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Metabolism and Target Organ Damage

Review

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Dysregulation of sphingolipid metabolism in liver fibrosis

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Abstract

The dysregulation of sphingolipid metabolism emerges as a pivotal factor in the development and progression of liver fibrosis, a condition marked by the overproduction and buildup of extracellular matrix proteins that can lead to liver cirrhosis and failure. Sphingolipids, a diverse class of lipids essential for cellular structure and signaling, are integral to numerous biological functions such as cellular proliferation, morphological differentiation, and programmed cell death. In the context of liver fibrosis, changes in sphingolipid metabolism have been associated with the activation of hepatic stellate cells, the primary cells responsible for fibrogenesis in the liver. These metabolic disruptions lead to an imbalance between profibrotic and antifibrotic sphingolipids, notably sphingosine-1-phosphate and ceramide, contributing to the pathophysiological mechanisms that drive fibrosis. The intricate relationship between sphingolipid metabolism and fibrotic pathways underscores the potential of targeting sphingolipid metabolic enzymes and receptors as therapeutic strategies to mitigate liver fibrosis. The core of this review delves into how disruptions in sphingolipid metabolism contribute to liver fibrosis, exploring biomarkers and potential therapeutic targets. Challenges in research and future directions for comprehensively understanding sphingolipid roles in liver fibrosis are discussed, aiming to open new pathways for therapeutic intervention.

Keywords: Sphingolipids, liver fibrosis, SphK, S1P

INTRODUCTION

Liver fibrosis is marked by a significant buildup of extracellular matrix (ECM) proteins, especially collagen, which represents a crucial phase in the advancement of chronic liver diseases toward cirrhosis and ultimate liver failure[1]. This pathological condition arises as a consequence of sustained liver injury, instigated by a plethora of insults, including chronic viral infections (e.g., hepatitis B and C), alcohol abuse, metabolic dysfunction-associated steatotic liver disease (MASLD), and autoimmune hepatitis[2]. The pathological significance of liver fibrosis stems from its ability to disrupt the liver's architecture and function, ultimately leading to cirrhosis—a stage defined by severe scarring, compromised liver function, and increased risk of liver cancer[3]. The transition from fibrosis to cirrhosis and liver failure highlights a pressing need for therapeutic interventions that can halt or reverse this progression[4].

Sphingolipids are essential components of cell membranes, functioning not merely as structural entities but also playing active roles in various cellular processes[5]. These lipids are characterized by their backbone, a long-chain amino alcohol known as sphingosine[6]. Acylation of sphingosine with a long-chain fatty acid forms ceramide, which serves as a central hub in sphingolipid metabolism, giving rise to various complex sphingolipids such as sphingomyelins (predominant in the myelin sheath of nerve cells) and glycosphingolipids (involved in cell-cell recognition and signaling)[7]. The diversity in sphingolipid structures corresponds to a broad spectrum of biological functions, including roles in apoptosis, cell proliferation, inflammation, and intracellular signaling[8]. The dynamic balance between ceramide, sphingosine, sphingosine-1-phosphate (S1P), and other sphingolipid metabolites is crucial for determining cell fate, underscoring the importance of regulated sphingolipid metabolism in maintaining cellular homeostasis[9].

The exploration of sphingolipid metabolism in the context of liver fibrosis is motivated by several pivotal considerations. Sphingolipids, particularly ceramides and S1P, play central roles in critical cellular processes, such as apoptosis, proliferation, and inflammation, which are vital in the pathogenesis of liver fibrosis[10-13]. Disruption in the balance of these sphingolipid metabolites has been implicated in activating hepatic stellate cells (HSCs), the key fibrogenic cells in the liver[10]. Upon liver injury, HSCs transform from a quiescent state into an activated, myofibroblast-like phenotype, proliferating and producing excessive ECM components[14]. This transition is influenced by sphingolipid cell signaling pathways, with ceramide promoting apoptosis and inhibiting proliferation and S1P favoring survival and proliferation, thus contributing to the fibrotic process[10,11]. Furthermore, sphingolipids regulate inflammation, a critical driver of fibrosis development and progression[15]. The interplay between different sphingolipid species and their receptors orchestrates inflammatory responses, influencing the recruitment and activation of immune cells in the liver[10].

Given these multifaceted roles, sphingolipid metabolism emerges as a compelling target for therapeutic intervention in liver fibrosis. Modulating sphingolipid pathways offers the potential to attenuate HSC activation, mitigate inflammation, and thus impede the fibrogenic process. Additionally, components of the sphingolipid metabolic pathway may serve as biomarkers for diagnosing and monitoring liver fibrosis, providing valuable tools for clinical management[16]. This review endeavors to discuss the complex interrelations between sphingolipid metabolism and liver fibrosis, aiming to shed light on the underlying mechanisms and explore the therapeutic and diagnostic potential of sphingolipids in this context. By doing so, it seeks to contribute to the broader effort to develop targeted therapies that can effectively combat liver fibrosis and prevent its progression to cirrhosis and liver failure.

SPHINGOLIPID METABOLISM

De novo synthesis of sphingolipids

The production of sphingolipids begins in the endoplasmic reticulum, where serine and a fatty acyl-CoA, typically palmitoyl-CoA, are condensed in a reaction catalyzed by serine palmitoyltransferase (SPT)[17]. This reaction forms 3-ketodihydrosphingosine, which is then reduced to dihydrosphingosine (sphinganine)[18], as shown in Figure 1. Dihydrosphingosine can be acylated by ceramide synthases to generate various dihydroceramides, differing by the acyl chain length[19]. The introduction of a trans double bond by dihydroceramide desaturase converts dihydroceramide into ceramide[20].

Complex sphingolipid formation

Ceramide acts as a foundational precursor in the synthesis of more complex sphingolipids. It is transported to the Golgi apparatus, either through vesicular transport or by the ceramide transport proteins, where it undergoes further transformation[7,15]. In the Golgi apparatus, ceramide is converted into sphingomyelin, a major component of the plasma membrane, by sphingomyelin synthase or into glucosylceramides and various glycosphingolipids through the addition of one or more sugar residues[21,22] [Figure 1]. The specific functions of these complex sphingolipids vary, influencing cell-cell communication, recognition, and signal transduction pathways[23].

Sphingolipid cycle and degradation

The transport of sphingomyelin and glucosylceramides to the plasma membrane is facilitated through vesicular transport[24]. Once in the plasma membrane, sphingomyelin undergoes hydrolysis by neutral sphingomyelinase (SMase) to produce ceramide[15]. Sphingomyelin, along with glycosphingolipids, can be incorporated into endocytic vesicles for transport to the lysosomal compartment. In the lysosome, sphingomyelin is hydrolyzed by acid SMase to produce ceramide, and glycosphingolipids undergo a stepwise removal of sugar residues until ceramide is generated[25]. Ceramide can undergo hydrolysis by ceramidases, generating sphingosine, which may be phosphorylated to form S1P by sphingosine kinases (SphKs)[26,27]. Sphingosine can also be recycled back into ceramide in the endoplasmic reticulum via the salvage pathway [Figure 1]. Additionally, S1P can be reverted to sphingosine through dephosphorylation or irreversibly degraded by S1P lyase (SPL), leading to an exit from the sphingolipid cycle[8,28].

Implications in disease

Dysregulation of sphingolipid metabolism has been implicated in numerous diseases. For instance, elevated ceramide levels have been linked to insulin resistance and hepatic steatosis in MASLD[29]. In viral hepatitis, alterations in sphingolipid metabolism have been observed to affect virus entry and replication[30]. Furthermore, alterations in sphingolipid metabolism have been linked to neurodegenerative disorders, such as Alzheimer's disease and Parkinson's disease, where the accumulation of specific sphingolipids contributes to neuronal dysfunction and death[31]. Similarly, cancer cells often exhibit altered sphingolipid metabolism, which supports tumor growth, proliferation, and resistance to apoptosis[32]. Sphingolipid dysregulation impact also extends to inflammatory and autoimmune diseases, where S1P plays a role in immune cell trafficking and inflammation[33]. In metabolic disorders, such as diabetes, disruptions in sphingolipid levels affect insulin signaling and glucose homeostasis[34,35]. Additionally, cardiovascular diseases have been linked to sphingolipid imbalances, with ceramide and S1P influencing processes like atherosclerosis and heart failure[36,37]. Sex-specific differences in serum sphingomyelin species may influence the progression of liver fibrosis differently in males and females, suggesting that these lipids could play distinct roles in disease based on sex[38].

Sphingolipid metabolism represents a vital aspect of cellular physiology with far-reaching implications for health and disease. In the context of liver disease, particularly hepatic fibrosis, dysregulated sphingolipid

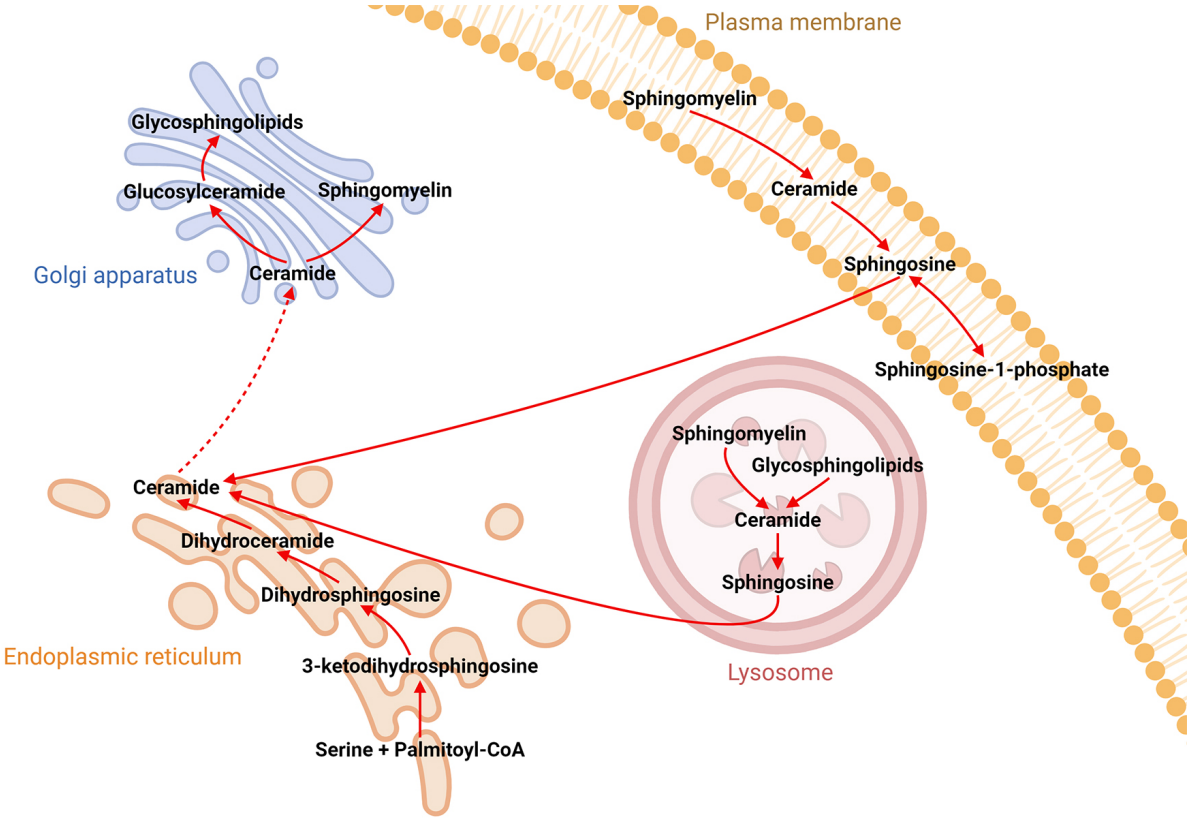

Figure 1. Key reactions of the sphingolipid metabolic pathway. This diagram outlines the sphingolipid metabolic pathway. Ceramide synthesis initiates in the endoplasmic reticulum and is then conveyed to the Golgi apparatus, where it is transformed into various complex sphingolipids. In addition to its formation via de novo synthesis, ceramide can also be derived from the hydrolysis of sphingomyelin and the removal of sugar residues from glycosphingolipids. Once produced, ceramide can be converted into sphingosine, which can either be phosphorylated to form sphingosine-1-phosphate or recycled back into ceramide within the endoplasmic reticulum. (Created with BioRender.com)

pathways contribute significantly to the progression of fibrosis through mechanisms involving inflammation and ECM deposition. As research continues to unravel the complexities of sphingolipid metabolism, targeting these pathways holds promise for developing novel treatments for fibrosis, offering hope for conditions like MASH that currently lack effective therapies.

LIVER FIBROSIS: PATHOGENESIS AND PROGRESSION

Liver fibrosis represents a critical stage in the progression of chronic liver diseases, which can culminate in cirrhosis, liver failure, or hepatocellular carcinoma. The pathogenesis of liver fibrosis is complex, involving the interplay of various cell types and molecular signaling pathways that respond to chronic liver injury. This intricate process revolves primarily around the excessive deposition of ECM components, disrupting liver architecture and function[2,39]. Liver fibrosis is linked with an increased risk of cardiovascular diseases, type 2 diabetes, and other metabolic complications[40]. Understanding the cellular and molecular mechanisms that drive liver fibrosis is paramount for developing effective therapeutic strategies.

Cellular contributors to liver fibrosis

HSCs

HSCs are central to the fibrogenic response in liver fibrosis. In their quiescent state, HSCs function in storing vitamin A and regulating sinusoidal blood flow[41]. However, upon liver injury, they undergo a

dramatic transformation into a myofibroblast-like phenotype, characterized by the loss of vitamin A, proliferation, enhanced contractility, and increased production of ECM components such as collagen types I and III[42]. This transformation is driven by various factors, including oxidative stress, hepatocyte apoptotic bodies, and paracrine signals from neighboring cells such as Kupffer cells and damaged endothelial cells[43].

Activated HSCs not only secrete ECM components but also express α-smooth muscle actin (α-SMA), a marker of myofibroblast activation, which contributes to the contractile properties that can exacerbate portal hypertension, a common complication of advanced liver fibrosis[44].

Kupffer cells

Kupffer cells, the liver's resident macrophages, are pivotal in initiating and perpetuating the fibrotic response[45]. Upon liver injury, Kupffer cells become activated and release many pro-inflammatory and profibrotic mediators. These include cytokines such as tumor necrosis factor-alpha (TNF-α), interleukins (IL-1β, IL-6), and chemokines that recruit additional inflammatory cells to the site of injury[46]. Moreover, Kupffer cells secrete growth factors, including transforming growth factor-beta (TGF-β), a key fibrogenic cytokine that directly stimulates HSC activation and collagen synthesis[47].

Cholangiocytes

Cholangiocytes, the epithelial cells lining the bile ducts of the liver, play a significant role in the pathogenesis of liver fibrosis, particularly in cholestatic liver diseases. These cells respond to bile duct injury or obstruction by undergoing phenotypic changes, proliferating, and secreting a range of pro-inflammatory and pro-fibrogenic cytokines that activate HSCs and portal fibroblasts. As a result, these activated cells produce excessive collagen and ECM components, contributing to the fibrotic tissue buildup[48,49]. Cholangiocytes also contribute to fibrosis through the dysregulation of bile acid homeostasis and increased expression of fibrogenic mediators such as connective tissue growth factor and TGF-β. Their involvement is pivotal in the progression of fibrosis, as they not only perpetuate inflammation but also directly influence the extent and severity of fibrotic changes within the liver, making them a critical target for therapeutic interventions aimed at mitigating fibrosis and promoting liver regeneration[50].

Mast cells

Mast cells play a significant role in the pathogenesis of liver fibrosis, primarily through their ability to release various mediators that influence fibrogenesis. When activated, mast cells release a range of substances, including histamine, cytokines such as TGF-β, synaptophysin 9, and tryptase, which directly stimulate HSCs to proliferate and produce collagen. The interaction between mast cells and HSCs promotes the deposition of the ECM, leading to progressive scarring of the liver[51].

Other cell types

Liver fibrosis is not solely the domain of HSCs, Kupffer cells, cholangiocytes, and mast cells; several other cell populations contribute to the fibrogenic process. Damaged hepatocytes release reactive oxygen species (ROS) and fibrogenic mediators, triggering the recruitment of inflammatory cells and enhancing the fibrogenic activities of liver myofibroblasts through apoptosis[52]. Portal fibroblasts can undergo a similar activation process as HSCs, contributing to ECM deposition, particularly around bile ducts and portal areas[53]. Moreover, bone marrow-derived fibrocytes can differentiate into collagen-producing myofibroblasts and migrate to the liver, further augmenting the fibrotic response[54]. Liver endothelial cells, through capillarization, lose their fenestrations and contribute to vascular abnormalities seen in advanced fibrosis[55]. Neutrophils exacerbate liver fibrosis by activating HSCs through ROS and myeloperoxidase production, which, in turn, secrete granulocyte-macrophage colony-stimulating factor and IL-15 to prolong

neutrophil survival and cytokine-induced chemoattractants to boost neutrophil recruitment. These pathophysiological processes create a self-sustaining feedback loop that amplifies the fibrotic response[56]. Dendritic cells may influence fibrosis by modulating the activity of other immune cells, such as natural killer cells and $CD8^+$ T cells, and through the release of metalloproteinases[57].

Molecular pathways in liver fibrosis

TGF-β Signaling

The TGF-β signaling pathway is a cornerstone in the development of liver fibrosis. It is a potent inducer of ECM production and inhibits ECM degradation, thus promoting fibrosis. When TGF-β binds to its receptors on HSCs, it activates both Smad-dependent and Smad-independent pathways. In the independent pathway, the Smad pathway translocates into the nucleus to promote the transcription of fibrogenic genes such as collagen and tissue inhibitors of metalloproteinases, tipping the balance toward ECM accumulation. TGF-β stimulates HSCs to proliferate, produce collagen, and transition to their activated state. It also downregulates matrix metalloproteinases and upregulates tissue inhibitors of metalloproteinases, tipping the balance toward ECM accumulation[58]. Notably, a recent investigation into thioacetamide-induced liver fibrosis in rats and HSCs revealed that aspirin mitigated liver fibrosis and decreased collagen production by inhibiting the TGF-β signaling pathway[59].

Platelet-derived growth factor

Platelet-derived growth factor (PDGF) is a critical mitogen for HSCs, driving their proliferation and migration. PDGF exerts its effects by binding to PDGFR-α and PDGFR-β on the surface of activated HSCs, leading to the activation of downstream signaling pathways such as PI3K/Akt and Ras/MAPK, which are crucial for cell survival, proliferation, and ECM production[60]. It is considered one of the most potent stimulators of HSC proliferation, acting through its receptors expressed on the surface of activated HSCs. PDGF signaling plays a crucial role in the expansion of the fibroblast population within the fibrotic liver[61].

Janus kinase/signal transducers and activators of transcription pathway

The Janus kinase/signal transducers and activators of transcription (JAK/STAT) pathway is another key cell signaling mechanism contributing to liver fibrogenesis. Activated by various cytokines and growth factors, this pathway promotes inflammation, HSC activation, and the expression of fibrogenic genes. Inhibitors of the JAK/STAT pathway have shown promise in reducing liver fibrosis in experimental animal models, underscoring its significance in fibrosis progression[62].

Wnt/β-Catenin signaling

The Wnt/β-catenin signaling pathway, which plays a crucial role in cell proliferation and differentiation, is implicated in the activation of HSCs and the progression of liver fibrosis. Activation of this pathway promotes the fibrogenic activity of HSCs, suggesting that targeting Wnt/β-catenin signaling could be a potential therapeutic strategy for liver fibrosis. Moreover, Wnt signaling interacts with other fibrogenic pathways, such as TGF-β, amplifying the overall fibrotic response in the liver[63,64].

Hedgehog signaling

Hedgehog signaling, which regulates tissue patterning and cell differentiation during development, is reactivated in adult liver diseases and promotes the fibrogenic response of HSCs. This pathway's involvement in liver fibrosis highlights the potential for developing antifibrotic therapies by inhibiting hedgehog signaling[65].

Correlation with cardiovascular risk

MAFLD is linked to increased cardiovascular disease risk, with liver fibrosis being a critical determinant of both liver-related and cardiovascular outcomes. Biomarkers and elastography techniques are shown to predict cardiovascular events and overall mortality in MAFLD patients. Studies indicate that advanced liver fibrosis correlates with a higher risk of cardiovascular events[40,66].

DYSREGULATION OF SPHINGOLIPID METABOLISM IN LIVER FIBROSIS

S1P-related signaling in liver fibrosis

Abnormal sphingolipid pathways have been found in different types of rodent liver fibrosis models. S1P is considered a key lipid mediator of organ fibrosis. The formation of S1P, catalyzed by SphKs, is balanced with its degradation by SPL and S1P phosphatase. The degradation process by SPL is irreversible. Five subtypes of S1P receptors (S1PRs) 1-5 have been identified; S1PR1, S1PR2, and S1PR3 are commonly expressed in various tissues *in vivo*, including hepatocytes and mesenchymal cells [Figure 2]. S1PR4 expression is limited to lymphoid and hematopoietic tissues, while S1PR5 expression is confined to the central nervous system. A study by Masaya Sato *et al*. found that sphingosine kinase 1 (SphK1) was elevated in advanced liver cirrhosis, whereas sphingosine kinase 2 (SphK2) showed no significant change. Moreover, the level of S1PR2 mRNA increased in liver fibrosis, rather than S1PR1 or S1PR3[67].

The effects of elevated S1P in MASH/hepatic fibrosis depend on the specific S1PR involved. Increased S1P levels in MASH livers contribute to fibrosis primarily via S1PR2[68]. In contrast, activation of S1PR1 and S1PR3 can have varied outcomes: S1PR1 promotes liver regeneration and reduces fibrosis in endothelial cells[69], but in HSCs, both S1PR1 and S1PR3 may drive fibrotic progression, depending on the signaling pathways that are activated[70].

Although SphK1 and SphK2 can catalyze the synthesis of S1P from sphingosine, they have different intracellular locations and distinct functions in cell survival. SphK1-induced HSC activation is essential for developing liver fibrosis in mice and is more significant than its regulatory effect on Kupffer cells. In HSCs, the direct targeting of miR-19b to the CCL2-CCR2 signaling pathway mediates SPHK1 activation and ECM deposition. TGF-β1 induces mouse bone marrow mesenchymal stem cells to differentiate into myofibroblasts by upregulating SphK1 and subsequently increasing the expression of S1PR1 and S1PR3. Inhibition of SphK1 reduces the proliferation of HSCs induced by TGF-β1[71]. Increased expression of SphK1 and components of the NOD-like receptor protein 3 (NLRP3) inflammasome were observed in liver fibrosis caused by chronic liver injury from diverse etiologies. In bone marrow-derived macrophages (BMM), S1P promotes the initiation and activation of the NLRP3 inflammasome through S1PR2 and fosters liver fibrosis through the release of inflammatory cytokines. Macrophage-specific knockout of S1PR2 alleviates liver inflammation and fibrosis in mice[72]. Hematopoietic stem cells have been used to treat liver fibrosis and are associated with increased numbers of neutrophils and macrophages in the liver. However, under these conditions, S1P levels rise in the liver, stimulating egress from the liver. Treatment with FTY720, which downregulates S1PRs 1, 3, 4, and 5, but not S1PR2, allows retention of these cells and reduces fibrosis[73].

SphK2 is highly expressed in the liver, primarily located in the nucleus. Activation of S1PR2 by bile acids upregulates the expression of SphK2 in hepatocytes and cholangiocytes via the ERK1/2 and AKT ignaling pathways. This process is involved in lipid metabolism, promotes the proliferation of bile duct cells, and contributes to liver fibrosis[74-77]. In contrast, S1PR2 inactivation accelerates regeneration, highlighting its crucial role in both regeneration and fibrosis following liver injury[78].

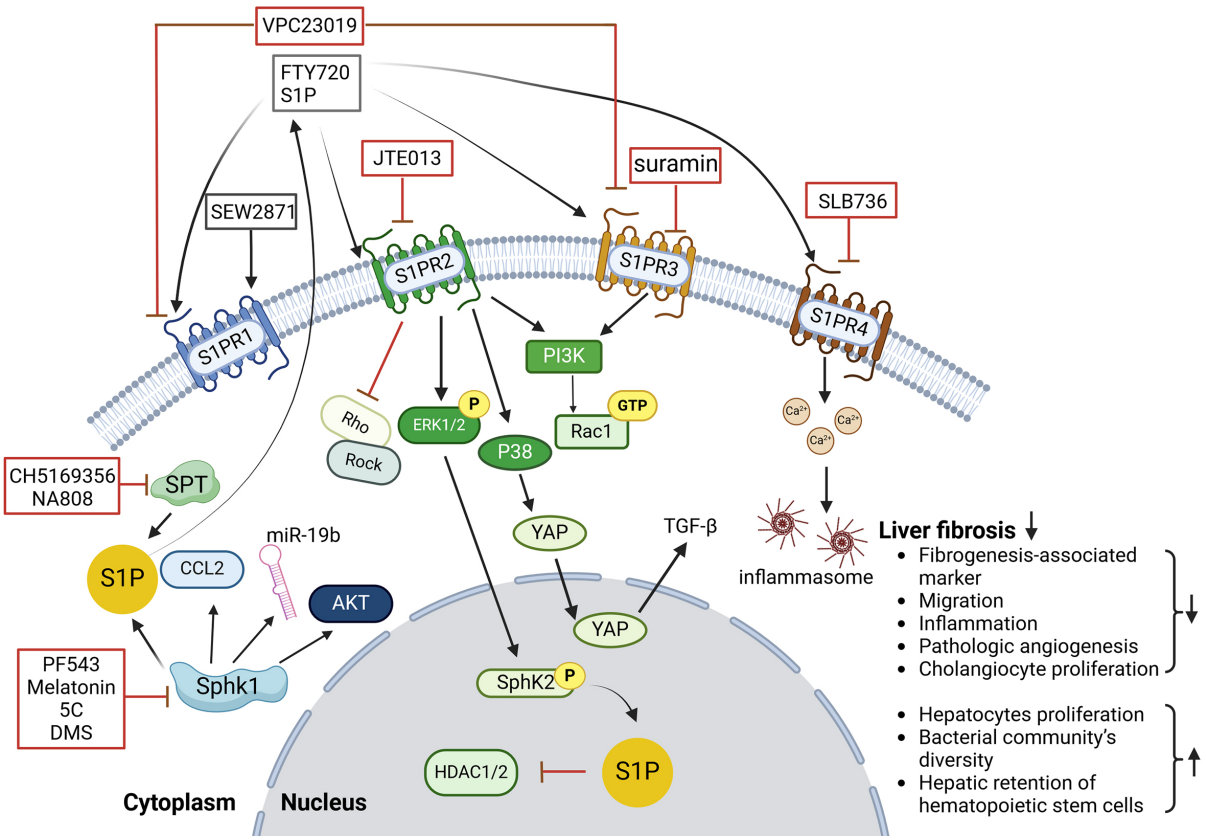

Figure 2. Drugs targeting the sphingolipid pathway in liver fibrosis. This figure illustrated how S1P modulators exert antifibrotic effects by acting on S1PR1-4. Additionally, key enzymes in S1P synthesis pathways, such as SPT and SphK, play a pivotal role in regulating fibrogenesis. ↑ represents upregulation, and ↓ represents downregulation. S1P: Sphingosine-1-phosphate; SPT: serine palmitoyltransferase; SphK: sphingosine kinase; TGF-β: transforming growth factor-beta; S1PRs: sphingosine-1-phosphate receptors; CCL2: monocyte chemoattractant protein 1; DMS: N,N-Dimethylsphingosine;, HDAC1/2: histone deacetylase 1 and 2; YAP: yes-associated protein; GTP: guanosine triphosphate; ERK1/2: extracellular signal-regulated kinase 1 and 2.

S1P promotes HSC migration via S1PR1 and S1PR3, while S1PR2 inhibits this process. In contrast, the migration of BMM to the injury site is mediated by S1PR2 and S1PR3. The roles of PDGFR and S1PRs in regulating fibroblast functions significantly overlap, with both receptors being activated by the same ligands to exert synergistic effects[79].

Upregulation of S1P expression is also observed in liver fibrosis caused by congestive hepatopathy. Elevated S1P is significantly associated with liver tumorigenesis in the context of congestive hepatopathy but is absent in liver tumorigenesis models of other etiologies by diethylnitrosamine and carbon tetrachloride[80]. In the congestive hepatopathy model, an increase in intestinal permeability raises intestinal-derived lipopolysaccharide, which promotes HSC activation through liver sinusoidal endothelial cell (LSEC) capillarization[80]. S1P stimulation enhances angiogenesis in fibrotic mice by inducing Ang1 expression through S1PR1 and S1PR3 and participates in liver fibrosis formation[81]. In contrast, when bound to HDL containing apolipoprotein M, S1P acts as a biased agonist of S1PR1 signaling in endothelial cells, a crucial step in liver regeneration and preventing fibrosis post-liver injury[69].

Since sphingolipids are closely linked with abnormal lipid metabolism, they are also closely associated with fibrosis in MAFLD. Unlike liver fibrosis caused by other injuries, increasing S1P synthesis in the liver is a

direct response to lipotoxicity. S1P released by hepatocytes into the ECM can promote the progression of fibrosis by enhancing the chemotaxis and enrichment of macrophages and HSC activation[82,83]. Activation of the SphK-S1P-S1PR pathway has been shown to be involved in the progression of chronic inflammation into liver fibrosis. The protective effect of SphK1-KO in hepatocytes against inflammation and fibrosis in MASLD was observed only in females. Estrogen stimulates the release of S1P from hepatocytes in female mice, which inhibits the expression of Col1a1 in HSCs stimulated by TGF-β1 via S1PR3. This partly explains why men with MASLD are more prone to developing metabolic dysfunction-associated steatohepatitis (MASH) and liver fibrosis than women[84]. Additionally, specific overexpression of S1PR2 in LSEC exacerbates liver fibrosis in mice by activating the yes-associated protein signaling pathway and inducing activation through paracrine TGF-β1 signlaing[85][Figure 2].

Hepatocytes communicate through exosomes containing neutral ceramidase and SphK2, which increase intracellular S1P synthesis to promote hepatocyte regeneration and repair after liver injury[86]. Sphingomyelin synthase 2, a key enzyme in synthesizing sphingomyelin from ceramide, when deficient, leads to the accumulation of S1P and aggravates liver fibrosis caused by choline-deficient, L-amino acid-defined, high-fat diet feeding[87].

The role of other sphingolipids in liver fibrosis

The pro-autophagy effect of dihydroceramide can promote the accumulation of lipid droplets in hepatocytes and indirectly promote the activation of HSC[88].

SPHINGOLIPIDS AS THERAPEUTIC TARGETS AND BIOMARKERS IN LIVER FIBROSIS

Sphingolipids as markers of liver fibrosis and inflammation

Thiele *et al.* discovered that plasma levels of sphingolipids and phosphatidylcholine were significantly altered in patients with alcoholic liver disease (ALD) as determined by lipidomics analysis. Specifically, sphingolipids showed a negative correlation with ALD-related liver fibrosis grading, and low plasma levels of sphingomyelin were associated with clinical events related to cirrhosis. The authors suggested that these changes in plasma lipids represent a connection between alcohol-related liver fibrosis and the gradual depletion of sphingomyelins, ceramides, and phosphatidylcholine in both the bloodstream and the liver, indicating a profibrotic and pro-inflammatory metabolic mechanism[89]. In cirrhotic patients, low levels of S1P are found particularly in those with hepatorenal syndrome, with more pronounced decreases observed in patients with severe pulmonary shunting. Furthermore, elevated levels of plasma deoxysphingolipids in patients with hepatic steatosis were not associated with further progression of MASLD to MASH or fibrogenesis, suggesting that deoxysphingolipin groups may be involved in the earlier steatosis development [90].

Therapeutic targeting of S1P for the treatment of liver fibrosis

The targeting of the SphK-S1P-S1PR signaling pathway has shown promise for treating a variety of diseases. Currently, several targeted S1P drugs have been employed to treat conditions like multiple sclerosis, ulcerative colitis, and other inflammatory diseases[91]. Etrasimod, a novel S1P receptor modulator, has been approved by the FDA for the treatment of ulcerative colitis[92]. Figure 2 outlines potential drug targets in the S1P signaling pathway, with Table 1 summarizing their effects on liver fibrosis across various models and cell types.

S1PR and SphK modulators

Fingolimod (FTY720): Known as the first-generation S1PR modulator, Fingolimod has lower receptor selectivity compared to other S1PR modulators. It is approved by both the FDA and the European Medicines Agency for the treatment of relapsing-remitting multiple sclerosis. Phosphorylated FTY720-P

Table 1. Potential target of S1P signaling in liver fibrosis

Target	Drug	Model/Cell Type	Result	Reference
Non-selective S1PR agonist	Fingolimod	CBDL/HPS	Arterial blood gas exchange↑, systemic and pulmonary inflammation↓, survival↑	[90]
	FTY720	HSC	Cell proliferation and migration↓	[79]
	FTY720	FFC diet	liver injury, inflammation, fibrosis↓	[93]
	FTY720	CCl_4	Hepatic retention of hematopoietic stem cells, reductions in fibrosis↑	[73]
Selective inhibitors of S1PR2	JTE013	pIVCL/HSC	Liver fibrosis↓, α-SMA expression of HSC↓	[80]
	JTE013	BDL	TCA- and S1P-induced cell proliferation and migration↓, cholangiocyte proliferation and cholestatic injury↓	[94]
	JTE013	DDC/HSC	Liver injury↓, collagen accumulation↓, fibrogenesis-associated genes↓, HSC proliferation, migration and contraction and ECM secretion↓	[77]
	JTE013	bone marrow-derived monocytes/macrophage	NLRP3 inflammasome priming↓, inflammatory cytokine (IL-1β and IL-18) secretion↓	[72]
	JTE013	BDL/ bone marrow-derived monocyte/macrophage	BMM recruitment↑, hepatic inflammation and fibrosis↓	[95]
	JTE013	isolated perfused liver	Portal pressure↓	[96]
	JTE013	BDL	Liver function↑, liver inflammation↓, hepatocyte apoptosis↓, NETosis↓, bacterial community's diversity↑,	[97]
S1PR1 specific agonist	SEW2871	PH/BDL/LSEC	Liver weight↑, survival↑, hepatic function recovery↑, regeneration of hepatic sinusoidal vasculature↑, fibrosis and thrombosis ↓	[69]
	SEW2871	HSC	SMA, procollagen a1(I) and a1(III) ↓	[70]
	SLB736	MAFLD induced by high-fat, high-cholesterol diet	MASH↓, hepatic fibrosis↓, NLRP3 inflammasome↓	[98]
SphK1 inhibitor	PF543	CCl_4-induced liver fibrosis	α-SMA, TGF-β1 and Col I↓	[99]
SPT inhibitor	CH5169356	MASH induced by atherogenic and high-fat content diet	hepatic fibrosis↓, HSC activation↓	[100]

Upregulation is represented by ↑ and downregulation is represented by ↓. S1P: Sphingosine-1-phosphate; S1PR: sphingosine-1-phosphate receptor; SphK1: sphingosine kinase 1; SPT: serine palmitoyltransferase; BDL: bile duct ligation; CBDL: common bile duct ligation; HPS: hepatopulmonary syndrome; HSC: hepatic stellate cell; FFC: fat, fructose, and cholesterol; CCl_4: carbon tetrachloride; pIVCL: partial inferior vena cava ligation; DDC: 3,5-diethoxycarbonyl-1,4-dihydrocollidine; PH: partial hepatectomy; LSEC: liver sinusoidal endothelial cell; MAFLD: metabolic dysfunction-associated fatty liver disease; MASH: metabolic dysfunction-associated steatohepatitis; α-SMA: α-smooth muscle actin; TCA: tricarboxylic acid; ECM: extracellular matrix; NLRP3: NOD-like receptor protein 3; BMM: bone marrow-derived macrophage; IL: interleukin; TGF-β: transforming growth factor beta; Col I: collagen type I.

binds to S1PR1, S1PR3, S1PR4, and S1PR5, but not S1PR2 on cell surfaces *in vivo*. Despite some reported cases of primary sclerosing cholangitis among patients treated with FTY720 for multiple sclerosis, its role in liver fibrosis remains incompletely understood[101]. Recent studies suggest that Fingolimod may exert protective effects against liver fibrosis through multiple targets, promoting hepatocyte proliferation, improving liver fibrosis, and reducing portal vein pressure. Its use has also been associated with alleviating hepatopulmonary syndrome in mice induced by common bile duct ligation (BDL). It reduces systemic and pulmonary inflammation, potentially related to changes in Treg and Th17 lymphocyte subpopulations[90]. FTY720 partially blocks S1P-mediated proliferation, migration, and proliferative responses of PDGF-activated HSCs in both a receptor-dependent and -independent manner[79]. Systemic administration of FTY720 enhances the antifibrotic effects of infused purified hematopoietic stem cells, aiding their therapeutic impacts[73]. Activation of lysophosphatidic acid (LPA) receptor 1 stimulated by LPA can augment the effects of S1P, enhancing the therapeutic efficacy of human adipose-derived mesenchymal stem cells and improving histological fibrosis and inflammation levels[102].

JTE-013: This selective S1PR2 receptor antagonist has been shown to decrease α-SMA, procollagen I, and relaxation in LX-2 cells[103]. Inhibition of the S1PR2 by JTE-013 significantly reduced portal vein pressure in a rat model of BDL-induced cirrhosis, potentially aiding in the elimination of liver fibrosis. By regulating the TCA/S1PR2/NOX2/NLRP3 pathway, JTE-013 improves liver function in BDL mice, reduces liver inflammation, and inhibits hepatocyte apoptosis and NETosis formation, while also altering the gut microbiome to favor a healthier bacterial balance[76,94,97].

SEW2871: As an agonist of S1PR1, SEW2871 can prevent liver parenchymal damage post-BDL and alleviate thrombosis and fibrosis in the damaged liver. It activates the S1P1 signal in LSECs post-injury, enhancing the regeneration of sinusoidal vessels and thus improving liver injury and related fibrosis outcomes[69]. Additionally, W146, an S1PR1 antagonist, can negate the effects of SEW2871 on HSC activation[70].

Other S1PR modulators like the S1PR4 selective functional antagonist SLB736 inactivate the NLRP3 inflammasome and block the development of high-fat, high-cholesterol diet-induced MASH and liver fibrosis[98]. By inhibiting S1PR1 and S1PR3, VPC23019 can significantly reduce angiogenesis and the degree of liver fibrosis[81].

Other liver fibrosis treatments based on the sphingolipid pathway

The SPT inhibitor CH5169356, an oral prodrug of NA808, significantly reduces the expression of α-SMA and collagen 1A1 mRNA in the liver, inhibiting the progression of liver fibrosis[100]. SKI-II(4-[[4-(4-chlorophenyl)-1, 3-thiazolyl] amino-] phenol), a non-selective inhibitor of SphK1, also attenuates the upregulation of α-SMA and collagen I expression in LX-2 cells and blocks angiogenesis and collagen deposition in liver fibrosis[81].

Studies have shown that Oleoylethanolamide induces significant changes in sphingolipid composition and ceramidase activity in the liver[104]. Oleoylethanolamide, a naturally occurring ethanolamide and a member of the fatty acid ethanolamide family, has demonstrated antifibrotic effects. These effects are primarily mediated through the modulation of peroxisome proliferator-activated receptor-alpha, which reduces hepatic stellate cell activation and collagen deposition[105]. Given its impact on the sphingolipid pathway and its potential to attenuate liver fibrosis, Oleoylethanolamide stands out as a promising therapeutic agent for the treatment of liver fibrosis.

CONCLUSION

The exploration of sphingolipid metabolic dysregulation within the context of liver fibrosis has significant therapeutic potential[10]. Current research highlights the intricate role of sphingolipids in the pathogenesis of liver fibrosis, offering novel insights into cellular mechanisms that could be targeted for therapeutic gains[12]. Despite these advancements, there remains a substantial need for further investigation to fully elucidate the complex interactions between sphingolipid metabolic pathways and fibrotic processes in the liver.

Most S1PR agonists currently activate multiple subtypes of S1PRs simultaneously. When endogenous S1P signaling is indiscriminately affected during disease therapy, this lack of specificity can undermine the efficacy of these ligands, limiting their potential applications and complicating efforts to minimize side effects. Consequently, there is an urgent need to develop highly selective agonists specifically for S1PR1 to enhance therapeutic outcomes and reduce adverse effects.

Future research directions should aim at developing a more comprehensive understanding of the regulatory mechanisms governing sphingolipid metabolism in healthy and fibrotic liver tissues. Additionally, the identification and validation of specific sphingolipid molecules as biomarkers for liver fibrosis could markedly improve diagnostic approaches, enabling earlier detection and intervention[106,107]. Ultimately, the development of targeted therapies that can modulate sphingolipid metabolism, restoring its balance within the liver, presents a promising frontier in the treatment of liver fibrosis. Such advancements will require concerted efforts in basic and translational research, possibly leading to the innovation of effective therapeutic strategies that can halt or even reverse the progression of liver fibrosis, offering hope to those affected by this challenging condition.

DECLARATIONS

Authors' contributions

Conception and design of this study, initiation of the literature search, initial title and abstract screening, full-text screening, and manuscript writing and editing: Wu N, Song M, Zhou H
Editing and review of the manuscript: Zhang F, Aseem SO, Hylemon PB

Availability of data and materials

Not applicable.

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Conflicts of interest

Huiping Zhou is an Editorial Board member of Metabolism and Target Organ Damage. The other authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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