associated with risk of incident CAD	5-year, incident CAD events (MI, resuscitated cardiac arrest, angina, cardiovascular death and revascularization)	Yeboah et al. (2009) [23]
Chinese, participants underwent coronary angiography for chest pain	732 adults	Plasma sphingomyelins	CAD, left ventricle systolic dysfunction	Chen et al. (2011) [21]
Caucasian, LURIC study	2538 CAD patients and 733 controls	Protetive sphingomyelins (23:0; 24:0); risky sphingomyelin species (16:0; 24:1) and risky ceramides (16:0; 24:1)	8-year, total and/ or CAD mortality	Sigruener et al. (2014) [24]
Human studies investigating the role of ceramides on T2D				
Obese T2D, adults	13 patients and 14 lean controls	Higher ceramide species (C18:1, 18:0, 20:0, 24:1, and 24:0)	T2D	Haus et al. (2009) [59]
Obese adults, T2D	13 lean, 5 obese, 12 T2D	Higher total ceramides	T2D	Boon et al. (2013) [62]
Obese female T2D, children, and adolescents	14 patients and 14 lean controls	Higher ceramide species (C18:0, 20:0, and 22:0), higher dihydroceramide (C24:1)	Obese T2D	Lopez et al. (2013) [60]
T2D, athletes, adults	15 T2D, 15 athletes, and 14 obese controls	Higher ceramide species (C18:0, 20:0, and 24:1) and total dihydroceramide	T2D	Bergman et al. (2015) [61]
Two cohorts: DESIR, western France; CoLaus, Switzerland	298, 300 participants without T2D	Higher dihydroceramides, higher ceramide species (C18:0, 20:0, and 22:0)	9-year, 5-year, incident T2D	Wigger et al. (2017) [65]
1557 multi-ethnic adults, the Dallas Heart Study	1557 participants without T2D	Short-chain saturated ceramide (C16:0, 18:0), longer chain polyunsaturated ceramides (C24:2, 30:10, and 32:11)	7-year, incident diabetes	Neeland et al. (2018) [64]
American Indian in SHFS	2086 participants without diabetes	Higher ceramide species (C16:0, 18:0, 20:0, and 22:0); sphingomyelin; GluCer; LacCer	5.4-year, pre-diabetes	Lemaitre et al. (2018) [63]
Human studies investigating the role of ceramides on CAD				
CAD patients	33 CAD patients	Higher ceramides	CAD	Mello et al. (2009) [135]
Chinese, CAD patients	304 CAD patients and 52 controls	Higher ceramides, higher sphingomyelins	ACS	Pan et al. (2014) [69]
German, CAD patients, LURIC	258 CAD patients and 187 controls	Higher ceramide species (C16:0 and 18:0); LacCer, GluCer, globotriaosylceramide	3-year, cardiovascular death	Tarasov et al. (2014) [71]
European, CAD patients, ATHEROREMO-IVUS	581 CAD patients	Higher ceramide species (C16:0, 24:0, and 16:0/24:0 ratio); LacCer (C18:0)	1-year, vulnerable plaque characteristics, MACE	Cheng et al. (2015) [66]
Chinese, CHF patients	423 CHF patients	Higher ceramides	4.4-year, mortality	Yu et al. (2015) [70]
Healthy volunteers, BLSA	433 participants	Higher ceramide species (C18:0, 20:0, and 24:1); higher dihydroceramides	Lower aerobic capacity	Fabbri et al. (2016) [72]

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Table 1 Human studies investigating the role of diverse sphingolipid species in atherosclerosis related diseases (Continued)

Study population	*n*	Correlating sphingolipids	Clinical end points	Reference
Three CAD cohorts: Corogene (Finnish); BECAC (Norway); PUM-ACS (Swiss)	80 stable CAD and 80 controls; 51 stable CAD and 1586 controls; 81 ACS and 1506 controls	Higher ceramide species (C16:0, 18:0, 24:1, and 16:0/24:0 ratio)	2.5-year; 4.6-year; 1-year. Cardiovascular death	Laaksonen et al. (2016) [67]
Finnish, healthy individuals	8101	Higher ceramide species (C16:0, 18:0, 24:1 and ratios with 24:0)	13-year, MACE	Havulinna et al. (2016) [73]
European Caucasians, the PREDIMED trial	980 participants	Higher ceramide species (C16:0, C22:0, C24:0 and C24:1)	4.5-year, non-fatal AMI, non-fatal stroke, or cardiovascular death	Wang et al. (2017) [74]
Participants before nonurgent coronary angiography	265 CAD and 230 No CAD	Higher ceramide species (C16:0, 18:0, 24:1 and ratios with 24:0)	12.8-year, MACE	Meeusen et al. (2018) [68]
Two cohorts: FHS and SHIP participants	2642 and 3134	Lower plasma C24:0/C16:0, C22:0/C16:0 ceramide ratios	6-year and 8.24-year, incident CAD and total mortality	Peterson et al. (2018) [15]
Human studies investigating the role of glycosphingolipids				
Autopsy (died with atherosclerosis)	3	Higher concentration of GluCer and LacCer in arterial tissues	Atherosclerotic plaque	Chatterjee et al. (1997) [105]
CAD patients	140 CAD patients and 80 controls	Higher dihexosylceramide	Unstable CAD	Meikle et al. (2011) [109]
Human studies investigating the role of S1P				
CAD patients	126 mild, 102 intermediate, and 90 severe CAD	Higher S1P	CAD	Deutschman et al. (2003) [136]
MI patients	22 MI patients and 21 controls	Lower S1P	MI	Knapp et al. (2009) [125]
CAD patients	83 MI, 95 stable CAD, and 85 healthy controls	Lower HDL-bound S1P, higher non-HDL-bound S1P	Stable CAD and MI	Sattler et al. (2010) [126]
Danes, CCHS	95 CAD and 109 No CAD	Lower HDL-bound S1P, dihydro-S1P and ceramide (C24:1)	CAD	Argraves et al. (2011) [127]
MI patients	32 MI and 32 controls	Lower S1P	MI	Knapp et al. (2013) [128]
CAD patients	59	Lower HDL-bound S1P	0.5-year, CAD	Katherine Sattler et al. (2014) [129]
Patients with ischemic heart disease	74	Lower S1P and sphingomyelins	Reduced left ventricular ejection fraction	Polzin et al. (2017) [130]

Abbreviations: ACS, acute coronary syndrome; ATHEROREMO-IVUS, Atherosclerosis Intravascular Ultrasound Study; BECAC, Bergen Coronary Angiography Cohort; BLSA, Baltimore Longitudinal Study of Aging study; CABG, coronary artery bypass grafting; CAD, coronary artery disease; CCHS, Copenhagen City Heart Study; CHF, chronic heart failure; CoLaus, Cohorte Lausannoise study; DESIR, Data from the Epidemiological Study on the Insulin Resistance Syndrome; FHS, Framingham Heart Study; LURIC, Ludwigshafen Risk and Cardiovascular Health; MACE, major adverse cardiac events (defined as all-cause mortality, ACS and unplanned coronary revascularization); MESA, Multi-Ethnic Study of Atherosclerosis; MI, myocardial infarction; PREDIMED, the Prevencion con Dieta Mediterranea; SHFS, Strong Heart Family Study; SHIP, Study of Health in Pomerania; SPUM-ACS, Special Program University Medicine-Inflammation in Acute Coronary Syndrome; T2D, type 2 diabetes.

Ceramides and atherosclerosis

Human studies investigating the role of ceramides

Ceramides are found accumulated in atherosclerotic lesions and in obesity [55]. They are involved in insulin resistance [56], lipoprotein uptake and aggregation [57], vascular tone [58], inflammation, oxidative stress, and apoptosis. Moreover, circulating ceramides are correlated strongly with diabetes and some specific species have served as predictive biomarkers of future adverse cardiovascular events [16]. Here, we summarize the related evidence.

Since 2009, several small cross-sectional studies showed diabetic patients had elevated plasma ceramide levels [59–62]. Lately, prospective studies based on large population were reported, further revealed that higher concentrations of several ceramide species (e.g. C16:0, 18:0, and dihydroceramides) were associated with fasting insulin levels [63,64] and an increased risk of future diabetes in individuals without diabetes [65]. However, after adjustment for age, sex, and race, none of the ceramide species was independently associated with incident type 2 diabetes [64].

In studies of patients with CAD, ceramide species (C16:0, 18:0, 22:0, 22:0, 24:0, and 24:1) were quantitated, and also used in ratios to perform risk estimation for plaque instability [66], adverse CAD incidents [67–69], and future mortality [15,70,71]. In studies of healthy individuals, serum ceramides were strongly associated with lower aerobic capacity [72] and could also forecast adverse cardiovascular outcomes [73,74]. Research findings from different groups

were not totally consistent, and whether C24-ceramides were cardioprotective remained controversial [24]. The differences may relate to patient selection and different quantitation methods. In general, these strongly supportive evidence of plasma ceramides driving cardiometabolic dysfunction provided the basis for developing ceramide-reducing interventions.

SPT as a potential therapeutic target for atherosclerosis

Myriocin, a commonly used SPT inhibitor (also known as thermozymocidin) inhibits the first step in the *de novo* synthesis pathway, originating from a traditional Chinese medicine called *Isaria sinclairii*, classified as a fungal species. Park et al. first investigated the beneficial effects of myriocin in ApoE$^{-/-}$ mice [75]. Myriocin administration could dramatically prevent the progression of atherosclerotic lesions and even regress the pre-existing plaques, with lower plasma lipid levels, including total cholesterols (TC), triglycerides (TG), ceramides, sphingomyelins, S1P, sphingosine, and glycosphingolipids [76–79]. Besides, myriocin was found to improve insulin sensitivity [80–82], ameliorate hepatic lipid accumulation and further reverse NAFLD [83,84]. Because myriocin inhibits the initial step in the synthesis of a number of sphingolipids, the identity of the critical species is unknown. Nevertheless, the experimental results supported the hypothesis that myriocin, an SPT inhibitor, could be a novel therapeutic drug for atherosclerosis and related diseases.

CerSs as potential therapeutic targets for atherosclerosis

Six fatty acyl selective CerSs (CerS1–6) exist in mammals, distributed in distinct tissues [85]. The regulation of CerSs is elaborate at multiple levels, and the enzyme activity may present inconsistently with the mRNA or protein expression levels. Mutations in CerSs genes or deregulation in the CerSs' contents and enzyme activity are all correlated with human disease. Over the past few years, each CerS knockout was established in mice, showing that specific CerS deactivation may cause serious impacts and may be lethal, such as CerS1-null mice exhibiting Purkinje cell death [86], CerS2-null mice generating myelin sheath defects and hepatocellular carcinomas [87], CerS3-null mice dying shortly after birth [88], and CerS4-null mice developing alopecia [89]. Relatively, CerS5/6-null mice showed a mild phenotype, presenting some behavioral abnormalities [90].

Turpin et al. [91] measured the gene expression of CerS1, 2, 4, 5, and 6 in human adipose tissues and identified that only CerS6 expression was positively correlated with obesity. Further, they generated conventional CerS6-deficient mice, as well as specific brown adipose tissue and liver CerS6 deletion mice, and then demonstrated CerS6 ablation could up-regulate β-oxidation and increase lipid utilization [91]. But so far, there are no conclusive data proving that targetting specifically unique CerS could benefit atherosclerotic degression, and no pharmacological inhibitors with a high degree selectivity for one certain CerS are available. Given that inhibiting CerS6 is good for obesity and diabetes, it will probably restrain atherosclerosis development, but needs further novel studies to provide favorable evidence.

DESs as potential therapeutic targets for atherosclerosis

DESs catalyze the last step in *de novo* ceramide biosynthesis, which is responsible for the conversion of dihydroceramide into ceramide. The dominant isoform is DES1, distributed in most tissues. In the last few years, multiple publications have demonstrated that dihydroceramides are implicated in a far wider spectrum of biological functions than previously thought [92]. Heterozygous deletion of DES1 in mice was also demonstrated to prevent diet-induced vascular dysfunction and hypertension in mice [93]. Importantly, pharmacological inhibition of DES1 protected humans from obesity and insulin resistance. The most notable inhibitory compound is fenretinide, which has been tested in several clinical trials [94]. Fenretinide treatment could positively balance the metabolic profile by improving insulin sensitivity in overweight premenopausal women [95]. Also, long-term therapy with fenretinide could alleviate diet-induced adiposity and dyslipidemia and prevent hepatic steatosis in mice [96–98]. Altogether, although there was no direct evidence on inhibited Des1 preventing atherosclerosis, it is reasonable to hypothesize DES1 as a effective target for normalizing vascular homeostasis by controlling ceramide production.

CDase as a potential therapeutic target for atherosclerosis

Since inhibitors targetting ceramide biosynthesis are potential means for the treatment of metabolic syndrome, promoting ceramide degradation may provide similar benefits. Deacylation of ceramide species is initiated by the family of enzymes called CDase. Chavez et al. [99] demonstrated that overexpression CDase negated the inhibitory effects of exogenous free fatty acids on muscle insulin sensitivity through blocking ceramide accumulation *in vitro*. Holland et al. [100] found that adiponectin, a protein hormone has antidiabetic and cardioprotective properties, could stimulate CDase activity and further lower cellular ceramides. CDase was found to have some homology with the adiponectin

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receptors, AdipoR1 and AdipoR2. *In vivo* studies, targetted induction of ceramide degradation in adipose tissue or liver by overexpressing transgenic CDase was found sufficient to recapitulate most adiponectin actions [14]. Moreover, overexpression of AdipoR1 or AdipoR2 in either the adipocyte or hepatocyte revealed enhanced CDase activation, improved hyperglycemia and glucose intolerance, while opposing hepatic steatosis [101]. Together, adiponectin exerted its metabolic improvement functions through CDase signaling [102]. These findings support the strategy of CDase replacement as a potential treatment for atherosclerosis.

Glycosphingolipids and atherosclerosis

Human studies investigating the role of glycosphingolipids

Glycosphingolipids are extremely diverse, composed of hydrophobic ceramide scaffolds and hydrophilic sugar chains. Glycosyl groups are different, such as D-glucose, D-galactose, D-acetylglucosamine, D-acetylgalactosamine, L-fucose, D-mannose, and sialic acid. Sphingoglycolipids can be generally divided into cerebrosides, sulphatides, globosides, and gangliosides. According to the number of glycosides, they can be divided into monohexosylceramide (MHC), dihexosylceramide (DHC), trihexosylceramide (THC), and tetrahexosylceramide.

Associating glycosphingolipids with atherosclerosis was based on the following observations: gangliosides [103], GluCer and LacCer accumulate in the atherosclerotic plaques [104,105]; GluCer and LacCer stimulate the proliferation of aortic smooth muscle [106]; GluCer and LacCer suppress apoE production in macrophages and cholesterol-loaded foam cells [107] and LacCer stimulates the recruitment of monocytes to the endothelium [108]. Recently, specific plasma glycosphingolipids were identified as discriminatory risk-associated lipids for unstable CAD and CAD mortality, such as DHC [109], GluCer and LacCer [66,71]. Our research team successfully separated GalCer and GluCer, a pair of isomers [110]. We discovered that the plasma GalCer levels were increasing in atherosclerotic patients, rather than GluCer. Although the enormous number of distinct glycosphingolipid species has made it difficult to determine which one is critical, it is suggested that inhibiting glycosphingolipid synthesis may be an effective approach for the treatment of atherosclerosis.

Glycosphingolipid synthase inhibitors

D-threo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol

D-threo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol (D-PDMP), an analog of GluCer, could inhibit both GCS and LCS activity [111]. Chatterjee et al. [112] confirmed that oral D-PDMP could dose-dependently ameliorate atherosclerosis and vascular stiffness in both ApoE$^{-/-}$ mice and rabbits fed a high-fat diet. They further proposed that D-PDMP could slow the progression of cardiac hypertrophy in ApoE$^{-/-}$ mice by inhibiting mitogen-activated protein kinase (MAPK) phosphorylation [113,114]. Thus, D-PDMP could be accepted as a potential desirable compound for treating cardiovascular diseases.

N-(5-adamantane-1-yl-methoxy)-pentyl-1-deoxynoijirimycin

The iminosugar N-(5-adamantane-1-yl-methoxy)-pentyl-1-deoxynoijirimycin (AMP-DNM) is another inhibitor, specifically inhibiting the activity of GCS. Aerts et al. [115] found this small molecule inhibitor could improve both muscle and hepatic insulin sensitivity in rodent models. Subsequent studies reported that AMP-DNM treatment could also reverse hepatic steatosis [116], improve adipocyte function, and reduce inflammation in leptin-deficient obese mice [117]. Bietrix et al. [118] evaluated the beneficial effect of AMP-DNM on atherosclerosis development in both APOE*3 Leiden mice and LDLr$^{-/-}$ mice. Collectively, AMP-DNM can be suggested as a possible valid approach for the prevention or treatment of atherosclerosis.

D-threo-1-ethylendioxyphenyl-2-palmitoylamino-3-pyrrolidino-propanol

D-threo-1-ethylendioxyphenyl-2-palmitoylamino-3-pyrrolidino-propanol (EtDO-P4), another specific GCS inhibitor, can reduce plasma and tissue glycosphingolipid concentrations [119]. Glaros et al. [120] assessed the impact of EtDO-P4 on atherosclerosis in apoE$^{-/-}$ mice. Unexpectedly, EtDO-P4 administration did not result in decreased lesion areas, although the plasma GluCer and LacCer concentrations were reduced. Unlike D-PDMP and AMP-DNM, EtDO-P4 did not affect plasma cholesterol or TG levels. At present, it is not clear whether one or more glycosphingolipids take part in atherosclerosis, and whether inhibition of glycosphingolipid synthesis *per se* has an antiatherogenic impact.

S1P and atherosclerosis

Human studies investigating the role of S1P

S1P is a bioactive lipid, primarily carried by apoM on HDL, and signals its G protein-coupled receptors, named S1PR1-5 [5], S1P is degraded by two pathways: dephosphorylation by S1P phosphatases (SPP1/2) and irreversible cleavage by S1P lyase (SPL). S1P has dual nature in the pathogenesis of atherosclerosis: S1P preserves endothelium via S1PR1/3 [121]; inhibits smooth muscle cells migration via S1PR2; and possesses anti-inflammatory properties via S1PR4 [122]; while S1P also promotes inflammatory monocyte/macrophage recruitment through S1PR2/3 [123,124]. Although it is yet not concensus, several clinical data reported that plasma S1P concentrations were negatively associated with prevalence and severity of CAD and myocardial infarction [125–130].

S1P receptor agonists

Nofer et al. [131] reported that 2-amino-2-[2-(4-octylphenyl)ethyl] propane-1, 3-diolhydrochloride (FTY720), a synthetic S1P analog targetting $S1PR_1$, $S1PR_3$, $S1PR_4$, and $S1PR_5$, could dose-dependently retard the progression of atherosclerosis in $LDLr^{-/-}$ mice. Another study team reached a similar conclusion with $ApoE^{-/-}$ mice [132]. As FTY720 is a non-selective S1P analog, Nofer et al. [131] further investigated the antiatherogenic effects of S1PR1-selective agonists, such as CYM5442 and KRP-203, and demonstrated that activating S1PR1 at least partially mediated atheroprotective effects [133,134]. Thus, S1P analogs may be promising bullets against atherosclerosis.

Conclusions

The worldwide burden of metabolic diseases, especially atherosclerotic disorder, is staggering. Understanding the precise roles of sphingolipid metabolites and related enzymes on the development of atherosclerosis will invite new available treatments. This brief review mainly focussed on seeking therapeutic targets for atherosclerosis from the complicated sphingolipids metabolism. The above possible targets or inhibitors shed significant light on those patients suffering from atherosclerosis as well as related diseases, although further investigation and refining is necessary.

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Competing interests

The authors declare that there are no competing interests associated with the manuscript.

Abbreviations

AMP-DNM, N-(5-adamantane-1-yl-methoxy)-pentyl-1-deoxynoijirimycin; Apo, apolipoprotein; CABG, coronary artery bypass grafting; CAD, coronary artery disease; CDase, ceramidase; CERK, ceramide kinase; CerS, ceramide synthase; CERT, ceramide transfer protein; CoA, coenzyme A; CPE, ceramide phosphoethanolamine; CPTP, C1P transfer protein; C1P, ceramide-1-phosphate; DES, dihydroceramide desaturase; DHC, dihexosylceramide; D-PDMP, D-threo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol; D-609, tricyclodecan-9-yl-xanthogenate; ER, endoplasmic reticulum; EtDO-P4, D-threo-1-ethylendioxyphenyl-2-palmitoylamino-3-pyrrolidino-propanol; FAPP2, four-phosphate adapter protein 2; FTY720, 2-amino-2-[2-(4-octylphenyl)ethyl] propane-1, 3-diolhydrochloride; GalCer, galactosylceramide synthase; GalT-2, glucosylceramide β1→4 galactosyltransferase; GCS, glucosylceramide synthase; GLTP, glycolipid transfer protein; GluCer, glucosylceramide; HDL, high-density lipoprotein; KSR, 3-ketosphinganine reductase; LacCer , lactosylceramide; LCS, lactose ceramide synthase; LDL, low-density lipoprotein; MAPK, mitogen-activated protein kinase; MHC, monohexosylceramide; MTP, microsomal triglyceride transfer protein; NAFLD, non-alcoholic fatty liver disease; SMase, sphingomyelinase; SMS, sphingomyelin synthase; SMSr, sphingomyelin synthase-related protein; SphK, sphingosine kinase; SPL , S1P lyase; SPNS2, spinster homolog 2; SPP, S1P phosphatase; SPT, serine palmitoyltransferase; S1P, sphingosine-1-phosphate; TC, total cholesterol; TG, triglyceride; THC, trihexosylceramide; VLDL, very-low-density lipoprotein.

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