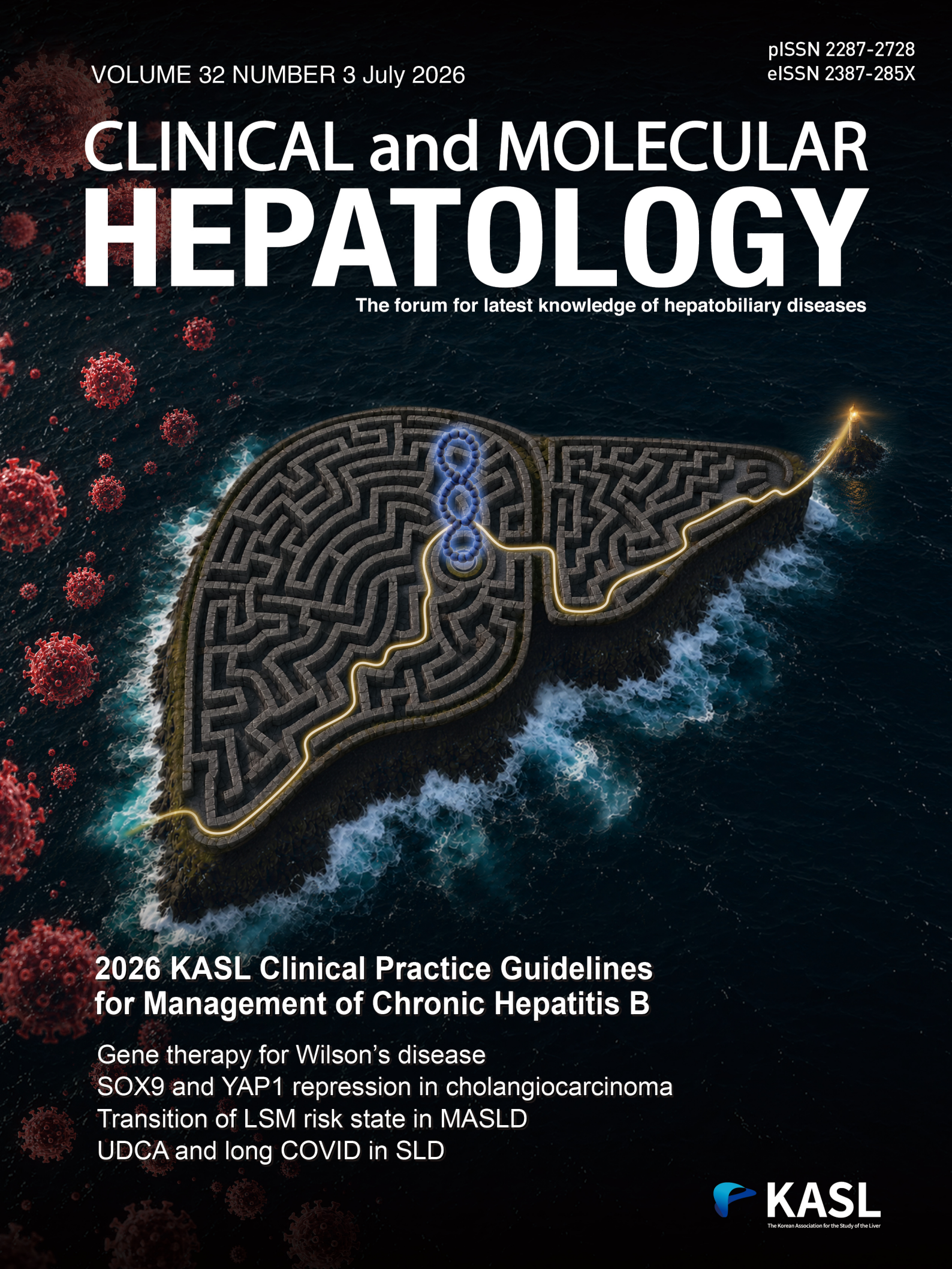
VOLUME 32 NUMBER 3 July 2026
pISSN 2287-2728
eISSN 2387-285X

CLINICAL and MOLECULAR HEPATOLOGY

The forum for latest knowledge of hepatobiliary diseases



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pISSN 2287-2728
eISSN 2287-285X

Clinical and Molecular Hepatology

https://doi.org/10.3350/cmh.2026.0053
Clinical and Molecular Hepatology 2026;32:1452-1456

Editorial

Beyond the canonical sequence: IsomiRs as modulators and drivers of MASLD progression: Editorial on "MicroRNA isomiRs reveal novel pathways linked to disease activity and fibrosis in MASLD"

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Keywords: MASH; miRNAs; miR-122; miR-34a; miR-21

See Article on https://doi.org/10.3350/cmh.2025.0933

The transition from non-alcoholic fatty liver disease (NAFLD) to metabolic dysfunction-associated steatotic liver disease (MASLD) reflects more than a nomenclatural shift; it signifies a deeper understanding of the systemic metabolic drivers of hepatic injury.[1] Despite significant advances in pharmacotherapy, such as the recent approval of resmetirom and the promising results of glucagon-like peptide 1 receptor agonists, MASLD remains a highly heterogeneous disorder.[2] This clinical and molecular diversity often obscures the precise regulatory mechanisms driving progression from simple steatosis to metabolic dysfunction-associated steatohepatitis (MASH) and advanced fibrosis.[2] In this issue of *Clinical and Molecular Hepatology*, Brion et al.[3] provide a landmark characterization of the hepatic isomiR landscape, demonstrating that the "canonical" microRNA (miRNA) sequences studied for decades may represent only a fraction of a far more complex regulatory architecture.[3]

For nearly two decades, liver epigenetics has focused on canonical miRNAs as master regulators of gene expression. Early profiling efforts, particularly the foundational work by Cheung et al.,[4] and our subsequent characterization of circulating miRNA signatures in NAFLD established that miRNAs such as miR-122, miR-34a, and miR-21 are key players linking liver histology to disease pathogenesis.[5] These studies, however, implicitly treated each miRNA as a single, uniform molecular entity.

Brion et al.[3] now challenge this paradigm by demonstrating that isomiRs, miRNA isoforms bearing 5′ or 3′ sequence variations, constitute approximately 67% of the total hepatic miRNA pool. This observation is conceptually transformative, suggesting that analyses limited to canonical sequences may have overlooked the majority of biologically relevant small RNA species in the liver. Notably, the study shows that while the 5′ end (and thus the seed re-

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Editor: Han Ah Lee, Chung-Ang University College of Medicine, Korea

Received : Jan. 13, 2026 / **Accepted :** Jan. 17, 2026

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gion) is conserved primarily across families, 3′ modifications, both templated and non-templated, are ubiquitous and are associated with disease severity.

At the same time, this quantitative dominance warrants careful methodological interpretation. Small RNA sequencing workflows are intrinsically shaped by RNA stability, end-chemistry capture, and size selection, all of which favor mature miRNAs relative to longer or structurally heterogeneous RNA fragments. Moreover, miRNAs, often protected within argonaute complexes, are comparatively resistant to degradation, potentially producing an apparent "pseudo-enrichment" when RNA integrity varies across samples.[5,6] Brion et al.[3] appropriately recognize this issue by identifying RNA integrity number (RIN) as a major driver of global variance and incorporating it as a covariate in downstream models. While this adjustment strengthens association analyses, it also underscores that proportional statements about RNA class abundance should be interpreted as conditioned by library preparation and mapping strategy, rather than as absolute cellular quantities.

The principal strength of this work lies in its rigorous integration of small RNA and mRNA sequencing with machine-learning approaches across the full histological spectrum of MASLD. Using ordinal regression and complementary analytical frameworks, the authors identify 173 isomiRs associated with disease activity (NAFLD activity score) and 58 with fibrosis stage,[3] highlighting a previously unappreciated layer of molecular stratification.

A particularly compelling innovation is the identification of a small subset of "seed-shifting" isomiRs that acquire distinct target repertoires. A notable example is a miR-122-5p isomiR carrying a 5′-templated deletion, predicted to target *INSIG1* and *CDKN1A*, genes not classically regulated by canonical miR-122. *INSIG1* plays a central role in cholesterol and lipid homeostasis via sterol regulatory element-binding protein (SREBP) signaling. *CDKN1A* encodes the cyclin-dependent kinase inhibitor p21, a key regulator of cell-cycle arrest downstream of p53. The identification of these targets provides a mechanistic bridge between altered miRNA processing and pathways governing metabolic control, cellular stress responses, and fibrogenesis in MASLD.[7,8]

Similarly, a miR-21-5p isomiR with a 5′ adenosine addition was found to target *PPARA* and *HMGCS2*, central nodes in fatty acid oxidation and ketogenesis in MASLD.[9,10] These examples illustrate how minimal alterations at the miRNA 5′ end can produce disproportionately large shifts in predicted regulatory networks, offering a molecular explanation for inter-individual heterogeneity in MASLD progression.

A critical conceptual question raised by this work is whether isomiRs represent regulated entities or are merely stochastic byproducts of imperfect miRNA biogenesis. If isomiRs were purely epiphenomenal, arising randomly from a shared precursor, one would expect them to track canonical miRNAs closely, changing in parallel across disease states.

The data presented by Brion et al.[3] largely support this expectation for the majority of isoforms: most isomiRs within a family show concordant directionality, consistent with regulation at the level of precursor transcription or processing. This observation argues that a substantial fraction of the isomiR landscape reflects structured variation around a common biological signal rather than independent regulatory actors.

Crucially, however, the study also documents biologically informative deviations from this pattern. Within the miR-122 family, for instance, most isomiRs decline with advancing fibrosis, yet a specific isoform increases significantly. Such discordance is challenging to reconcile with purely stochastic processing and instead suggests selective post-biogenetic mechanisms, such as differential trimming, tailing, argonaute loading, stability, or cellular export. Thus, the emerging picture is not one of universal functional novelty, but of a bimodal system in which most isomiRs fine-tune canonical miRNA activity. At the same time, a minority acquires context-specific regulatory influence.

This bimodal framework also resolves the apparent paradox of target diversity within miRNA families. Brion et al.[3] report that seed-altering isomiRs represent a numerical mi-

Abbreviations:

lncRNAs, long non-coding RNAs; MASH, metabolic dysfunction-associated steatohepatitis; MASLD, metabolic dysfunction-associated steatotic liver disease; miRNA, microRNA; ncRNA, non-coding RNA; NAFLD, non-alcoholic fatty liver disease; RIN, RNA integrity number; SREBP, sterol regulatory element-binding protein; UMI, unique molecular identifiers

nority (about 12%), while internal substitutions within the seed region are exceedingly rare. Accordingly, for the vast majority of seed-preserved isomiRs, predicted target repertoires should largely overlap with those of canonical miRNAs, differing mainly in binding strength, stability, or regulatory kinetics rather than in target identity.

The expansions in predicted targetomes observed in the study are therefore driven primarily by rare seed-shifting isoforms rather than by the bulk of isomiRs. In silico network analyses naturally amplify the impact of these variants, as even a one-nucleotide 5′ shift redefines seed-based targeting rules. While such findings are mechanistically provocative, they also highlight the need to distinguish between numerical dominance and functional leverage when interpreting isomiR landscapes.

Given the prominence assigned to specific seed-shifting isoforms, the possibility of technical artefact deserves careful consideration. Several features of the study mitigate this concern. First, the authors estimate polymerase chain reaction duplication using unique molecular identifiers (UMI),

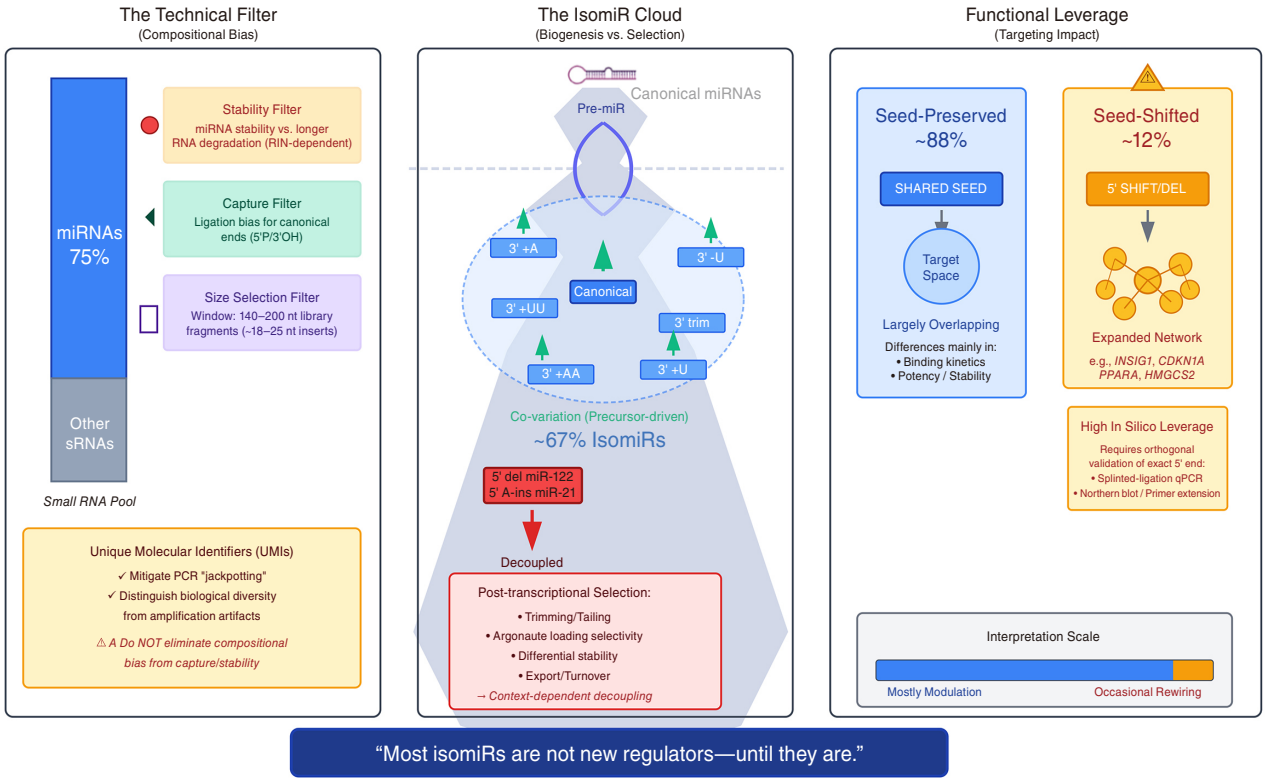

Figure 1. The IsomiR Landscape in MASLD. Conceptual framework for the technical and biological interpretation of hepatic isomiR landscapes in MASLD. The figure illustrates the multi-layered complexity of microRNA (miRNA) isoform analysis. (Left box) The Technical Filter highlights compositional biases in small RNA sequencing, where miRNA "pseudo-enrichment" can occur due to differential stability (RIN-dependent degradation of longer RNAs) and capture bias (ligation preference for 5′-P/3′-OH ends). UMIs are widely regarded as essential tools to mitigate PCR stochasticity, although they do not eliminate upstream capture artifacts. (Center box) The IsomiR Cloud represents the diversity of variants arising from a single precursor. These include 3′ trimming (shortening of the canonical sequence), 3′ non-templated additions (tailing, typically A/U-tailing via terminal nucleotidyltransferases), and 5′ end variation (isomiRs with shifted or deleted 5′ termini). While most variants co-vary with the canonical form (precursor-driven), specific isoforms, such as the 5′-del miR-122 and 5′-A-ins miR-21, can decouple from the dominant strand, suggesting regulated post-transcriptional selection or differential argonaute (AGO) loading. The background iceberg symbolizes the hierarchical structure of the miRNA pool. The visible "tip" represents the canonical miRNAs, the traditionally recognized, dominant sequences. The submerged, much larger mass represents the isomiR ecology: a vast, often overlooked diversity of isoforms that, while less abundant individually, collectively dominate the hepatic small-RNA landscape and provide a deeper layer of post-transcriptional regulation in MASLD. (Right box) Functional Leverage distinguishes between seed-preserved and seed-shifted variants. Seed-preserved isomiRs (the majority) largely modulate the canonical target space through altered binding kinetics or stability. In contrast, 5′ end variation (seed-shifting) can fundamentally rewire the regulatory network by targeting novel transcripts (e.g., *INSIG1*, *PPARA*), necessitating orthogonal validation of the exact 5′ terminus to exclude sequencing artifacts. RIN, RNA integrity number; sRNA, small RNA; PCR, polymerase chain reaction; qPCR, quantitative PCR; MASLD, metabolic dysfunction-associated steatotic liver disease.

https://doi.org/10.3350/cmh.2026.0053

thereby distinguishing true molecular diversity from amplification "jackpotting." Second, the scarcity of internal base substitutions argues against widespread sequencing error as a driver of isomiR diversity. Third, the highlighted miR-122 variant involves a templated 5′ deletion, a modification compatible with alternative RNase III cleavage rather than random error.

Nevertheless, as with all isoform-centric claims, definitive resolution will require orthogonal validation. Demonstrating persistence of the signal after collapsing reads by UMI and confirming the precise 5′ terminus using targeted, ligation-independent assays would conclusively exclude trimming or library-preparation artefacts. Such validation represents not a weakness of the study, but the natural next step in translating computational insight into mechanistic certainty. Figure 1 summarizes all the discussed aspects.

The dominance of miR-122 in the liver cannot be overstated, accounting for over 40% of hepatic miRNA reads in this cohort. Our prior work[5] consistently identified miR-122 as a sentinel of liver integrity, with declining hepatic levels and rising circulating concentrations marking advanced MASLD and fibrosis. Brion et al.[3] refine this paradigm by revealing that miR-122 is not a single molecular signal, but a constellation of isoforms whose relative balance shifts with disease stage.

This insight dovetails with broader observations regarding non-coding RNA (ncRNA) crosstalk in MASLD. The interaction between isomiRs and other non-coding species, particularly long non-coding RNAs (lncRNAs) and circular RNAs, remains an underexplored regulatory layer. As we have previously proposed, this "dark matter" of the MASLD genome may critically shape disease heterogeneity and therapeutic response.[11]

One of the most provocative avenues opened by this work lies in the competitive endogenous RNA network. Emerging evidence suggests that lncRNAs, some potentially derived from or induced by viral or endogenous retroviral elements, can act as sponges for specific miRs, modulating their bioavailability.[12] Such interactions could create patient-specific regulatory bottlenecks, where sequestration/regulation of protective miRs/isomiRs accelerates progression toward liver steatosis and fibrosis. A clear example is the hepatitis C virus sequestering miR-122.[13]

Although it deserves focused investigation in future studies, the viral-host ncRNA interplay represents a compelling, if still speculative, framework for understanding the heterogeneity of MASLD and other complex diseases.[14,15]

Brion et al.[3] have expanded the conceptual boundaries of hepatic miRNA biology. By demonstrating that isomiRs dominate the liver miRNA landscape and that a minority of seed-shifting variants may exert outsized regulatory effects, they provide both a cautionary tale against oversimplifying the canonical view and a roadmap for precision hepatology (Fig. 1). As the field advances toward personalized interventions in MASLD, embracing the full complexity of isomiR biology, while maintaining rigorous methodological scrutiny, will be essential.

Authors' contribution

Carlos Jose Pirola is responsible for the interpretation of data, the drafting, the critical revision of the manuscript for important intellectual content, and the approval of the final version of the article.

Conflicts of Interest

The author declares that he has no competing interests.

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