

Letter

Hepatic Stellate Cell Phenotypes in Metabolic Dysfunction-Associated Steatohepatitis (MASH)

Ralf Weiskirchen^{1,*} and Amedeo Lonardo^{2,*}

¹ Institute of Molecular Pathobiochemistry, Experimental Gene Therapy and Clinical Chemistry (IFMPEGKC), RWTH University Hospital Aachen, D-52074 Aachen, Germany

² Department of Internal Medicine, Azienda Ospedaliero-Universitaria of Modena, 41100 Modena, Italy

* Corresponding author. E-mail: rweiskirchen@ukaachen.de (R.W.); a.lonardo@libero.it (A.L.)

Received: 25 March 2025; Accepted: 2 April 2025; Available online: 7 April 2025



© 2025 The authors. This is an open access article under the Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>).

To the Editor:

We have read with great interest the paper by Kisseleva et al., recently published in *Gastroenterology* [1], and we would like to comment on it.

Metabolic dysfunction-associated steatohepatitis (MASH) features the ectopic accumulation of fat within hepatocytes in close and bi-directional association with metabolic dysregulation, triggering chronic inflammation, ballooning degeneration of liver cells, impaired mitochondrial dynamics, and progression to liver fibrosis [2]. The ensuing fibrotic scarring disrupts normal hepatic architecture, compromises liver function, and heightens the risk of hepatocellular carcinoma in a proportion of individuals. Given the burgeoning burden of MASH due to the rise in obesity, insulin resistance, and other metabolic disorders [3], the Perspective article by Kisseleva and colleagues provides valuable insights into how hepatic stellate cells (HSCs) drive the initiation and progression of MASH [1].

Under physiologic conditions, quiescent HSCs (qHSCs) in the perisinusoidal space (Space of Disse) store vitamin A, help maintain sinusoidal blood flow, and secrete various cytokines and growth factors that sustain hepatocyte viability and liver sinusoidal endothelial cell (LSEC) function [4]. In MASH, however, a convergence of lipotoxic stress, inflammation, gut-derived signals, and hepatocyte injury leads to HSC activation [5]. These perturbations are part of a systemic disorder in the context of the cardiovascular-kidney metabolic syndrome, which associates systemic metabolic dysfunction with target organ damage [6]. Activated HSCs (aHSCs) acquire a myofibroblast-like phenotype, downregulating genes involved in lipid metabolism and vitamin A storage while upregulating collagens (particularly types I and III), α -smooth muscle actin, and tissue inhibitors of metalloproteinases [7]. Notably, Kisseleva and colleagues emphasize that this activation is not monolithic. In line with this assumption, single-cell and single-nucleus sequencing have demonstrated that aHSCs encompass multiple subclusters: some are intensely fibrogenic, others show proliferative or inflammatory signatures, and a distinct subgroup exhibits intermediate levels of activation [5]. These diverse phenotypes highlight the complexity of HSC involvement in the pathogenesis of MASH and may potentially contribute to accounting for the widely recognized pathogenic heterogeneity of the disease [8].

Another central theme of Kisseleva's work is metabolic reprogramming, which supports HSC activation. Activated HSCs switch from oxidative phosphorylation to glycolysis and lactate production, often under the influence of altered hedgehog and HIF-1 α signaling. Additionally, allosteric changes of key metabolic enzymes such as pyruvate kinase M2 (PKM2) promote HAC activation in its dimeric form but not in its tetrameric form [9]. Previous work has shown that PKM2 directly interacts with the HIF-1 α subunit and enhances transactivation of HIF-1 target genes by increasing HIF-1 binding and p300 recruitment to hypoxia response elements [10]. The excess lactate can further amplify fibrogenic gene expression through histone lactylation. In parallel, aHSCs increase glutaminolysis and, in some cases, *de novo* lipogenesis or fatty acid oxidation to ensure a robust energy supply for extracellular matrix (ECM) synthesis and persistent fibrosis. The authors discuss that inhibiting key metabolic enzymes like hexokinase 2, glutaminase, or

carnitine palmitoyltransferase 1A has proven to attenuate liver fibrosis in preclinical MASH models, highlighting potential therapeutic strategies.

Beyond intracellular signals, the hepatic microenvironment strongly influences HSC function. Injured hepatocytes release damage-associated molecular patterns (DAMPs) and iron-rich exosomes that heighten HSC reactivity. Macrophages, including Kupffer cells and infiltrating monocytes, secrete fibrogenic mediators, such as TGF- β 1, IL-6, and prostaglandin E2 [11]. In healthy conditions, macrophages provide protective microRNAs that help maintain HSC quiescence. However, in MASH, this protective mechanism is lost, and macrophages instead support fibrogenesis [12]. Additionally, LSECs become “capillarized”, losing their fenestrations, and producing molecules like VCAM1, VEGF, and inflammatory cytokines that further spur HSC activation [13]. Kisseleva and her coauthors link these changes to an intricate network where each cell type in the MASH liver contributes to the fibrotic response.

Even so, MASH does not always progress to an irreversible fibrotic state in all patients. Once the causal insults are removed or diminished, some aHSCs undergo apoptosis, others become senescent (ceasing proliferation but secreting inflammatory cytokines), and yet others may transition to an “inactivated” state (iHSC) similar to, but not identical with, the original quiescent phenotype. Mouse models more readily demonstrate iHSCs and senescent HSCs, whereas in human MASH, these subsets appear less clearly defined and may overlap with partially activated or inflammatory HSC populations [1]. Nonetheless, the existence of such phenotypes suggests opportunities to boost fibrosis regression, either by selectively inducing myofibroblast apoptosis, clearing senescent cells, or reprogramming them toward quiescence.

In this connection, we would like to highlight the role of sex/gender as significant contributors to disease heterogeneity in liver fibrosis and as a potential key to exploring innovative approaches to our understanding and manipulation of liver fibrosis. Accumulating evidence indicates that liver fibrosis in metabolic dysfunction-associated steatotic liver disease (MASLD) is significantly influenced not only by age and reproductive status but also by biological sex and gender, a socio-cultural construct [14]. Sex and gender impact the pathobiology of liver fibrosis through various genetic, hormonal, immunological, metabolic, and lifestyle-related factors. These factors include alcohol consumption, tobacco smoking, diet, sedentary behavior, and hormonal therapy [14]. These studies may pave the way for innovative therapeutic approaches in the MASLD arena [15].

Liver fibrosis is a complex phenomenon that reflects systemic health, leading us to define liver fibrosis as a “barometer” of systemic health [16]. Failure to recognize these metabolic determinants of liver fibrosis in MASLD has been suggested as one of the contributing factors to failures in MASH trials, which could potentially be addressed with a more holistic approach [17,18].

Additionally, the potential role of liver fibrosis in influencing extrahepatic outcomes should be emphasized. For instance, recent research has identified the hepato-retinal axis in the context of eye-kidney-liver complications that are increasingly seen in clinics and resemble ciliopathies and the Senior-Løken syndrome [19]. This could open up new research paths, such as exploring the involvement of the *NPH3* gene [20]. In addition to these micro-vascular connections to liver fibrosis, it is important to note that liver fibrosis is now recognized as an independent risk factor for major adverse cardiovascular events [21,22], and possibly for abdominal aortic aneurysms [23].

In MASH, epigenetic modifications in the fibrogenic activation of HSCs include DNA methylation, covalent histone modification, chromatin remodeling, and non-coding RNAs. Collectively, these reversible and dynamic epigenetic modifications regulate HSC gene activity without altering the coding sequence, persist once the causal factor disappears, and may be the molecular target of innovative therapeutic strategies against MASH-associated liver fibrosis [24]. There is also a strong interaction associated with immunity and metabolism. For example, the immune system senses metabolic dysfunction, and hepatic growth factor levels are associated with basal metabolic rate, which is mediated by the proinflammatory cytokine IL-16. These findings pinpoint the spleen’s influence in the development and progression of MASLD/MASH, a role which has been alluded to as the “spleen-liver axis” [25].

Overall, Kisseleva’s group emphasizes that a better understanding of HSC heterogeneity in MASH opens new possibilities for innovative therapies. New approaches are emerging that target HSC-derived ECM components, such as inhibiting collagen synthesis or lysyl oxidase-like 2 (LOXL2) activity, disrupting key metabolic processes (by blocking glycolysis, glutaminolysis, or fatty acid oxidation), or promoting HSC clearance (through immunotoxins or apoptosis-inducing pathways). While complete HSC ablation can reduce fibrosis in experimental models, the loss of normal HSC functions, including their ability to store vitamin A and support liver regeneration, suggests the need for more precise targeting methods. Ultimately, the findings highlight the complex and multifaceted roles of HSCs in MASH, influenced by intricate transcriptional and epigenetic regulation as well as interaction with hepatocytes, macrophages, and LSECs. Understanding these changing HSC states and finding ways to redirect them holds great promise in reducing and reversing the fibrotic burden in metabolic liver disease. However, these approaches should be

viewed as just one piece of the complex puzzle of systemic metabolic dysfunction, within a holistic framework that considers factors such as sex, gender, reproductive status, epigenetic modifications, the role of the spleen, and extrahepatic outcomes.

Author Contributions

Both authors contributed equally to Conceptualization, Methodology, Data Curation, Writing, Review & Editing of this manuscript.

Ethics Statement

Not applicable.

Informed Consent Statement

Not applicable.

Data Availability Statement

The statement is required for all original articles which informs readers about the accessibility of research data linked to a paper and outlines the terms under which the data can be obtained.

Funding

This research received no external funding.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. Kisseleva T, Ganguly S, Murad R, Wang A, Brenner DA. Regulation of hepatic stellate cell phenotypes in MASH. *Gastroenterology* **2025**, *in press*. doi:10.1053/j.gastro.2025.03.010.
2. Steinberg GR, Valvano CM, De Nardo W, Watt MJ. Integrative metabolism in MASLD and MASH: Pathophysiology and emerging mechanisms. *J. Hepatol.* **2025**, *in press*. doi:10.1016/j.jhep.2025.02.033.
3. Younossi ZM, Razavi H, Sherman M, Allen AM, Anstee QM, Cusi K, et al. Addressing the high and rising global burden of metabolic dysfunction-associated steatotic liver disease (MASLD) and metabolic dysfunction-associated steatohepatitis (MASH): From the growing prevalence to Payors' perspective. *Aliment Pharmacol. Ther.* **2025**, *in press*. doi:10.1111/apt.70020.
4. Acharya P, Chouhan K, Weiskirchen S, Weiskirchen R. Cellular mechanisms of liver fibrosis. *Front. Pharmacol.* **2021**, *12*, 671640. doi:10.3389/fphar.2021.671640.
5. Kim HY, Rosenthal SB, Liu X, Miciano C, Hou X, Miller M, et al. Multi-modal analysis of human hepatic stellate cells identifies novel therapeutic targets for metabolic dysfunction-associated steatotic liver disease. *J. Hepatol.* **2024**, *in press*. doi:10.1016/j.jhep.2024.10.044.
6. Ndumele CE, Rangaswami J, Chow SL, Neeland IJ, Tuttle KR, Khan SS, et al. Cardiovascular-Kidney-Metabolic Health: A Presidential Advisory from the American Heart Association. *Circulation* **2023**, *148*, 1606–1635. doi:10.1161/CIR.0000000000001184; Erratum in *Circulation* **2024**, *149*, e1023. doi:10.1161/CIR.0000000000001241.
7. Gressner AM, Weiskirchen R. Modern pathogenetic concepts of liver fibrosis suggest stellate cells and TGF-beta as major players and therapeutic targets. *J. Cell. Mol. Med.* **2006**, *10*, 76–99. doi:10.1111/j.1582-4934.2006.tb00292.x.
8. Arrese M, Arab JP, Barrera F, Kaufmann B, Valenti L, Feldstein AE. Insights into Nonalcoholic Fatty-Liver Disease Heterogeneity. *Semin. Liver Dis.* **2021**, *41*, 421–434. doi:10.1055/s-0041-1730927.
9. Zheng D, Jiang Y, Qu C, Yuan H, Hu K, He L, et al. Pyruvate kinase M2 tetramerization protects against hepatic stellate cell activation and liver fibrosis. *Am. J. Pathol.* **2020**, *190*, 2267–2281. doi:10.1016/j.ajpath.2020.08.002.
10. Luo W, Hu H, Chang R, Zhong J, Knabel M, O'Meally R, et al. Pyruvate kinase M2 is a PHD3-stimulated coactivator for hypoxia-inducible factor 1. *Cell* **2011**, *145*, 732–744. doi:10.1016/j.cell.2011.03.054.
11. Liu Y, Zheng Y, Yang Y, Liu K, Wu J, Gao P, et al. Exosomes in liver fibrosis: The role of modulating hepatic stellate cells and immune cells, and prospects for clinical applications. *Front. Immunol.* **2023**, *14*, 1133297. doi:10.3389/fimmu.2023.1133297.

12. Yu W, Wang S, Wang Y, Chen H, Nie H, Liu L, et al. MicroRNA: role in macrophage polarization and the pathogenesis of the liver fibrosis. *Front. Immunol.* **2023**, *14*, 1147710. doi:10.3389/fimmu.2023.1147710.
13. Guo Q, Furuta K, Islam S, Caporarello N, Kostallari E, Dielis K, et al. Liver sinusoidal endothelial cell expressed vascular cell adhesion molecule 1 promotes liver fibrosis. *Front. Immunol.* **2022**, *13*, 983255. doi:10.3389/fimmu.2022.983255.
14. Jamalnia M, Lonardo A, Weiskirchen R. Sex and gender differences in liver fibrosis: Pathomechanisms and clinical outcomes. *Fibrosis* **2024**, *2*, 10006. doi:10.70322/fibrosis.2024.10006.
15. Smiriglia A, Lorito N, Bacci M, Subbiani A, Bonechi F, Comito G, et al. Estrogen-dependent activation of TRX2 reverses oxidative stress and metabolic dysfunction associated with steatotic disease. *Cell Death Dis.* **2025**, *16*, 57. doi:10.1038/s41419-025-07331-7.
16. Lonardo A, Ballestri S, Baffy G, Weiskirchen R. Liver fibrosis as a barometer of systemic health by gauging the risk of extrahepatic disease. *Metab. Target Organ Damage* **2024**, *4*, 41. doi:10.20517/mtod.2024.42.
17. Tilg H, Byrne CD, Targher G. NASH drug treatment development: challenges and lessons. *Lancet. Gastroenterol. Hepatol.* **2023**, *8*, 943–954. doi:10.1016/S2468-1253(23)00159-0.
18. Lonardo A, Ballestri S, Mantovani A, Targher G, Bril F. Endpoints in NASH clinical trials: Are we blind in one eye? *Metabolites* **2024**, *14*, 40. doi:10.3390/metabo14010040.
19. Lonardo A. Liver fibrosis: More than meets the eye. *Ann. Hepatol.* **2024**, *29*, 101479. doi:10.1016/j.aohp.2024.101479.
20. Olbrich H, Fliegauf M, Hoefele J, Kispert A, Otto E, Volz A, et al. Mutations in a novel gene, NPHP3, cause adolescent nephronophthisis, tapeto-retinal degeneration and hepatic fibrosis. *Nat. Genet.* **2003**, *34*, 455–459. doi:10.1038/ng1216.
21. Vieira Barbosa J, Milligan S, Frick A, Broestl J, Younossi Z, Afdhal N, et al. Fibrosis-4 index can independently predict major adverse cardiovascular events in nonalcoholic fatty liver disease. *Am. J. Gastroenterol.* **2022**, *117*, 453–461. doi:10.14309/ajg.0000000000001606.
22. Stefan N, Lonardo A, Targher G. Role of steatotic liver disease in prediction and prevention of cardiometabolic diseases. *Nat. Rev. Gastroenterol. Hepatol.* **2024**, *21*, 136–137. doi:10.1038/s41575-023-00880-2.
23. Jamalnia M, Lonardo A, Weiskirchen R. Abdominal aortic aneurysm and liver fibrosis. Clinical evidence and molecular pathomechanics. *Preprints* **2025**, doi:10.20944/preprints202503.0790.v1.
24. Garbuzenko DV. Mechanisms of epigenetic regulation in the fibrogenic activation of hepatic stellate cells in non-alcoholic fatty liver disease. *Gene Exp.* **2024**, *23*, 31–43. doi:10.14218/GE.2023.00090.
25. Tarantino G, Citro V, Capone D. Nonalcoholic fatty liver disease: A challenge from mechanisms to therapy. *J. Clin. Med.* **2019**, *9*, 15. doi:10.3390/jcm9010015.