The role of SPP1 in MASLD pathogenesis: Therapeutic insights into ursolic acid's mechanisms of action

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Running title

Ursolic acid targets SPP1 against MASLD

Abbreviations

NAFLD, non-alcoholic fatty liver disease; MASLD, metabolic dysfunction-associated steatotic liver disease; ECM, extracellular matrix; SPP1, secreted phosphoprotein 1; ITGB1, integrin β1.
In the current global health landscape, the prevalence of non-alcoholic fatty liver disease (NAFLD) is escalating annually, establishing it as the most common chronic liver disease. Consequently, a consensus statement formulated by the NAFLD Consensus Consortium in 2022, has designated NAFLD as a chronic metabolic public health issue, underscoring its significance and highlighting the urgent need to address this metabolic disorder.¹ Despite the rapid evolution of intervention strategies for NAFLD in recent years, substantial technological gaps persist, making the development of related therapeutic drugs a major focus of international academic and pharmaceutical research.

In 2023, a consensus group composed of multiple societies opted to replace the term "nonalcoholic fatty liver disease" with "metabolic dysfunction-associated steatotic liver disease" (MASLD) to more accurately reflect its underlying pathophysiology, emphasizing that metabolic dysfunction is fundamental to its etiology.² Within this consensus framework, the term 'steatohepatitis' is considered a crucial pathophysiological concept that should be retained, signifying that immune inflammation is the pivotal factor in the progression of MASLD.

Our previous research has demonstrated that ursolic acid, a pentacyclic triterpenoid compound naturally occurring in various plants, exerts multiple beneficial effects in the treatment of MASLD, including lipid-lowering, anti-inflammatory, and antioxidant activities, with these pharmacological mechanisms being closely linked to SPP1-mediated immune inflammation.³ We express our sincere gratitude to Dr. So Jung Kim and Dr. Jeongeun Hyun for their interest in our research.⁴ And in response to their editorial, we have some recent findings to share by chance.

Recent studies indicate that during MASLD progression, persistent metabolic overload in liver tissue may alter the immune microenvironment, triggering chronic low-grade inflammation, and that various
secreted factors mediate intercellular communication, further promoting the inflammatory cascade.\textsuperscript{5} The extracellular matrix (ECM) provides structural support for liver tissue, maintains cellular architecture, facilitates nutrient transport, waste clearance, liver regeneration, and microenvironment stability. In MASLD liver, significant alterations occur in the composition and structure of the ECM, where various secreted factors and proteins deposit, further promoting the activation and infiltration of inflammatory cells through multiple signaling pathways. Notably, ECM-targeted strategies have shown promising therapeutic potential for MASLD, as demonstrated by Dr. Hongliang Li's findings that supplementing selected connexins can promote the ubiquitination and proteasomal degradation of fatty acid synthase, thereby improving lipid metabolism, suggesting that modulating secreted proteins to remodel the ECM may represent a new approach for MASLD intervention.\textsuperscript{6}

Secreted phosphoprotein 1 (SPP1), also known as osteopontin, is a multifunctional protein widely expressed in the ECM. While it is initially identified as a crucial bone matrix protein involved in cell adhesion and migration, recent research has also underscored its significant role as an immune-active protein within the liver microenvironment. Increasing evidence suggests that SPP1 is critically involved in various stages of MASLD progression and correlates with disease severity.\textsuperscript{7} Furthermore, Dr. Yeon-Su Lee and Dr. Jong Hoon Park recently proposed that SPP1 have significant potential as a critical marker for diagnosis.\textsuperscript{8}

Our research revealed that ursolic acid can directly target SPP1, exerting therapeutic effects. However, the mechanisms by which ursolic acid operates in the liver and inhibits SPP1 remain unclear. As noted by Dr. Kim and Dr. Hyun, due to ursolic acid's limited permeability, it likely acts primarily on extracellular rather than intracellular SPP1. Therefore, we dissociated and filtered mice liver tissues to eliminate
interference from intracellular protein components, confirming that intervention with ursolic acid regulates SPP1 in the ECM. Moreover, as highlighted by Dr. Kim and Dr. Hyun, identifying the cell types that secrete SPP1 is vital. Thus, in our recent studies, we employed single-cell sequencing to detect SPP1 expression in different cells, and the results showed that in MASLD mice liver tissues, the primary source of SPP1 is cholangiocytes.

Cholangiocytes, single-layer columnar or goblet cells within the liver, form the inner lining of the intrahepatic bile duct tree. With their tight cellular connections, these cells produce bile and transport it to the gallbladder and duodenum, which is essential for fat digestion, absorption, and metabolic waste excretion. During MASLD progression, abnormal bile acid metabolism stimulates cholangiocytes to release large quantities of chemokines and pro-inflammatory cytokines, attracting and activating immune cells, thereby exacerbating liver inflammation, oxidative stress, and cellular damage. Numerous studies have shown that targeting FXR to regulate bile acid metabolism is an effective MASLD treatment strategy. However, a common issue with these drugs is the increased risk of gallstones, cholecystitis, and pruritus. Notably, gallstone incidence is already elevated in MASLD patients. Therefore, identifying alternative downstream targets to mitigate adverse effects may be key for advancing therapeutic strategies.

Given that cholangiocytes are primary effector cells in abnormal bile acid metabolism and that SPP1 is a pro-inflammatory cytokine released upon stimulation, altering the liver microenvironment as a vital ECM component, along with recent literature indicating that secreted factor-mediated intercellular communication plays a vital role in metabolic inflammation, we further analysed single-cell sequencing data to explore SPP1-mediated intercellular communication in MASLD mice liver tissues. The results showed that in the overall cell communication network, macrophages are the primary signal receivers of...
SPP1, while the receptors include integrin α4, α5, α8, α9, β1, β5, and CD44. Among these, integrin β1 (ITGB1) and CD44 are the highest-weighted nodes in the integrin and non-integrin receptor networks, respectively, and are highly expressed in cholangiocyte-macrophage communication. Meanwhile, recent studies have highlighted SPP1 as a crucial marker and key target for macrophage polarization. Therefore, we propose that SPP1-mediated cholangiocyte-macrophage communication is a critical interaction between metabolic stress and immune inflammation, serving as an effective downstream therapeutic target for bile acid metabolism. Just as Dr. Kim and Dr. Hyun mentioned, other immune cells, not just CD4+ T cells, may respond to extracellular SPP1 via ITGB1 (or other integrins) or CD44, with macrophages being a key example.

An issue in our research is that after further subdividing macrophages into subpopulations, we found that in the cell subpopulation co-expressing ITGB1 and CD44, SPP1 is also highly expressed with IL1b and CD86, suggesting this subpopulation comprises pro-inflammatory macrophages capable of secreting SPP1 in a positive feedback loop to promote the inflammatory cascade. Moreover, pseudotime analysis revealed differential SPP1 expression at macrophage differentiation branch points, indicating its role in macrophage stimulation and differentiation. However, Dr. Natalia Nieto’s research suggested that SPP1 can trigger macrophage polarization into the M2 phenotype, which presents a certain confusion with our findings. Regarding this, we currently speculate that SPP1 may be a key secreted factor in macrophage differentiation, and its different phenotype structures, cell sources, or locations may result in distinct inflammatory characteristics. Thus, further experiments are needed to explore its exact role in the progression of MASLD.

In summary, we propose that during metabolic dysfunction in MASLD, SPP1 may be secreted by
cholangiocytes and deposited in the ECM, where it activates macrophages, thereby playing a critical role in modulating metabolic immune inflammation. Ursolic acid, as a natural therapeutic agent, targets SPP1 and exerts multiple beneficial effects in treating MASLD, warranting its application and promotion in MASLD management strategies (Fig. 1).

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Author contributions

Y.Z. and Z.X. designed the study, performed the animal experiments and data analyses; L.Z., C.H., and Q.Y. assisted in conducting animal experiments; D.F. drafted the manuscript; F.L. and Y.L. conceived the idea. All authors read and approved the final manuscript.

Conflicts of Interest

The authors have no conflicts to disclose.
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Figure 1. Schematic diagram of the mechanism by which ursolic acid targets SPP1 to regulate metabolic inflammation against MASLD. The schematic diagram was created by Figdraw. MASLD, metabolic dysfunction-associated steatotic liver disease; ECM, extracellular matrix; SPP1, Secreted phosphoprotein 1; ITGB1, integrin β1.