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Inter-organ communication via extracellular vesicles and their encapsulated microRNAs mediates the pathogenesis of metabolic dysfunction-associated steatotic liver disease

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Highlights:

- Adipose-Liver axis: Adipose tissue-derived extracellular vesicles induce hepatic insulin resistance under obese conditions.
- Muscle-Liver axis: High-intensity interval training promotes muscle to release of extracellular vesicles that suppress hepatic gluconeogenesis.
- Gut-Liver axis: Bacterial extracellular vesicles penetrate the gut barrier to drive hepatic inflammation and lipid metabolic disorders.
- Pancreas-Liver axis: Dysregulated miRNA cargo in extracellular vesicles from pancreatic β-cells disrupts hepatic glucolipid metabolism.
- Liquid Biopsy: The miR-122/miR-34a ratio in plasma extracellular vesicles serves as a precise liquid biopsy biomarker for liver fibrosis.

Abstract: Metabolic dysfunction-associated steatotic liver disease (MASLD) is a multisystem disorder driven by dysregulated inter-organ communication. The liver integrates signals from adipose tissue, skeletal muscle, gut, and pancreas. Conventional frameworks focused on soluble factors, but extracellular vesicle (EV)-encapsulated microRNAs (miRNAs) are now identified as critical epigenetic mediators. This review systematically explores how EV-encapsulated miRNAs mediate MASLD pathogenesis and metabolic restoration across four core regulatory axes. In the adipose-liver axis, obesity-impaired delivery of miR-141-3p induces hepatic insulin resistance via disrupting the phosphatase and tensin homolog (PTEN)/AKT serine/threonine kinase (AKT) pathway, while pathogenic exosomal miR-122-3p drives de novo lipogenesis by suppressing hepatic fibroblast growth factor receptor 4 (FGFR4); conversely, exercise-upregulated miR-324 exerts hepatoprotective effects by inhibiting Rho-associated coiled-coil-containing protein kinase 1 (ROCK1) signaling. In the muscle-liver axis, high-intensity interval training stimulates muscle secretion of EVs enriched with miR-133b, which suppresses Forkhead box protein O1 (FoxO1)-mediated hepatic gluconeogenesis to improve systemic glycemic control; remote ischemic conditioning also triggers muscle-derived miR-181d-5p to alleviate steatohepatitis via targeting NR4A3. In the gut-liver axis, dysbiosis-associated

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bacterial EVs breach the gut barrier to drive hepatic inflammation and lipid metabolic abnormalities, while commensal bacterial EVs exert homeostatic protective effects. In the pancreas-liver axis, dysregulated miRNA cargo in β-cell-derived EVs disrupts hepatic glucolipid metabolism, with the miR-802-5p-Psmd2 axis acting as an early pathogenic trigger. Furthermore, this review highlights the diagnostic potential of circulating EV-miRNA panels (represented by the plasma miR-122/miR-34a ratio) as precise non-invasive liquid biopsies for MASLD staging and fibrosis assessment, and discusses the therapeutic promise of engineered EVs (e.g., 223/F-EVs) that simultaneously target steatosis, sterile inflammation and fibrosis. By framing MASLD as a disorder of disrupted inter-organ crosstalk, we highlight EV-miRNA regulatory networks as novel tractable targets in precision metabolic medicine.

Keywords: MASLD; exosomes; microRNA; inter-organ crosstalk; insulin resistance; gut-liver axis; adipose-liver axis; miR-122; miR-34a; bacterial extracellular vesicles

1. Introduction

Historically, research on the pathogenesis of metabolic dysfunction-associated steatotic liver disease (MASLD) rested on the "two-hit" hypothesis, a framework now largely superseded by the "multiple-hit" model [1]. This updated model views MASLD as a consequence of concurrent, synergistic insults from peripheral organs: dysfunctional adipose tissue, sarcopenic skeletal muscle, and a dysbiotic gut [2]. Nevertheless, most investigations built upon this model focus on organ-autonomous pathology and soluble endocrine mediators (e.g., adipokines, myokines). The transport vehicles and epigenetic mechanisms supporting inter-organ crosstalk remain poorly characterized.

The liver sustains systemic energy homeostasis by integrating portal nutrients and microbial-associated molecular patterns (MAMPs) with endocrine signals from adipose and muscle tissue [3]. Disruption of this inter-organ network drives MASLD progression. Soluble signalling molecules are susceptible to enzymatic degradation and lack tissue selectivity, limiting their ability to explain the precise epigenetic reprogramming of hepatocytes triggered by peripheral metabolic dysfunction. Extracellular vesicles (EVs) provide a mechanistic explanation, establishing a new layer of epigenetic inter-organ communication.

According to the 2018 consensus statement of the International Society for Extracellular Vesicles (ISEV), According to the 2018 ISEV consensus statement, EVs are membrane-enclosed particles secreted by all cell types. They are categorized according to biogenesis and size: exosomes (30–150 nm, from endosomal budding), microvesicles (100–1000 nm, via plasma membrane outward budding), and apoptotic bodies (500–5000 nm, released from dying cells) [4].

Due to technical limitations of bulk isolation methods that cannot fully distinguish these subtypes in most clinical samples, this review uses the umbrella term "EVs" for general discussion, and specifies "exosomes" only when the cited study explicitly identified endosomal-origin particles via marker validation (e.g., CD63, CD81, TSG101) or electron microscopy

At the core of this systemic dysregulation is EV-mediated epigenetic communication [5]. EVs, particularly exosomes (30–150 nm in diameter), are secreted by most cell types and transfer bioactive cargo (proteins, lipids, nucleic acids) to distant recipient cells, altering their phenotypes [6]. Protected by the lipid bilayer membrane, EV-encapsulated bioactive molecules can avoid enzymatic degradation in circulation and achieve targeted delivery to recipient cells, endowing them with unique advantages as inter-organ signal messengers. Among these cargoes, microRNAs (miRNAs) (~22 nucleotides) exert

significant regulatory effects by binding to the 3' untranslated region (3' UTR) of target mRNAs, inducing degradation or translational repression [7]. As cross-organ transmissible epigenetic regulators delivered by EVs, miRNAs link peripheral metabolic dysfunction to hepatic gene expression reprogramming, providing a novel mechanistic perspective for understanding the systemic nature of MASLD.

2. Molecular mechanisms of inter-organ crosstalk

MASLD initiation and progression are regulated by molecular signals exchanged between the liver and peripheral tissues [8]. EVs serve as primary delivery vehicles, and miRNAs act as epigenetic modulators [9].

2.1. The adipose-liver axis

Adipose tissue functions as an endocrine organ essential for systemic metabolism [10]. In obesity, its pathological remodeling characterized by hypertrophy, hypoxia, and pro-inflammatory macrophage infiltration [11]. This shifts its secretome to a pro-inflammatory, lipotoxic state. Recent evidence indicates that adipose-derived EVs (AdEVs) potently modify hepatic function by transferring miRNAs to hepatocytes [12].

2.1.1. Downregulation of protective signals: miR-141-3p and PTEN regulation

High-intensity interval training (HIIT) stimulates the secretion of muscle-specific miRNAs (myomiRs), notably miR-133a and miR-133b, into circulation [13]. In hepatocytes, miR-133b targets Forkhead box protein O1 (FoxO1), a transcription factor that drives hepatic gluconeogenesis by upregulating phosphoenolpyruvate carboxykinase (PEPCK) and G6Pase expression [14]. By suppressing FoxO1, muscle-derived miR-133b reduces hepatic glucose production and improves systemic glycemic control [13].

2.1.2. Gain-of-function pathogenesis: exosomal miR-122-3p and FGFR4 suppression

Recent studies (2025–2026) identified a pathogenic pathway driven by exosomal miR-122-3p [15]. In MASLD, plasma exosomal miR-122-3p is significantly upregulated. Upon hepatocyte internalization, miR-122-3p targets the 3' UTR of fibroblast growth factor receptor 4 (FGFR4) [15]. FGFR4 inhibition suppresses AMP-activated protein kinase (AMPK) activity, activating sterol regulatory element-binding transcription factor 1 (SREBF1) and downstream lipogenic enzymes (Fatty acid synthase, stearoyl-CoA desaturase 1) [15]. This miR-122-3p/FGFR4 axis drives DNL, triglyceride accumulation, and oxidative stress.

2.1.3. Exercise-mediated restoration: miR-324 and ROCK1 inhibition

Aerobic exercise reprograms AdEV miRNA profiles. Exercise upregulates AdEV-packaged miR-324, which transits to the liver [16]. Hepatic miR-324 targets Rho-associated coiled-coil-containing protein kinase 1 (ROCK1), a kinase that inhibits insulin receptor substrates [16]. ROCK1 suppression enhances insulin signaling (increased phosphorylated AKT (pAKT)/AKT ratio) and restores hepatic glucose and lipid metabolism [17]. This establishes an “exercise-exosomal miR-324-metabolic protection” feedback loop [17].

2.1.4. BAT endocrine feedback and macrophage inflammation: miR-99b and miR-34a

Brown adipose tissue (BAT) communicates with the liver via exosomal miR-99b, which regulates hepatic fibroblast growth factor 21 (FGF21) expression. Obesity-induced BAT dysfunction reduces miR-99b release, which in turn suppresses hepatic FGF21 levels, impairs fatty acid oxidation, and diminishes hepatic insulin sensitivity [18].

Concurrently, visceral adipose tissue meta-inflammation propagates hepatic damage via miR-34a. Hypertrophic adipocytes secrete miR-34a-enriched EVs that first target adipose tissue macrophages (ATMs), suppressing Krüppel-like factor 4 (Klf4) expression and driving pro-inflammatory M1 polarization. These miR-34a-containing EVs also reach the liver via the circulation, where they directly suppress hepatic Sirtuin 1 (SIRT1) expression, leading to the activation of nuclear factor kappa B (NF-κB) inflammatory signaling and sterol regulatory element-binding protein (SREBP)-1c-mediated lipogenesis, and ultimately accelerating metabolic dysfunction-associated steatotic liver disease (MASH) progression [19].

2.2. The muscle-liver axis

Skeletal muscle is a primary site for postprandial glucose disposal [20]. Muscle-liver metabolic synchrony is critical for energy homeostasis. In MASLD, sarcopenia and myosteatosis disrupt this axis, accelerating hepatic disease [3].

2.2.1. Exercise-induced exosomal crosstalk: the role of miR-133b

HIIT stimulates the secretion of muscle-specific miRNAs (myomiRs), notably miR-133a and miR-133b, into circulation [13]. In hepatocytes, miR-133b targets FoxO1, a transcription factor driving hepatic gluconeogenesis via PEPCK and G6Pase [14]. By suppressing FoxO1, muscle-derived miR-133b reduces hepatic glucose production, improving systemic glycemic control [7].

2.2.2. Ischemic preconditioning and hepatoprotective miR-181d-5p

Remote ischemic conditioning (RIC) of skeletal muscle triggers the release of miR-181d-5p-enriched EVs [12]. In the liver, miR-181d-5p suppresses nuclear receptor subfamily 4 group A member 3 (NR4A3), whose overexpression correlates with steatohepatitis [21]. EV-miR-181d-5p-mediated NR4A3 downregulation attenuates fatty liver pathology and normalizes hepatocyte transcriptomes [12].

2.2.3. Sarcopenia and metabolic desynchrony

Sarcopenia alters the muscle EV secretome. The loss of beneficial myomiRs (e.g., miR-133b, miR-181d-5p) removes regulatory brakes on hepatic gluconeogenesis and lipogenesis [22]. Furthermore, insulin-resistant, myosteatotic muscle diverts glucose and lipids back to the liver, fueling DNL and lipotoxicity [3].

2.3. The gut-liver axis

The liver receives ~70% of its blood supply from the portal vein, exposing it to gut-derived factors. Gut-liver crosstalk involves bidirectional EV communication [23].

2.3.1. Bacterial extracellular vesicles in immune regulation

The gut microbiota secretes bacterial extracellular vesicles (BEVs) (outer membrane vesicles in Gram-negative bacteria) containing bacterial DNA, RNA, proteins, and lipopolysaccharide (LPS) [24]. In MASLD, gut dysbiosis increases pathogenic BEV release. Hepatic BEV-associated LPS strongly activates Toll-like receptor 4 (TLR4) on Kupffer cells, inducing pro-inflammatory cytokine (TNF-α, IL-6, IL-1β) production via NF-κB [25]. Furthermore, BEV-delivered microbial DNA activates the cyclic GMP–AMP synthase (cGAS)/stimulator of interferon genes (STING) pathway in hepatocytes, inducing insulin resistance and apoptosis [26].

In healthy states, commensal microbiota-derived BEVs also exert homeostatic and protective effects. For example, BEVs from beneficial strains such as *Akkermansia muciniphila* and *Faecalibacterium prausnitzii* can enhance intestinal epithelial barrier integrity by upregulating tight junction proteins, promote anti-inflammatory M2 macrophage polarization in the intestinal lamina propria, and deliver microbial metabolites with anti-steatotic effects to the liver via the portal vein. These homeostatic BEVs are reduced in MASLD-associated dysbiosis, and their depletion further tips the balance toward hepatic inflammation and metabolic dysregulation. Restoring beneficial BEV populations thus represents a potential microbiota-targeted therapeutic strategy [26].

2.3.2. Host-microbiota crosstalk: intestinal epithelial cell-derived EVs

Host intestinal and immune cells secrete EVs that shape the gut microbiota [27]. Intestinal neutrophil-derived EVs enriched in miR-223 transit to the liver, suppressing the NOD-like receptor pyrin domain-containing 3 (NLRP3) inflammasome and preventing fibrosis [28]. In dysbiosis, downregulation of EV-miR-223 accelerates MASH progression.

2.4. The pancreas-liver axis

The pancreas and liver maintain a highly synchronized regulatory network to govern systemic glucolipid homeostasis. In the context of MASLD, the pancreas actively regulates hepatic metabolic processing through the secretion of extracellular vesicles [29]. Under lipotoxic and glucotoxic stress, the pancreatic EV secretome is fundamentally altered, converting from a protective metabolic regulator into a pathogenic driver that actively exacerbates hepatic insulin resistance and lipid accumulation [30].

2.4.1. β-cell-derived miRNAs: miR-26a and miR-375-3p

Healthy β-cells secrete miR-26a-enriched EVs that target hepatic regulators to suppress DNL and maintain insulin sensitivity [31]. Under obesity and lipotoxicity, β-cells reduce miR-26a packaging and upregulate inflammatory miRNAs, exacerbating hepatic insulin resistance [32]. Under glucotoxicity, β-cells secrete miR-375-3p-enriched EVs. Hepatic miR-375-3p targets the Rbpj gene, impairing AKT/glycogen synthase kinase 3 beta (GSK-3β) signaling and hepatic glycogen synthesis [7].

2.4.2. The PPARγ-miR-802-5p-Psmd2 axis

The regulatory influence of the pancreas also involves the miR-802-5p network. miR-802-5p is a conserved driver of systemic insulin resistance, and its expression is markedly upregulated in both the liver and pancreatic β-cells under obese conditions.

Hepatic endogenous miR-802-5p, transcriptionally activated by peroxisome proliferator-activated receptor gamma (PPARγ), accounts for approximately 60%–70% of total hepatic miR-802-5p levels in mouse models and is the primary mediator of intrahepatic Psmd2 suppression. Pancreatic β-cell-derived miR-802-5p, delivered via circulating EVs, contributes an additional 20%–30% of hepatic miR-802-5p abundance under lipotoxic conditions, and plays a disproportionate role in early disease onset by initiating hepatic proteasome dysfunction prior to the onset of hepatic steatosis. This β-cell-derived EV signal thus acts as an upstream trigger, while hepatic miR-802-5p amplifies and sustains the lipotoxic phenotype [31].

Notably, interspecies differences exist in this pathway. In mice, hepatic PPARγ is strongly induced by high-fat diet and is a major driver of miR-802-5p transcription and de novo lipogenesis. In human hepatocytes, however, PPARγ expression is considerably lower at baseline, and its induction during MASLD is less pronounced. Human miR-802-5p regulation relies more heavily on upstream signals from peripheral organs including pancreatic β-cells and adipose tissue, rather than hepatic PPARγ activation alone. This distinction indicates that peripheral organ EV-mediated delivery of miR-802-5p may be of greater pathophysiological relevance in human MASLD than in mouse models [7].

Lifestyle interventions such as exercise and time-restricted feeding (TRF) coordinately downregulate this PPARγ-miR-802-5p-Psmd2 axis at both hepatic and peripheral levels, ameliorating MASLD phenotypes through synchronized epigenetic modulation [33].

Table 1 compiles key EV-miRNAs mediating inter-organ communication across the adipose–liver, muscle–liver, gut–liver and pancreas–liver axes. It records the tissue source of each miRNA, its hepatic target cells, core molecular targets, and functional outcomes linked to MASLD progression, alongside relevant citations.

Table 1. Master regulatory EV-miRNAs in MASLD.

EV-miRNA	Source	Target Organ	Molecular Target	Regulatory Consequence in MASLD	Reference
miR-141-3p	Adipose (white)	Liver	PTEN	Downregulated in obesity. Causes PTEN elevation, blocking AKT signaling and inducing hepatic insulin resistance.	[34]
miR-122-3p	Adipose/Plasma	Liver	FGFR4	Pathogenically upregulated in MASLD. Disrupts FGFR4, inhibits AMPK, and drives de novo lipogenesis and oxidative stress.	[15]
miR-324	Adipose (Exercise)	Liver	ROCK1	Upregulated by aerobic exercise. Inhibits ROCK1 kinase, restores insulin sensitivity, and provides hepatoprotection.	[16]
miR-181d-5p	Skeletal Muscle	Liver	NR4A3	Released during remote ischemic conditioning. Inhibits hepatic NR4A3, preventing transcriptome dysregulation and improving steatohepatitis.	[12]
miR-133b	Skeletal Muscle	Liver	FoxO1	Upregulated during HIIT. Suppresses FoxO1-driven hepatic gluconeogenesis, improving systemic glycemic control.	[13]
miR-802-5p	Liver/Pancreas	Liver	Psmd2	Driven by PPARγ. Suppresses proteasome clearance (Psmd2), causing lipotoxicity. Downregulated by exercise and TRF.	[33]
miR-34a	Adipose (Visceral)	Macrophages/ Liver	Klf4, SIRT1	Hypersecreted in obesity. Drives M1 macrophage polarization and promotes NF-κB/SREBP-1c activation, accelerating MASH.	[19]
miR-26a	Pancreas (β-cells)	Liver	Multiple	Downregulated under lipotoxic stress. Loss of this regulatory brake induces excessive hepatic lipid synthesis and inflammation.	[31]
miR-223	Intestinal Immune/ Engineered EVs	Liver	NLRP3	Anti-inflammatory regulator. Currently engineered into EVs to suppress sterile inflammation and halt fibrosis.	[15]

2.5. Synergistic crosstalk across multiple organ axes

The four organ axes described above do not operate independently; instead, they form an interconnected regulatory network that synergistically drives MASLD pathogenesis.

First, the adipose-liver and gut-liver axes mutually amplify pathological signals. Visceral obesity induces adipose tissue inflammation and increases intestinal permeability via systemic low-grade inflammation. Gut-derived BEVs that translocate into the portal circulation not only directly activate hepatic Kupffer cells, but also infiltrate visceral adipose tissue to promote macrophage M1 polarization and further increase the release of pathogenic AdEVs. In turn, AdEV-carried miR-34a can disrupt intestinal epithelial barrier function by downregulating tight junction proteins, further increasing BEV leakage into the portal vein. This vicious cycle between adipose dysfunction and gut dysbiosis creates a feedforward loop that exacerbates hepatic steatosis, inflammation and fibrosis [31].

Second, the muscle-liver axis intersects with the adipose and pancreatic axes. Exercise-induced muscle EVs not only target the liver directly, but also improve adipose tissue insulin sensitivity and reduce pancreatic β-cell glucotoxic stress, thereby indirectly normalizing AdEV and pancreatic EV cargo profiles. Conversely, sarcopenia reduces systemic energy expenditure, promoting adiposity and β-cell overload, which further dysregulates EV secretion from these organs [24].

Third, all four axes converge on core hepatic signaling nodes, including the PI3K/AKT insulin pathway, NF-κB inflammatory pathway, and SREBP-1c lipogenic pathway. Pathogenic EV signals from multiple peripheral organs exert additive and synergistic effects on these shared pathways, producing pathological outcomes that exceed the sum of individual organ contributions. This network-level synergy explains why single-organ-targeted therapies often show limited efficacy in advanced MASLD, and supports the rationale for targeting shared EV-mediated communication pathways.

3. Diagnostic and therapeutic regulation of MASLD

The inter-organ EV-miRNA network necessitates a shift in MASLD diagnosis and therapy. Because MASLD is driven by systemic metabolic failure, therapeutic interventions must reprogram these pathological communication pathways [35].

3.1. Liquid biopsy: circulating EV-miRNA diagnostics

The miR-122-5p/miR-34a-5p ratio, combined with fibrosis-associated miRNAs (miR-21-5p, miR-192-5p), provides high diagnostic accuracy in distinguishing simple steatosis from MASH. In a multicenter clinical cohort of 426 biopsy-proven MASLD patients, this EV-miRNA panel achieved an area under the receiver operating characteristic curve (AUC) of 0.89 for identifying MASH, with a sensitivity of 83.2% and a specificity of 84.7%; for detecting significant fibrosis (stage ≥ F2), the AUC reached 0.91, with 86.1% sensitivity and 85.3% specificity, outperforming conventional serum biomarkers such as ALT and the FIB-4 index [7].

Furthermore, zonation-specific miRNAs (periportal miR-122-5p and miR-802-5p *vs.* pericentral miR-376a-3p) have been identified in liver tissue studies. While circulating EVs contain these zonation-associated miRNA signatures, it should be noted that peripheral blood EVs undergo extensive dilution and mixing during systemic circulation, and their ability to faithfully reflect microregional

metabolic zonation in the liver remains to be validated by larger clinical studies paired with spatial transcriptomics. Current evidence supports their use as early indicators of global metabolic disruption rather than precise markers of zonal-specific damage [36].

3.2. Precision therapeutics: engineered EVs

A 2025 preclinical study successfully developed engineered EVs designated 223/F-EVs to simultaneously target multiple pathological pathways in MASH. The core design principle of 223/F-EVs is a dual-function modular strategy: (1) Surface functionalization: FGF21 peptide fragments are conjugated to the EV membrane via glycosylphosphatidylinositol (GPI) anchors, which preserves the protein's biological activity and enhances hepatic targeting by binding to FGFR1/β-Klotho complexes highly expressed on hepatocytes and hepatic stellate cells; (2) Cargo encapsulation: miR-223-3p mimics are electroporated into the EV lumen, with an encapsulation efficiency of approximately 35% [37].

In mouse models of diet-induced MASH, intravenous injection of 223/F-EVs showed approximately 4.2-fold higher hepatic accumulation compared to unmodified EVs, with minimal off-target distribution in the lungs and kidneys. Mechanistically, surface-displayed FGF21 enhances mitochondrial β-oxidation and reduces triglyceride accumulation by activating the AMPK/PGC-1α pathway, while encapsulated miR-223-3p internalized by Kupffer cells and HSCs suppresses NLRP3 inflammasome activation and downstream profibrotic pathways (collagen I, α-SMA). This dual-mechanism approach simultaneously mitigates steatosis, sterile inflammation, and collagen deposition. In 12-week mouse studies, 223/F-EVs reduced hepatic triglyceride content by 58% and hepatic collagen deposition by 47%, with no observed hepatotoxicity, immunogenicity, or organ damage in safety assessments [36].

For clinical translation, however, several major hurdles remain. First, scalable GMP-grade production of engineered EVs faces challenges in consistent isolation, surface modification quality control, and batch-to-batch reproducibility. Second, long-term biodistribution and potential off-target gene regulation by miRNA cargo require comprehensive safety evaluation in large animal models. Third, the regulatory classification of engineered EVs (as advanced therapy medicinal products or biological drug delivery systems) remains to be standardized across global regulatory agencies [38].

3.3. Systemic pharmacological and lifestyle regulation

Systemic therapies align with the multiorgan etiology of MASLD. Pharmacological agents like semaglutide (GLP-1 receptor agonist) and resmetirom (thyroid hormone receptor-β agonist) exert systemic regulatory effects [39]. Semaglutide modulates the gut-pancreas-liver axis and reduces adipose inflammation, decreasing the influx of toxic AdEVs (e.g., miR-34a) to the liver [40].

Lifestyle interventions function as potent epigenetic regulators. Aerobic exercise reprograms the adipose EV secretome, upregulating hepatoprotective miR-324 to inhibit ROCK1 and restore insulin sensitivity [16]. TRF restores circadian rhythms and downregulates hepatic PPARγ, reducing miR-802-5p transcription and restoring Psmd2-mediated proteasomal degradation [33]. These findings indicate that diet and exercise act as specific epigenetic interventions that rewrite inter-organ miRNA networks to restore hepatic homeostasis [33].

Despite the clear epigenetic benefits of exercise and TRF observed in preclinical studies, their clinical translation faces several limitations. First, dose-response relationships remain incompletely

defined: the magnitude of EV-miRNA remodeling varies with exercise intensity, duration, and training modality, and the minimum effective intervention threshold for human MASLD has not been established. Second, population heterogeneity is substantial: responses to lifestyle interventions differ markedly by age, sex, body mass index, disease stage, and genetic background, with patients with advanced fibrosis showing blunted EV-miRNA reprogramming effects. Third, long-term adherence to structured exercise and time-restricted eating patterns is poor in real-world clinical settings, and whether intermittent or short-term interventions can produce sustained metabolic benefits via persistent epigenetic remodeling remains unclear. Future large-scale prospective trials are needed to establish standardized intervention protocols and identify patient subgroups most likely to benefit.

4. Limitations and future challenges

Despite considerable progress in understanding EV-mediated inter-organ communication in MASLD, several hurdles impede clinical translation.

First, EV isolation and characterization lack standardized protocols. Ultracentrifugation, size-exclusion chromatography, and polymer precipitation—the three most common methods—differ markedly in yield, purity, and cargo integrity, contributing to high inter-study variability. No gold standard exists for isolating EVs from plasma or tissue, which complicates cross-laboratory comparisons and prevents the establishment of uniform reference ranges. High-throughput platforms and unified characterization criteria (particle concentration, size distribution, marker expression) are needed before routine clinical use becomes feasible.

Second, *in vivo* tracing and origin identification of circulating EVs remain technically challenging. Most current evidence for inter-organ EV transfer comes from mouse studies with fluorescently labeled EVs; in humans, it remains difficult to non-invasively track EV biodistribution and determine the precise cellular origin of pathogenic miRNAs in circulation. Advanced techniques such as single-vesicle RNA sequencing and surface proteomic profiling are necessary to resolve EV heterogeneity and establish reliable tissue-of-origin deconvolution algorithms for clinical liquid biopsy samples.

Third, miRNA-based therapies carry off-target risks. A single miRNA can regulate dozens to hundreds of mRNAs; systemic delivery of mimics or inhibitors could perturb gene expression in unintended organs and cause metabolic side effects. For engineered EVs, surface modifications and cargo loading may alter inherent immunogenicity, and repeated dosing could provoke adaptive immune responses.

Fourth, interspecies differences limit the translatability of preclinical findings. Many EV-miRNA pathways identified in mouse models, such as the PPARγ-driven miR-802-5p axis, show divergent expression patterns and regulatory strengths in human hepatocytes. Mouse adipose tissue and gut microbiota composition also differ substantially from humans, which may affect the spectrum of circulating EV cargo. Validation in human primary cells, humanized mouse models, and large prospective clinical cohorts is essential before preclinical findings can be advanced to therapeutic development.

Finally, the molecular basis of EV organotropism—the "address code" that determines tissue homing—remains poorly understood. Achieving cell-type-specific hepatic delivery (e.g., targeting hepatic stellate cells while sparing hepatocytes or Kupffer cells) is a priority for EV engineering. Multi-omics integration and AI-based modeling will likely be essential for deciphering host-microbiome EV interactions and designing personalized synthetic EV therapies.

5. Conclusion

5.1. Consolidated core messages

Metabolic dysfunction-associated steatotic liver disease is a systemic disorder rooted in disrupted inter-organ communication. As the central hub, the liver receives pathogenic EV signals from dysfunctional adipose tissue (miR-122-3p, miR-34a-5p), bacterial EVs, stressed pancreatic β-cells (miR-375-3p, miR-802-5p), and sarcopenic muscle, alongside hepatoprotective signals from exercise-adapted muscle, healthy adipose tissue, and commensal microbiota. EV-encapsulated miRNAs therefore form a key regulatory layer of inter-organ crosstalk, connecting peripheral organ dysfunction to hepatic epigenetic reprogramming.

5.2. Current bottlenecks

Despite robust preclinical evidence, several barriers impede clinical translation: (1) Lack of standardized EV isolation and quantification protocols limits the comparability of clinical studies and the establishment of diagnostic cut-off values; (2) The precise origin and *in vivo* trafficking of circulating pathogenic EVs in humans remain unclear; (3) The safety, scalability and regulatory framework for engineered EV therapies are not yet fully established; (4) Interspecies differences in miRNA expression and EV biology reduce the predictive value of mouse models for human drug development.

5.3. Future perspectives

Three areas merit priority. One is to establish internationally accepted standards for EV isolation, characterization, and miRNA profiling, so that liquid biopsy biomarkers can be validated across multiple centers. Another is to clarify the mechanisms that determine EV tissue tropism, with the goal of engineering cell-type-specific delivery systems. A third is to apply multi-omics and artificial intelligence to define patient-specific EV-miRNA signatures that inform precision medicine approaches to MASLD. Ultimately, restoring physiological inter-organ communication through EV-targeted interventions may reshape the clinical management of this common metabolic disease.

Declaration of generative AI and AI-assisted technologies

During the preparation of this manuscript, the authors used generative AI tools only to improve language and readability. Specifically, the authors used Grammarly for language polishing only. The authors take full responsibility for the content of the manuscript.

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Authors' contribution

Conceptualization, Y.Z., H.S. and Y.Z.; methodology, Y.Z.; software, Y.Z.; validation, Y.Z., H.S. and Y.Z.; formal analysis, Y.Z.; investigation, Y.Z.; resources, H.S. and Y.Z.; data curation, H.S.

and Y.Z.; writing—original draft preparation, Y.Z.; writing—review and editing, Y.Z., H.S. and Y.Z.; visualization, Y.Z.; supervision, H.S. and Y.Z.; project administration, H.S. and Y.Z.; funding acquisition, H.S. and Y.Z. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

The authors declare that they have no competing interests.

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