Ursolic acid: A promising therapeutic agent for MASLD via inhibition of SPP1-induced Th17 cell differentiation

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Running title: Ursolic acid inhibits pro-inflammatory SPP1 in MASLD

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Metabolic dysfunction-associated steatotic liver disease (MASLD) is the most prevalent chronic liver disease, encompassing a spectrum of pathological conditions from simple steatosis to metabolic dysfunction-associated steatohepatitis (MASH), with or without hepatic fibrosis. The global prevalence and incidence of MASLD are rising in parallel with the epidemics of obesity and metabolic syndrome.[1] If MASLD worsens, it can lead to life-threatening liver damage, including cirrhosis and liver cancer, necessitating proper clinical management in advance. The first-line treatment for MASLD is weight loss through lifestyle changes, which can reduce hepatic fat and inflammation.[2] However, not all patients succeed in losing weight, and MASLD can occur in both obese and non-obese populations. Although several medicines for metabolic syndrome, such as those for diabetes, obesity, and hyperlipidemia, have been used for MASLD treatment, these drugs have shown only partial efficacy.[3] Currently, only one medicine, Resmetirom (Rezdiffra), which obtained U.S. Food and Drug Administration's approval in 2024, is available for the treatment of non-cirrhotic MASH with moderate to severe liver fibrosis. The global drug market for MASLD is actively working to develop new medications and obtain approval for a wide spectrum of MASLD, addressing the significant clinical unmet needs.

Ursolic acid, discovered in apple feels in the early 1920s and found abundantly in various fruits and vegetables, is a natural pentacyclic triterpenoid with multiple beneficial effects against oxidative stress, inflammation, and tumorigenesis in various organs, including the skin, colon, breast, and liver.[4] Recent studies have shown that ursolic acid can ameliorate insulin resistance, hepatic steatosis, inflammation, and liver hypoxia, which can induce metabolic dysfunctions, and liver fibrosis in preclinical models.[5] However, the underlying mechanisms by which ursolic acid exerts therapeutic effects against MASLD and liver fibrosis remain to be fully elucidated.

In this issue of *Clinical and Molecular Hepatology*, Zheng et al. consistently observed that ursolic acid alleviated increases in liver and body weights, serum and hepatic levels of triglycerides and cholesterol, blood glucose levels, MASLD activity score (NAS), and hepatic lipid accumulation in a mouse model of MASLD induced by a high-fat diet (HFD) feeding for 12 weeks, compared to untreated controls.[6] Further assessment showed significant improvements in hepatic gene expression of pro-inflammatory cytokines, including transforming growth factor (TGF)-β, interleukin (IL)-1β, IL-6, IL-17A, and IL-23 after ursolic acid treatment in a dose-dependent manner. In this study, the authors investigated the immune-modulatory role of ursolic acid against the progression of MASLD by conducting a comprehensive analysis combining in vitro and in vivo studies.

To explore the key factors that play critical roles in the progression of MASLD (as an early stage of MASLD) to MASH, the authors re-analyzed two microarrays (GSE49541 and GSE89632) performed on human MASLD and MASH tissues. They built a protein-protein interaction network using the STRING database for the 106 differentially expressed genes between MASLD and MASH. Pathway enrichment analysis revealed that the extracellular matrix (ECM)-receptor interaction pathway is highly involved in the progression from MASLD to MASH. Importantly, secreted phosphoprotein 1 (SPP1), also known as osteopontin, emerged as the central player in this network. SPP1 is a glycoprotein and an important component of the ECM. It acts as a signaling mediator to regulate intercellular

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communications, affecting cell adhesion, migration, and inflammatory response.[7] In MASLD patients and mouse models, serum and plasma levels of SPP1 are elevated and correlate with the severity of liver fibrosis.[8,9] Previous studies have shown that whole-body knockout of SPP1 in mice fed HFD for 24 weeks significantly reduces hepatic triglycerides, lipid accumulation, and inflammation.[10] Zheng et al. further revealed that adeno-associated virus-mediated knockdown of SPP1 reduces liver weight, serum and hepatic levels of triglycerides, cholesterol, alanine/aspartate aminotransferases, and blood glucose level, and improves NAS score, lipid accumulation, and inflammation in HFD-fed mice.

The dynamic equilibrium between helper T (Th) 17 and regulatory T (Treg) cells, both differentiating from naïve $CD4^+$ T cells, is crucial for maintaining immune homeostasis.[11] Th17 cells are pro-inflammatory, while Treg cells are anti-inflammatory. Th17 cell populations increase in the livers of HFD-induced MASLD mice, inducing hepatocyte damage. IL-17, secreted by Th17 cells, influences hepatic steatosis and inflammation, which can eventually lead to MASH. The presence of Th17 cells correlates with the severity of MASLD in patients.[12] Treating primary $CD4^+$ T cells from healthy mouse spleens with SPP1 promoted differentiation into Th17 cells in a dose-dependent manner[6], suggesting an immune-modulatory role of SPP1. Zheng et al. showed that SPP1 binds to cell surface receptors like integrin β1 (ITGB1) and CD44 on naïve $CD4^+$ T cells, activating the downstream extracellular signal-regulated kinase (ERK) pathway and upregulating retinoic acid-related orphan receptor gamma t (RORγt) to induce Th17 cell differentiation and IL-17 production **(Figure 1)**. Notably, ursolic acid inhibited the SPP1-mediated Th17 cell differentiation in a dose-dependent manner. To investigate the effect of ursolic acid on SPP1, Zheng et al. conducted a human proteome microarray and found that SPP1 is a direct protein target of ursolic acid. SPP1 levels and Th17 cell population in liver tissues were reduced in ursolic acid-treated mice with HFD-induced MASLD. These findings indicate that ursolic acid protects the liver from inflammatory damage by inhibiting Th17 cell differentiation through interrupting SPP1 binding with ITGB1 and CD44, consequently suppressing MASLD progression.

Although this study demonstrates how ursolic acid alleviates MASLD progression by modulating the Th17 cell differentiation-promoting signaling pathway (SPP1-ITGB1/CD44-ERK), several limitations exist. First, the mechanisms by which ursolic acid operates in the liver and inhibits SPP1 remain unclear. Since ursolic acid has poor permeability, it likely acts primarily on extracellular SPP1 rather than intracellular SPP1. Zheng et al. showed that ursolic acid acts on secreted SPP1, an integral ECM component recognized by ITGB1 or CD44, by excluding interference from intracellular protein components when analyzing SPP1 content. In addition, the study indicates that ursolic acid directly binds to SPP1. However, the specific mechanism by which ursolic acid inhibits SPP1 expression needs further clarification. It remains to be determined whether ursolic acid induces SPP1 proteolysis, promotes SPP1 discharge from the liver, or inhibits SPP1 activity by capturing it, thereby disrupting the positive feedback that amplifies SPP1 production and secretion by SPP1-responsive cells.

Second, the cell type responsible for producing and secreting SPP1 in MASLD remains unclear. Previous studies have shown that SPP1 is mainly expressed by non-parenchymal cells[13] while another study claims that lipid-injured hepatocytes release SPP1.[14] Given that previous studies on the

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contribution of SPP1 to MASLD using SPP1-knockout mice showed inconsistent results attributable to the cellular and tissue source of SPP1[15], understanding which cell types release SPP1 is critical. Macrophages, in particular, play crucial roles in MASLD progression, and they are reported as a major cell type promoting liver fibrosis by releasing SPP1 during MASH.[16] Conditional knockout of myeloid-specific SPP1 was reported to worsen MASH, although SPP1 expression in macrophages was upregulated in MASLD patients and mice fed a high-fat, fructose and cholesterol diet[17], suggesting a protective role of macrophage-derived SPP1 against MASH. Intriguingly, the expression of arginase 2 (Arg2), which enhances fatty acid oxidation in hepatocytes and leads to a protective effect against MASH, is not affected by direct SPP1 treatment in macrophages. This might implicate the importance of intracellular SPP1 in mediating signaling pathways and suggest a cell type-dependent role of SPP1 in MASLD.

Third, other immune cells, not just $CD4^+$ T cells, may respond to extracellular SPP1 via ITGB1 (or other integrins) or CD44. SPP1 promotes the migration of bone marrow-derived macrophages into damaged tissues and binds to α4 integrin and CD44 to inhibit macrophage apoptosis, thereby sustaining the inflammatory response.[18] Additionally, SPP1 can trigger the polarization of macrophages into M2 phenotype, known as tumor-associated macrophages, via CD44 on macrophages, which was demonstrated in patients with hepatocellular carcinoma and in vitro experiments.[19] Further studies would enhance understanding of the immune-modulatory effect of ursolic acid. Finally, SPP1 is well known for its pro-fibrotic effect[20], and Th17 cells contribute to liver fibrosis. Since liver fibrosis requires careful management during MASH progression, as it can lead to cirrhosis, one of the main complications of MASH, the potential anti-fibrotic effect of ursolic acid via SPP1 inhibition should be explored in liver fibrosis models with significant implications.

In summary, the findings by Zheng et al. underscore the critical role of ursolic acid in regulating SPP1 and subsequent Th17 differentiation during MASLD progression. Despite several unresolved limitations and questions, this study provides valuable insight into MASLD pathogenesis, laying the groundwork for understanding the anti-inflammatory effects of ursolic acid in MASLD livers and suggesting its application in MASLD management.

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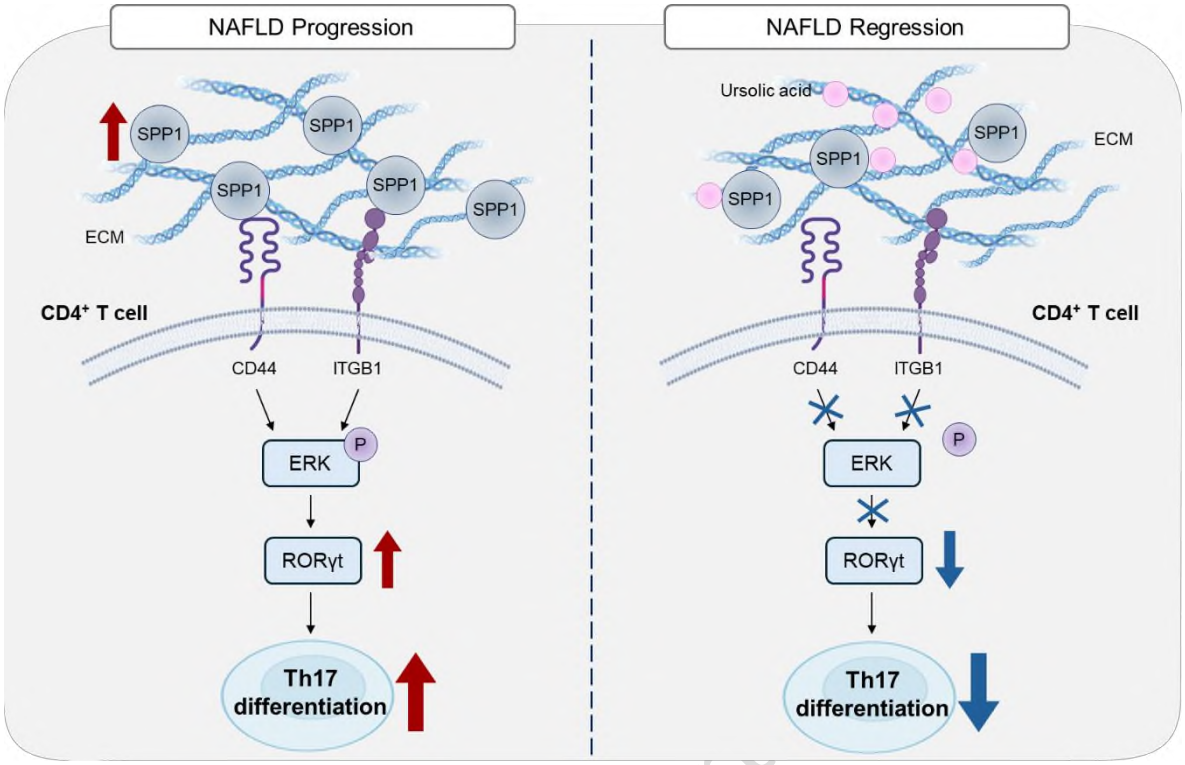

Figure 1. The anti-inflammatory mechanism of ursolic acid in MASLD. In the progression of MASLD (left panel), there is an elevation in extracellular SPP1 levels, which can bind directly to surface receptors CD44 and ITGB1 on naïve CD4+ T cells. This binding triggers the phosphorylation of ERK and subsequent expression of RORγt, promoting the differentiation of pro-inflammatory Th17 cells. These Th17 cells exacerbate inflammation and lipid accumulation, accelerating MASLD progression. Conversely, ursolic acid induces regression of MASLD (right panel) by interrupting the binding of SPP1 with CD44/ITGB1. This disruption inhibits the differentiation of Th17 cells, thereby ameliorating insulin resistance, lipid accumulation, and inflammatory responses. These findings suggest that ursolic acid holds promise as a therapeutic agent for MASLD. Abbreviations: MASLD, non-alcoholic fatty liver disease; SPP1, secreted phosphoprotein 1; ECM, extracellular matrix; ITGB1, integrin β1; ERK, extracellular signal-regulated kinase; RORγt, retinoic acid-related orphan receptor gamma t; Th17, Helper T 17.

Authors' contribution

SJK and JH conceived and wrote the manuscript. S.J.K. drew the figure. All authors have read and approved the article.

Conflicts of Interest

The authors have no conflicts to disclose.

Abbreviations

MASLD, metabolic dysfunction-associated steatotic liver disease; MASH, metabolic dysfunction-associated steatohepatitis; NAS, MASLD activity score; HFD, high-fat diet; TGF, transforming growth factor; IL, interleukin; ECM, extracellular matrix; SPP1, secreted phosphoprotein 1; Th, helper T; Treg, regulatory T; ITGB1, integrin β1; ERK, extracellular signal-regulated kinase; RORγt, retinoid-related orphan receptor gamma t; Arg2, arginase 2.

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