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REVIEW

Epigenetic regulation in MASLD – insight for therapeutic target discovery

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ABSTRACT

Metabolic dysfunction-associated steatotic liver disease (MASLD) is the most prevalent chronic liver disease. It encompasses metabolic affections that arise primarily from lifestyle and diet, independent of alcohol consumption. Particularly, the global rise in obesity constitutes a major risk factor and comorbidity, contributing to the prevalence and severity of MASLD. Despite its widespread impact and potential progression to severe liver pathologies, effective therapies remain limited, underscoring the urgency to better understand its underlying molecular mechanisms.

Epigenetic regulation is essential for maintaining hepatic metabolic function and cellular identity in response to metabolic cues but becomes critically dysregulated during MASLD. Alterations in DNA methylation, histone modifications, and non-coding RNAs contribute to disease progression, promoting metabolic dysfunction and lipid accumulation. Recent evidence indicates that epigenetic dysregulation in MASLD also occurs in a spatial manner where changes in chromatin accessibility, zone-enriched microRNAs, and transcription factor activity can disrupt gene expression along the periportal-pericentral axis, leading to zone-specific disturbances in metabolic specialization, which emerges as a critical novel factor influencing the onset and progression of MASLD. Furthermore, this review explores the role of microRNAs and extracellular vesicles in mediating systemic metabolic dysfunction observed in MASLD and highlights emerging epigenetic strategies with therapeutic potential in MASLD.

PLAIN LANGUAGE SUMMARY

Obesity is a condition when consumed calories exceed the amount of calories burned. Obesity numbers are rising, and it drives the development of diseases challenging to diagnose or to treat. One of such diseases is the metabolic dysfunction-associated steatotic liver disease (MASLD). MASLD is a condition where the organism starts to store fat in the liver tissue since the natural fat reservoirs (fat tissue) are overflowing. This fat accumulation causes impairments of liver function until complete liver failure if allowed to progress. Even though fat cells and liver cells share the same inherited genetic information in the form of DNA, the cell types respond differently to their stored fat content and show a distinguishable shape and function. This difference in shape and response to fat is regulated by mechanisms that lay "above" (Greek: epi) the genetic information and are therefore termed epigenetics. Alterations in epigenetics, usually assessed by comparing the diseased-state with the pre-diseased state of an organ, are drivers for a variety of diseases, including MASLD. Identifying those epigenetic alterations and restoring the pre-diseased state displays great therapeutic potential to treat MASLD. This review aims to summarize the current knowledge and drug research in this topic.

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1. Introduction

1.1. Definition and pathogenesis of MASLD

Metabolic dysfunction-associated steatotic liver disease (MASLD), formerly known as nonalcoholic fatty liver disease (NAFLD), represents an umbrella term for metabolically driven liver diseases. MASLD is characterized by fat accumulation in at least 5% of hepatocytes accompanied by at least one cardiometabolic risk factor such as insulin resistance, hypertension, dyslipidemia, and elevated BMI, with alcohol consumption below 140 g/week for women and 210 g/week for men [1]. The global prevalence of MASLD has increased dramatically over the past three decades, currently affecting approximately 30–38% of adults and 7–14% of children and adolescents worldwide [2].

MASLD can progress to more severe and life-threatening conditions, particularly metabolic dysfunction-associated steatohepatitis (MASH, formerly NASH), which leads to fibrosis, cirrhosis, or hepatocellular carcinoma (HCC) [3]. Until recently, no pharmacological treatment was approved for either MASLD or MASH. The approval of resmetirom, a selective thyroid hormone receptor beta (THRβ) agonist, provided the first therapy for MASH [4]. Additionally, semaglutide, a glucagon-like peptide 1 receptor agonist, received FDA approval for MASH treatment in August 2025 [5]. However, therapies targeting MASLD onset or progression to more severe manifestations remain limited due to the disease's complexity and heterogeneity [6].

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Article highlights

- In this review article we give a comprehensive overview of the most recent epigenetic drug developments for treating Metabolic dysfunction-associated steatotic liver disease (MASLD).
- We provide a detailed description of the current cell type-specific delivery strategies including hit-and-run epigenome editing which achieved durable reductions in serum cholesterol after a single administration as demonstrated preclinically by targeted DNA methylation and histone modifications at the hepatic PCSK9 locus, establishing proof of principle for epigenome-based liver therapies.
- Epigenetic dysregulations associated with fatty liver and MASLD are critically summarized and compared between humans and mice. The deduced novel miRNA-based therapeutics, including miRNA mimics and inhibitors targeting dysregulated metabolic and inflammatory pathways, represent a promising and actively developing approach for MASLD treatment.
- We highlight that epigenetic dysregulation in MASLD is not only global but spatially organized along the periportal–pericentral axis of the liver lobule, with zone-specific gradients in chromatin accessibility, DNA methylation, microRNA expression, and transcription factor activity maintaining metabolic zonation in healthy liver.
- Advances in cell type-specific delivery systems, including nanoparticles, extracellular vesicles, and GalNAc-conjugated oligonucleotides enabling carrier-free, liver-directed delivery, are summarized as they are facilitating the clinical translation of epigenetic therapies.

1.2. Scope of this review

In this review, we summarize current knowledge on how epigenetic mechanisms shape liver zonation, contribute to MASLD development and progression, and offer potential therapeutic targets. Furthermore, we highlight current developments in epigenetic treatment therapies for MASLD, recognizing that the liver has a unique bidirectional role in producing and relying on epigenetic substrates. Understanding these processes is critical, as the lack of effective treatments for MASLD underscores the urgent need to identify new strategies to address this disease. DNA methylation, non-coding RNAs, and histone modifications.

2. Epigenetic mechanisms: overview and principles

Epigenetics refers to the sum of mechanisms that are mitotically and/or meiotically heritable and regulate gene expression without altering the underlying genetic information [7]. These mechanisms are cell- and tissue-specific [8] and determine cell fate while exhibiting high plasticity in response to external and internal stimuli, allowing organisms to adapt dynamically to specific environmental circumstances [9]. Three major epigenetic mechanisms are best understood: histone modifications, DNA methylation, and non-coding RNAs [10]. While RNA modifications are increasingly recognized as part of the epigenetic regulatory landscape (termed epitranscriptomics or RNA epigenetics), their effects are predominantly transient, and debate continues regarding whether they constitute classical epigenetic mechanisms. For clarity, we focus here on the three established epigenetic mechanisms and their effects on gene regulation.

2.1. Histone modifications

Histone modifications predominantly modulate chromatin structure and accessibility or recruit transcriptional enhancers/repressors, thereby modulating gene expression. These post-translational chemical modifications to histone proteins can alter the charge of histones and affect their binding intensity to negatively charged DNA. Modifications are added to specific amino acid residues on the "tails" of histone proteins and include acetylation, methylation, phosphorylation, ubiquitination, and citrullination.

Histone acetylation and phosphorylation reduce the positive histone charge, disrupting electrostatic interactions with negatively charged DNA and causing a less compact chromatin structure, thereby increasing chromatin accessibility and gene transcription. Acetylation occurs on lysine residues, whereas phosphorylation predominantly takes place at serines, threonines, or tyrosines [11]. In contrast, histone methylation occurs at lysine and arginine residues without altering histone charge; thus, the chromatin structure is not directly affected. Lysine residues are often mono-, di-, or trimethylated, while arginine residues are mono- or demethylated [12]. The effect of methylation on transcription depends on its location (which lysine or arginine of a histone), the number of methylations (mono, di, tri), and which factors are recruited. For example, H3K4me3 (Histone 3, Lysine residue 4, trimethylation) is associated with active transcription and is found in promoters [13], whereas H3K9me3 (Histone 3, Lysine residue 9, trimethylation) is associated with repressive heterochromatin through heterochromatin protein 1 (HP1)-mediated gene silencing (Figure 1(a)) [14]. A common but sparsely studied modification is histone ubiquitination. Estimations suggest that up to 15% of histone 2A (H2A) and 2% of histone 2B (H2B) are conjugated to ubiquitin in vertebrate cells. The former is associated with transcriptional activation, whereas the latter is associated with transcriptional repression [15].

2.2. DNA methylation

DNA methylation involves the transfer of a methyl group onto the fifth carbon of cytosine within cytosine-guanine sites, also known as CpG sites [16]. This process is catalyzed by DNA methyltransferases (DNMTs), where S-adenosylmethionine (SAM), generated in the methionine cycle, serves as the main methyl group donor within eukaryotic cells [17]. Three DNMTs are currently recognized: *DNMT1*, *DNMT2*, and *DNMT3* (comprising *DNMT3a*, *DNMT3b*, and *DNMT3L*). Despite binding to DNA, there is no evidence proving methylation activity of *DNMT2*, which is theorized to serve as a marker of specific DNA sequences [18]. Therefore, *DNMT1*, *DNMT3a*, and *DNMT3b* are assumed to be the predominant DNA methyltransferases (Figure 1(a)).

DNMT1 preferentially methylates hemimethylated cytosines-a state where only one strand of double-stranded DNA is methylated, while the newly synthesized strand remains unmethylated after DNA replication. *DNMT1* recognizes the 'missing' methylation on the unmethylated strand and transfers a methyl group to restore the methylation pattern on both strands. Therefore, *DNMT1* is often referred to as the maintenance DNMT [19]. *DNMT3a* and *DNMT3b* are capable of methylating hemimethylated CpGs but show no preference for unmethylated or hemimethylated CpGs. Since DNMT3s can introduce new methylation on 'naked,' unmethylated DNA, they are referred to as de novo DNMTs [20,21]. DNA methylation regulates gene expression by

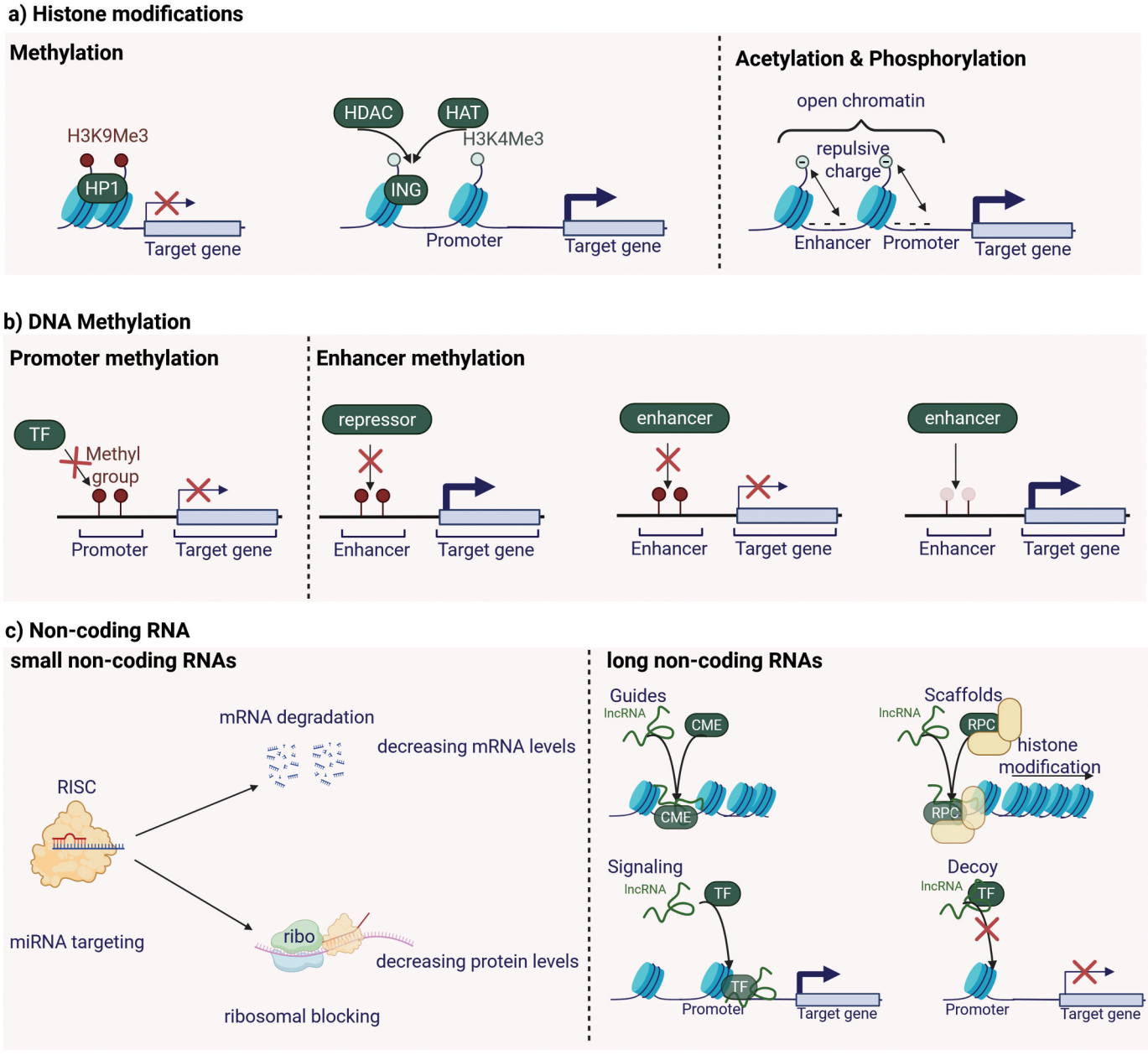

Figure 1. Overview of the three best described epigenetic mechanisms.

Histone modifications involve methylation, acetylation, and phosphorylation. Since the latter two introduce a negative charge to the histone, repulsive electrostatic effects between the negatively charged histone and DNA create an opened, chromatin accessible for transcription factors. Histone methylation either represses gene expression by recruitment of HP1 or enhances gene expression. DNA methylation occurs at CpG sites, and promoter methylation is commonly associated with repression of the target gene. In enhancer regions, DNA methylation either enhances gene expression by interfering with repressor binding or represses gene expression by interfering with enhancer binding. Non-coding RNAs are majorly categorized into small non-coding RNAs to which miRNAs belong to and long non-coding RNAs (lncRNAs). MiRNA binding to a target gene mRNA induces either mRNA degradation or translational inhibition. LncRNAs serve guiding, scaffolding, signaling, or decoy functions.

Abbreviations: H3K9Me3 = trimethylation at lysine 9 of histone 3, HP1 = heterochromatin protein 1, HDAC = histone deacetylases, HAT = histone acetyltransferases, ING = inhibitor of growth proteins, TF = transcription factor, RISC = RNA-induced silencing complex, miRNA = microRNA, ribo = ribosome, mRNA = messenger RNA, lncRNA = long non-coding RNA, CME = chromatin modifying enzyme, RPC = ribonucleoprotein complex.

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interfering with DNA–protein interactions and is capable of gene silencing or activation depending on the location where methylation occurs [22,23]. DNA methylation of the proximal promoter region and the first exon commonly inhibits gene expression [24]. However, methylation of the gene body is considered to enhance gene expression [25,26]. DNA methylation of other regulatory elements, such as enhancers, highly depends on the factors binding to these specific regions (Figure 1(b)).

2.3. Non-coding RNAs

The category of non-coding RNAs can be subdivided into long non-coding RNAs (lncRNAs) and small non-coding RNAs, comprising endogenous small interference RNAs (endo-siRNAs), Piwi-interacting RNAs (piRNAs), small nucleolar RNAs (snoRNAs), micro RNAs (miRNAs), transfer RNAs (tRNAs), and circular RNAs [27–29]. Among these, lncRNAs and miRNAs are mechanistically well described. miRNAs regulate gene expression at the post-

transcriptional level through RNA interference (RNAi), either inhibiting ribosomal mRNA translation or initiating mRNA degradation. In contrast, lncRNAs regulate gene expression through various mechanisms [30,31]. lncRNAs can modulate gene expression through direct interaction with transcription factors (TFs) by acting as decoys or scavenger molecules preventing TF binding, by recruiting TFs, or by affecting chromatin structure (Figure 1(c)) [31].

3. Metabolic sources of epigenetic substrates and their dysregulation in MASLD

Epigenetic modifications depend critically on the availability of specific metabolic substrates, particularly methyl donors for DNA and histone methylation, and acetyl groups for histone acetylation. The liver, as the central metabolic organ, plays a pivotal role in generating and regulating these epigenetic substrates through interconnected metabolic pathways. Understanding the interplay between nutritional intake, hepatic metabolism, and epigenetic regulation is essential for elucidating the molecular mechanisms underlying MASLD.

3.1. Hepatic one-carbon metabolism and methyl donor availability

The one-carbon metabolism serves as the liver's primary system for generating methyl groups used in epigenetic modifications. This metabolic network converts dietary nutrients-including methionine, folate, choline, and betaine-into S-adenosylmethionine (SAM), the universal methyl donor for all biological methylation reactions. The process integrates three interconnected pathways: the folate cycle, the methionine cycle, and transsulfuration pathway [32]. In the methionine cycle, the essential amino acid methionine is converted by methionine adenosyltransferase (MAT) into SAM, which serves as the direct substrate for DNA methyltransferases (DNMTs) and histone methyltransferases (HMTs) [33,34]. After donating its methyl group to chromatin, SAM is converted to S-adenosylhomocysteine (SAH), which acts as a competitive inhibitor of methyltransferases, creating a feedback mechanism that links methylation potential to the SAM/SAH ratio (Figure 2) [35]. Dietary sources that support this pathway include leafy greens (spinach, kale), cruciferous vegetables (broccoli, cauliflower), eggs, liver, fish, legumes, whole grains, berries, nuts, and seeds. Multiple analyses of the National Health and Nutrition Examination Survey (NHANES) database have demonstrated that serum folate and 5-methyltetrahydrofolate levels are reduced in adults with MASLD and that individuals with higher serum folate levels have a lower risk of developing MASLD [36–38]. These findings suggest that enhancing folate status through dietary or pharmacological interventions might represent a valuable therapeutic strategy for MASLD (Figure 2).

3.2. Acetyl-CoA metabolism and histone acetylation

Acetyl-CoA, the primary acetyl group donor for histone acetylation, is derived from multiple dietary macronutrients. Glucose metabolism through glycolysis generates pyruvate, which is

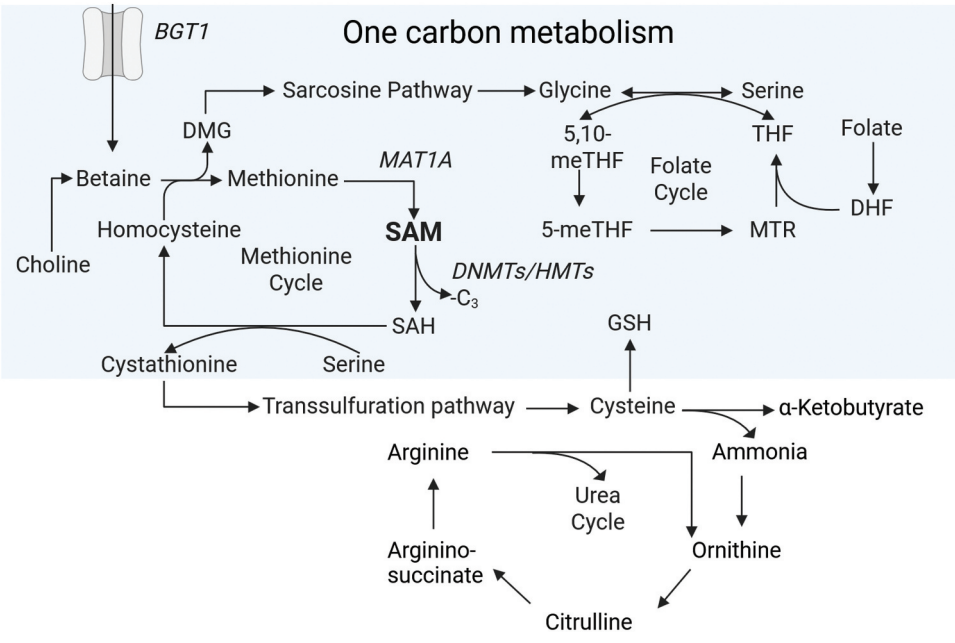

Figure 2. Overview of the one-carbon metabolism, which comprises the interlocked methionine- and folate cycles highlighted in blue background and the connected urea cycle.

Betaine is imported into the hepatocytes via the betaine/GABA transporter 1 (BGT1, encoded by SLC6A12). Intracellular conversion of choline into betaine is an additional source for betaine levels. A methyl group transfer from betaine to homocysteine forms methionine and dimethylglycine (DMG). DMG is further metabolized in the sarcosine pathway into glycine, which enters the folate cycle, whereas methionine is channeled into the methionine cycle to form S-adenosylmethionine (SAM) catalyzed by MAT1A. From SAM, a methyl group is transferred either onto CpG in the DNA via DNMTs or onto histone tails by HMTs, which forms the remaining degradation product S-adenosyl homocysteine (SAH). SAH is broken down into homocysteine, which enters again either the methionine cycle or is condensed with serine to form cystathionine, the educt of the transsulfuration pathway to form cysteine, which is necessary for ammonia detoxification into urea within the urea cycle.

Abbreviations: DNMTs = DNA methyltransferases, HMTs = histone methyltransferases, THF = tetrahydrofolate, DHF = dihydrofolate, GSH = glutathione, GSSG = glutathione disulfide.

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subsequently converted to acetyl-CoA by pyruvate dehydrogenase in mitochondria [39]. Fatty acid β-oxidation represents another major source of acetyl-CoA, particularly during fasting states or ketogenic conditions [40]. Additionally, certain amino acids, including leucine, isoleucine, lysine, and threonine, can be catabolized to generate acetyl-CoA [41]. Acetate, obtained from dietary fiber fermentation by gut microbiota or directly from alcoholic beverages, can be converted to acetyl-CoA by acetyl-CoA synthetase 2 (*ACSS2*), providing an alternative acetyl source for nuclear histone acetylation [42]. Under conditions of nutrient excess, particularly high carbohydrate availability as observed in many individuals with MASLD, hepatic acetyl-CoA levels increase substantially, promoting histone acetylation and the expression of lipogenic genes [43].

3.3. Western diet-induced disruption of epigenetic substrate metabolism

The Western diet, characterized by high fat content, refined carbohydrates, and low micronutrient density, profoundly disrupts hepatic metabolism and alters the availability of epigenetic substrates. Chronic consumption of high-fat diets induces hepatic insulin resistance, leading to dysregulated glucose and lipid metabolism [44]. This metabolic dysfunction is accompanied by alterations in one-carbon metabolism, with studies demonstrating decreased hepatic SAM levels and reduced SAM/SAH ratios in animal models fed high-fat diets [45]. The mechanism underlying this depletion involves increased oxidative stress and inflammation, which impairs the activity of MAT and other enzymes in the methionine cycle [46]. High-fat diet feeding also perturbs choline and betaine metabolism, as these nutrients are preferentially directed toward phospholipid synthesis for very-low-density lipoprotein (VLDL) assembly rather than serving as methyl donors [47]. This metabolic shift can result in relative choline deficiency and reduced betaine-homocysteine methyltransferase (*BHMT*)-mediated homocysteine remethylation, further compromising methylation capacity. Additionally, western diet-induced gut dysbiosis alters the production of microbial metabolites, including short-chain fatty acids and trimethylamine, which influence hepatic one-carbon metabolism and acetyl-CoA availability [48]. The relationship between epigenetic dysregulation and liver disease is thus bidirectional, with altered epigenetic modifications both resulting from and contributing to hepatic pathology. The progression from simple steatosis to MASH, fibrosis, and eventually HCC is accompanied by characteristic epigenetic signatures. Conversely, the inflammatory and oxidative stress environment characteristic of MASLD and advanced liver disease further disrupts epigenetic substrate metabolism, creating a vicious cycle of metabolic and epigenetic dysfunction.

4. Epigenetic regulation of liver zonation

4.1. Structural organization and functional specialization in liver zonation

Epigenetic mechanisms are essential for driving cell identity and function. Thus, it is not surprising that epigenetic mechanisms are involved in liver development, metabolism, regeneration, differentiation, and zonation. To develop epigenetic drugs that ameliorate MASLD, it is important to better understand the physiological role of epigenetics in normal liver development. One prominent example of a liver-specific epigenetic mark is miR-122, which is predominantly expressed in hepatocytes and is reported to be upregulated in liver of patients with MASLD, but decreases with more severe MASLD state, whereas circulating miR-122 levels are increased throughout all stages [49]. It is important for maintaining hepatocyte identity by suppressing non-hepatic genes and regulating immune responses. However, it is also essential for hepatitis C virus tropism toward hepatocytes and is crucial for viral replication [50,51]. The anti-miR-122 compound miravirsen was among the first miRNA drugs to enter clinical trials for HCV infection treatment. Nevertheless, all trials were terminated due to limited efficacy, and other anti-miR-122 compounds have not yet received FDA approval [52].

In the context of metabolic disorders and acquired epigenetic changes during disease development, understanding the structural organization of liver metabolism is important for investigating and developing pivotal treatment strategies. The liver is a major central metabolic organ, regulating the uptake, conversion, and distribution of nutrients, along with the detoxification of endogenous and exogenous compounds. Its metabolic and functional heterogeneity relies on its intricate cellular diversity and spatial organization, which becomes altered in MASLD [53].

The functional metabolic unit of the liver is the lobule, which comprises four major cell types. Hepatocytes constitute approximately 70% of liver cells, performing the majority of metabolic functions. Liver sinusoidal endothelial cells (LSECs) form the vasculature, ensuring nutrient exchange and signaling. Hepatic stellate cells (HSCs) comprise about 5% of liver cells, regulating extracellular matrix deposition. Kupffer cells (KCs), the resident macrophages of the liver, are involved in immune responses. Minor populations of T-cells, B-cells, dendritic cells (DCs), myeloid-derived suppressor cells (MDSCs), natural killer cells (NK cells), and small proportions of progenitor cells are also present in the lobule [54]. The lobule is further divided across the periportal-pericentral axis into three spatial zones: the periportal zone I, midzonal zone II, and pericentral zone III (Figure 3(a)) [55]. This zonation underlies metabolic specialization, with periportal hepatocytes exhibiting enhanced gluconeogenesis, urea cycle activity, and detoxification, while pericentral hepatocytes specialize in glycolysis, lipogenesis, and glutamate synthesis. Advances in single-cell RNA sequencing, spatial transcriptomics, and spatial single-cell mass spectrometry have confirmed that hepatocyte function is plastic and responsive to environmental cues [56]. Okada and colleagues demonstrated how pericentral hepatocytes upregulate gluconeogenesis genes when mice were food restricted, a process predominantly occurring in periportal hepatocytes during fed conditions. Furthermore, evidence suggests that in early MASLD development, lipid accumulation starts in the pericentral area and expands from there across the complete zonation [57,58].

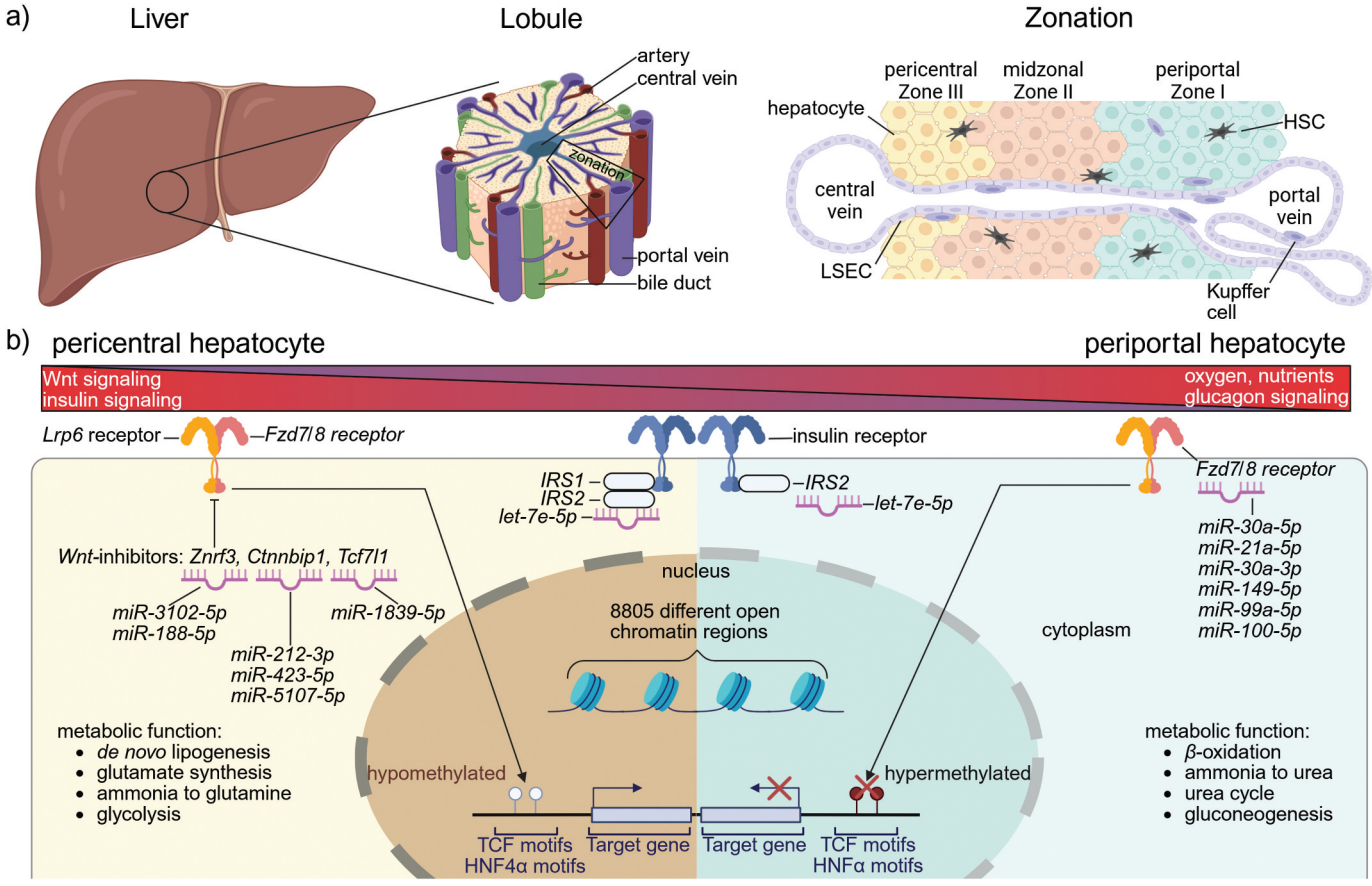

Figure 3. Schematic overview of liver zonation.

(a) The liver is structured into small-metabolic compartments called liver lobule. Within the liver lobule, hepatocytes are organized into three different zones, pericentral, midzonal, and periportal area. Aside from hepatocytes, HSC, LSEC, and Kupffer cells display zonated function. (b) The zonation is established by Wnt/β-catenin signaling and oxygen availability. Epigenetic modifications support the establishment of zonation. For instance, miRNA expression differs in pericentral and periportal hepatocytes, which target Lrp/Fzd receptors to regulate Wnt-signaling. Also, hypo- and hypermethylation of specific zonation-mediating transcription factor-binding motifs enhance or repress downstream Wnt-signaling. Furthermore, differential open chromatin regions are reported.

Abbreviations: LSEC = liver sinusoidal endothelial cells, HSC = hepatic stellate cells, Lrp6 = low-density lipoprotein receptor related 6, Fzd = frizzled receptors, IRS1/2 = insulin receptor substrate 1/2, Znf3 = zinc finger protein 3, Ctnnbip1 = catenin beta interacting protein 1, Tcf7l1 = transcription factor 7 like 1, TCF = T-cell factor, HNF4A = hepatocyte nuclear factor 4 alpha.

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4.2. Wnt/β-catenin signaling and miRNA-mediated zonation

Since hepatocytes share the same genetic information across zones, epigenetic mechanisms are crucial for establishing and maintaining pericentral or periportal hepatocyte identity. A central factor establishing pericentral and periportal regions is a gradient in Wnt/β-catenin signaling, potentially regulated by miRNAs. The activity of this pathway mainly depends on the local Wnt ligand availability, which is high in the pericentral region and low in the periportal region. In addition to the Wnt ligand gradient, the expression of the adenomatous polyposis coli (APC), the canonical inhibitor of the Wnt/β-catenin pathway, is higher in periportal hepatocytes [59].

When *Wnt* ligands are abundant, they bind to Frizzled (*Fzd*) receptor subtypes, together with low-density lipoprotein receptor-related protein (*LRP*), inhibiting β-catenin degradation and resulting in its translocation into the nucleus, where it activates gene expression [60]. Ben-Moshe and colleagues showed that miRNAs display zone-specific expression patterns in spatially sorted primary mouse hepatocytes (PMHs). Among zonated miRNAs, miR-122-5p, miR-100-5p, miR-149-5p, miR-30a-3p, miR-31-5p, miR-99a-5p, let-7e-5p, and miR-802-5p are highly expressed in the periportal area, whereas miR-376a-3p, miR-3102-5p, miR-188-5p, miR-423-5p, miR-212-3p, and miR-5107-5p are highly expressed in the pericentral area. Furthermore, several miRNAs show inverted expression patterns relative to their predicted target genes within the Wnt-signaling pathway in PMHs, indicating a regulatory role of miRNAs in this pathway consistent with the concept of RNAi [61]. Other studies reveal miR-504 and miR-542-3p as negative regulators of *Fzd* mRNA levels, blunting *Wnt* signaling in hepatocellular cancer cell lines [62,63]. Furthermore, miR-182-5p is upregulated in the liver of obese patients with type 2 diabetes (T2D) compared to obese patients without T2D. miR-182-5p targets the *Fzd* coreceptor *Lrp6* in both mouse and human systems, as demonstrated through overexpression of miR-182-5p in mouse liver and in vitro HepG2 cell culture [64]. Whether miR-182-5p also affects liver zonation by interfering with *Wnt* signaling requires further investigation.

4.3. DNA methylation patterns in liver zonation

β-catenin interacts with T-cell factor (*TCF*) transcription factors to drive gene expression downstream of *Wnt* signaling [65,66]. Reduced representation bisulfite sequencing from laser-dissected human liver biopsies of normal controls and patients with varying degrees of steatosis, separated into periportal and pericentral zones, revealed important methylation patterns. Sequence enrichment analysis showed that differentially methylated regions (DMRs) spanning *TCF* binding motifs (*TCF12* and *TCF7L2*) are hypomethylated within pericentral hepatocytes, leading to higher signaling efficiency for *Wnt* in those cells. Binding sites for hepatocyte nuclear factor 4 alpha (*HNF4α*), another crucial transcription factor involved in liver zonation that can be activated by *Wnt* signaling, are also hypomethylated in pericentral hepatocytes in the same dataset [67]. In total, the authors identified 271 genes involved in liver zonation that could potentially be regulated by DNA methylation within the binding sites of 46 different transcription factors. These described patterns of DNA methylation in liver zonation provide a deeper understanding of liver function, including in the context of liver diseases.

4.4. Insulin and glucagon signaling in zonation

Besides *Wnt*-signaling, the antagonistic relationship between insulin and glucagon signaling contributes to liver zonation [68,69]. Whereas glucagon stimulation favors the periportal phenotype, insulin stimulation favors the pericentral phenotype. This is unsurprising given the metabolic roles attributed to these two hormones, which align with the metabolic tasks of the two zones. Insulin increases gene expression for de novo lipogenesis and glycolysis in hepatocytes, which are pericentral tasks [70]. Conversely, glucagon stimulates gluconeogenesis and urea cycle gene expression, both tasks assigned to periportal hepatocytes [71]. Interestingly, the response of hepatocytes within different zones is guided by the differential expression and distribution of insulin receptor substrates *IRS1* and *IRS2* across the zones. *IRS2* is uniformly distributed, whereas IRS1 is more abundant within pericentral hepatocytes [69].

4.5. Chromatin accessibility and zonation

A combined single-cell multiomics analysis using single-nuclei RNA sequencing (snRNA-seq) and single-nuclei ATAC sequencing (snATAC-seq, indicating chromatin accessibility) of major cell types from murine livers (zone-specific hepatocytes, LSECs, Kupffer cells, HSCs, and immune subpopulations) showed that information acquired from snATAC-seq identified the same cell clusters as snRNA-seq data. Moreover, chromatin status analyzed with snATAC-seq is sufficiently informative to differentiate between pericentral and periportal hepatocytes. Furthermore, the authors demonstrate that of the 6823 genes expressed within hepatocytes, 2697 are differentially expressed across zones, accompanied by differential chromatin accessibility within 8805 regions (out of 14,005 hepatocyte-specific regions) [72]. Overall, epigenetic mechanisms are evidently involved in the structural and metabolic organization of the liver, though this interaction remains poorly understood (Figure 3(b)). Notably, known metabolic changes occurring during MASLD progression are connected to liver zonation and overlap with epigenetic changes. However, whether epigenetic mechanisms can be targeted during MASLD progression to prevent zonation loss or disturbances and thereby maintain liver function requires further investigation.

5. Epigenetic dysregulation in MASLD progression

With epigenetic mechanisms governing hepatic zonation and metabolic regulation characterized under basal and physiologically healthy conditions, it is increasingly evident that disruptions to these regulatory processes contribute to liver disease progression. In this section, we examine how DNA methylation, histone modifications, and non-coding RNAs become dysregulated during MASLD development and progression.

5.1. Global DNA methylation changes in MASLD

Amino acid metabolism, especially methionine metabolism, has been shown to be crucially linked to MASLD progression, and methionine/choline-deficient diets are commonly used to model hepatic fibrosis [73]. As discussed above, methionine metabolism is crucial for SAM synthesis, the main methyl donor, and substrate used in methylation processes [74]. During MASLD progression, inflammatory processes increase, and immune cells infiltrate liver tissue, causing inflammation and cirrhosis [75]. Assessment of hepatic whole-genome DNA methylation using the Illumina HumanMethylation450K Bead Chip coupled with HuGene Affymetrix transcriptome analysis reveals a total of 467 differentially methylated CpGs annotated to 294 genes across different steatosis degrees. Applying stricter filtering reveals a subset of 9 differentially expressed that gradually decreased in DNA methylation (*GALNTL4*, *ACLY*, *PLCG1*, *PRKCE*, *IP6K3*) or increased (*IGFBP2*, *IGF1*, *PC*, *GRID1*) with steatosis degree. These effects are partially reversible through bariatric surgery and persist up to 9 months post-surgery in the liver of patients [76]. Another study identified 185 differentially methylated CpGs in the liver of obese subjects with T2D compared to obese subjects without T2D. The identified CpG-associated genes have not been previously reported in the context of T2D or MASLD [77]. In HCC patients, representing the end-stage of MASLD progression, genome-wide methylation loss occurs (4,528 hypomethylated regions vs. 2654 hypermethylated regions) compared to non-tumor MASLD patients [78]. In another human cohort study, patients are stratified into mild (Fib-0/1) and advanced (Fib-3/4) MASLD, and global DNA methylation is assessed with the Illumina HumanMethylation450K Bead Chip. This analysis reveals overall methylation loss (hypomethylation) in promoter regions in patients with advanced MASLD. A total of 1741 CpG sites are hypomethylated and 624 are hypermethylated [79]. These findings consistently demonstrate that MASLD progression is associated with global DNA hypomethylation,

particularly in promoter regions, suggesting widespread epigenetic dysregulation.

5.1.1. Regulation of lipid uptake: CD36 as a model target

Hepatic lipid accumulation, the main feature of MASLD, and other metabolic dysregulations occurring in MASH are frequently regulated by DNA methylation and histone modifications, which may serve as potential prognostic/diagnostic markers or treatment targets. The lipid transporter *CD36*, which governs fatty acid uptake into hepatocytes, is tightly regulated by DNA methylation in the liver of several MASLD mouse models. For instance, the *Cd36* promoter is hypomethylated in the liver of diet-induced obesity (DIO) mice, which is associated with increased *Cd36* gene expression. Furthermore, *Cd36* is among the top downregulated genes in *Tet1*$^{-/-}$ mice, which additionally are reduced liver weight when fed high-fat diet (HFD). *Tet1*$^{-/-}$ mice also show reduced hepatic triglyceride accumulation and cholesterol levels and increased *Igfbp2* expression. These findings are confirmed by knockdown of *TET1* in immortalized human hepatocytes (OUMS29 cell line) with shRNA, which leads to increased *IGFBP2* and decreased *CD36* expression combined with decreased intracellular lipid accumulation. Liver-specific *Tet1* knockdown in mice during HFD feeding replicates *Cd36* downregulation [80].

Acquired prenatal or postnatal changes in DNA methylation are linked to MASLD onset in adulthood, though this is sparsely investigated [81]. Early-life stress through neonatal maternal separation (NMS) induces adulthood MASLD at 25 weeks of age in mice, even when fed standard chow diet. This phenotype is reinforced by high-fat, high-sucrose diet (HFS) and is more pronounced in male offspring. This is associated with hypomethylation in the *Cd36* promoter region in NMS-chow, NMS-HFS, and HFS mice and increased *Cd36* hepatic expression, which again reflects a stronger effect in males than females [82]. Hepatic methylation also indirectly regulates *Cd36* expression via the mediator C-Maf inducing protein (*Cmip*), as shown in DIO mouse MASLD models by HFS feeding. *CMIP* expression in human livers correlates with *CMIP* promoter methylation. *Cmip* is hypomethylated in MASLD, leading to increased *Cmip* expression and consequently inducing *Pparg* activity and *Cd36* expression, which is confirmed in primary human hepatocytes and the AML12 hepatocyte line. Interestingly, *DNMT1* and *TET2* enzymes bind alternatingly to the *CMIP* promoter during MASLD in vitro cell culture models after treatment of AML12 cells with palmitate/oleate (PO) mixture. In the presence of PO, less *DNMT1* and more *TET2* bind to the *Cmip* promoter. Less *TET2* binding to the *Cmip* promoter is also observed in genetic mouse models for obesity (*ob/ob* mice), resulting in *Cmip* demethylation, increased *Cmip* expression, and downstream upregulation of *Pparg* and *Cd36*. Knockdown of *Cmip* ameliorates hepatic lipid accumulation and downregulates *Pparg* and *Cd36* [83]. These findings indicate a strong influence of DNA methylation on specific hepatic MASLD-inducing genes and involvement in MASLD progression, rendering *Cd36* as a potential epigenetic target for therapeutic interventions.

5.1.2. Transcriptional regulators of lipid metabolism

Once fatty acids are imported by *CD36*, they activate transcriptional regulators such as sterol regulatory element-binding transcription factors (*SREBFs*), peroxisome proliferator-activated receptors (*PPARs*, especially *PPARγ*), liver X receptors (*LXRs*), hepatocyte nuclear factors (*HNFs*), and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (*PGC1α*) [84]. Although only the *SREBF1* binding motif comprises a potential central CpG methylation site, all of these transcription factors are reported to be DNA methylation-sensitive and affected by methylation of proximal CpGs [85–87]. Interestingly, *HNF4α* is not only methylation-sensitive but also plays an active role in hepatocyte differentiation by occupying active chromatin regions and establishing hypomethylation around *HNF4α* binding motifs. This is demonstrated by comparing DNA methylation profiles (MeDIP-seq & hMeDIP-seq) and chromatin accessibility (H3K4me1, H3K4me3, and H3K27ac ChIP-seq) of isolated primary murine hepatoblasts at embryonic day 14.5 (E14.5) with isolated primary murine hepatocytes. *HNF4α* binding in enhancer regions during hepatocyte differentiation is necessary to maintain active H3K27ac marks and governs *Tet3* expression in HPPL cells [88]. Given the influence of *HNF4α* on the global hepatocyte epigenome and its necessity for the hepatocyte phenotype, targeting *HNF4α* therapeutically might represent a potential strategy to rescue aberrant acquired epigenetic changes. However, reports also suggest that *HNF4α* activity might decrease hepatocyte plasticity based on the forced 'lock-in' to the hepatocyte phenotype, whereas higher plasticity is needed for liver regeneration. This is shown through knockdown of *HNF4α* in HepG2, HepRG cell lines, and liver organoid models, where downregulation of *HNF4α* enables transcriptomic reprogramming [89]. This may also provide a potential explanation for the connection between insulin resistance/T2D and MASLD.

5.1.3. De novo Lipogenesis and insulin signaling

SREBF1 and *PPARγ* are master regulators of *DNL* in the liver, and downstream target genes such as *FASN*, *ACACA*, *SCD1*, and *ACLY* are commonly upregulated in patients with MASLD. Since *DNL* contributes to lipid accumulation in the liver, loss of DNA methylation or altered histone modifications within *SREBF1* or *PPARγ* binding motifs in target gene promoters/enhancers is responsible for target gene upregulation.

Stimulation of HepG2 cells or primary rat hepatocytes (PRH) with insulin leads to increased H3K4 methylation, H3, and H4 acetylation in the *FASN* promoter-histone modifications associated with active gene expression. Consequently, the *FASN* expression is elevated. Insulin stimulation also leads to translocation of *SREBF1* from the cytoplasm into the nucleus in both cell cultures (HepG2, PRH). This pattern is reversed upon insulin withdrawal. Interestingly, knockdown of *SREBF1* in both cell types results in reduced insulin-induced H3K4 methylation, suggesting that *SREBF1* is not only involved in activation of *FASN* gene expression but also participates in shaping the epigenetic requirements that lead to *FASN* induction [90]. Whether similar processes occur in the liver of patients with MASLD requires further investigation to estimate potential treatment strategies.

Interestingly, *SREBF1* not only acts as a transcriptional activator of *DNL* genes but also functions as a transcriptional repressor for genes involved in insulin signaling. *IRS2*, a crucial mediator of insulin signaling, is downregulated in the liver of obese patients with T2D, which is mediated by increased DNA methylation in the promoter region at a specific SNP (cg25924746) but is also associated with hypomethylation of an intron 1-located CpG. Methylation-sensitive reporter gene assays in HepG2 cells confirm that increased methylation at cg25924746 leads to decreased IRS2 expression. Notably, this chromosomal region displays binding sites for *SREBF1* and *SP1* [64]. DNA methylation in *PPARγ* target genes of the liver in MASLD remains sparsely studied.

5.1.4. Fatty acid oxidation and thyroid hormone signaling

Reducing lipid accumulation within hepatocytes is one mechanism to prevent MASLD progression to more severe forms or improve MASH outcomes since lipid toxicity contributes to liver fibrosis. Increased fatty acid β-oxidation reduces lipid accumulation and is induced by thyroid hormone signaling via thyroid hormone receptor β (THRβ) activation [91]. Resmetirom, a liver-specific THRβ agonist and approved drug for MASH treatment, employs this axis. In the liver of MASH patients compared to MASLD patients, the first intron of the *THRB* gene is hypermethylated, as assessed by pyrosequencing. Hypermethylation of the *THRB* promoter is associated with decreased *THRB* expression in the liver of MASH patients. Methylation in *THRB* is strongly positively correlated with HbA1c, AST, ALT, liver fat, and MASH score [92]. Whether hypermethylation is indeed causative for *THRB* downregulation requires confirmation. Nevertheless, blunted resmetirom action in MASH patients exhibiting higher *THRB* methylation and decreased *THRB* expression could explain resmetirom non-responders, and rescuing *THRB* DNA methylation in co-treatment with resmetirom might amplify resmetirom efficiency since predictors of non-response to resmetirom remain elusive [93].

5.1.5. Methionine cycle dysregulation

As mentioned previously, the methionine cycle is essential for SAM production, which sustains methylation processes in the cell. Genes involved in the methionine cycle may similarly undergo epigenetic regulation in MASLD. For instance, promoter methylation of *MAT1A*, the rate-limiting enzyme responsible for catalyzing methionine into SAM, is hypermethylated in the liver of patients with advanced MASLD compared to normal controls. This also applies to *AHCY*, the enzyme catalyzing the reaction from SAH into homocysteine. Hypermethylation in both genes is negatively correlated with gene expression, respectively [79]. Similar loss of global DNA methylation in the liver of patients with MASLD and gradual loss of DNA methylation with increasing disease severity is reported [94].

In general, recent studies assessing the global DNA methylome with specific focus on differences in methylation of transcription factor-binding motifs from human patients with MASLD compared to patients without MASLD are lacking. This information would generate valuable knowledge for future epigenetic therapeutics. Restoring aberrant DNA methylation acquired in MASLD to its native condition at specific chromosomal regions enriched for transcription factors binding motifs involved in MASLD progression might have a significant impact. Furthermore, recurring reports of gradual DNA methylation loss in MASLD and drastic epigenome changes outline promising therapeutics that liver-specifically inhibit *TET* enzymes or activate *DNMTs*, which may normalize aberrant DNA methylation to prevent disease progression (Figure 4).

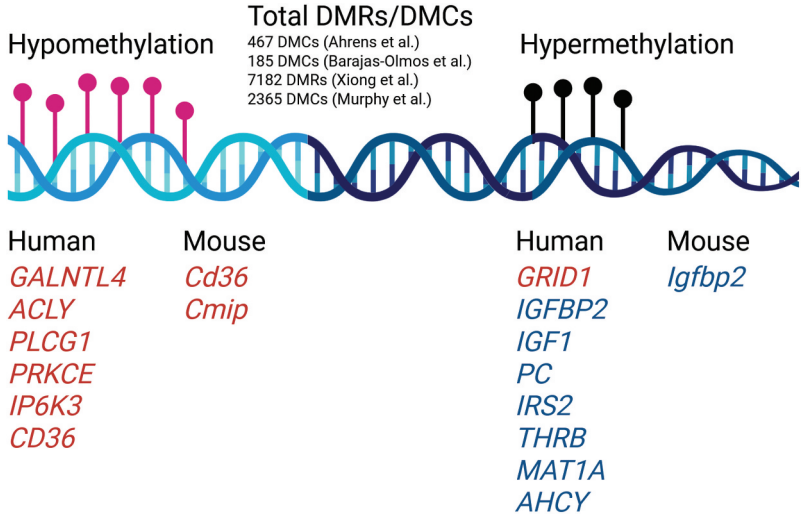

Figure 4. DNA methylation in MASLD. The schematic overview summarizes the key findings.

Genes are sorted by either hypermethylated or hypomethylated state in MASLD. Red genes indicate an associated increase in gene expression, whereas blue colored genes indicate an associated decrease in gene expression.

Abbreviations: DMR = differentially methylated region, DMC = differentially methylated cytosine, GALNTL4 = GalNAc T-like protein 4, ACLY = ATP citrate lyase, PLCG1 = phospholipase c gamma 1, IP6K3 = inositol hexakisphosphate kinase 3, CD36 = cluster of differentiation 36/fatty acid translocase, Cmip = C-Maf inducing protein, GRID1 = glutamate ionotropic receptor delta-type subunit 1, IGFBP2 = insulin-like growth factor-binding protein 2, IGF1 = insulin-like growth factor 1, PC = pyruvate carboxylase, IRS2 = insulin receptor substrate 2, THRB = thyroid hormone receptor beta, MAT1A = methionine adenosyltransferase 1A, AHCY = adenosyl homocysteinase.

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6. miRNA regulation of metabolic pathways in MASLD

Currently, no genome-wide study analyzing differentially expressed miRNAs in the liver of MASLD patients exists. However, we have shown with an array-based expression analysis that a total of 28 mature miRNAs are differentially expressed in obese patients with T2D compared to obese patients without T2D. Of those 28 miRNAs, 19 are associated with different metabolic traits (NAS score, insulin levels, fasting glucose, and serum triglycerides) [95]. Bridging this knowledge gap in the literature of a genome-wide miRNA study of patients with MASLD might drastically improve the understanding and involvement of miRNAs in MASLD progression and development but also facilitate the identification of novel drug targets. Majorly, in the literature, single miRNA studies are presented.

6.1. miRNA targeting of lipid uptake and de novo lipogenesis

The metabolic pathways altered by DNA methylation or histone modifications in MASLD are often additionally targeted by non-coding RNAs. In this section, we specifically discuss the roles of miRNAs contributing to dysregulation of metabolic processes in MASLD. While lncRNAs have a broader range of gene expression modulation compared to miRNAs, which rely on RNAi, delivery of short RNA molecules in a liver-specific manner through conjugations with GalNAc is more efficient and decreases with RNA molecule length. Therefore, development of miRNA therapeutics is currently more advanced compared to lncRNA modulation and is prioritized in this review. Several miRNAs have been found to regulate *Cd36* expression. miR-20a-5p decreases in the serum of patients with MASLD when compared with non-MASLD patients. This observation is confirmed in the serum of HFD-fed mice compared with chow-diet mice. The reported gene assays reveal a direct regulation of *Cd36* by miR-20a-5p in HepG2 and HEK 293T cells [96]. Although hepatic expression levels of miR-20a-5p and *CD36* are not assessed in human MASLD patients, HFD feeding of mice decreases miR-20a-5p expression in the liver, which is accompanied by upregulation of *Cd36*. However, the effects of miR-20a-5p on MASLD pathology still need to be investigated in vivo [96]. miR-411-5p overexpression in steatosis-induced hepatocytes *in vitro* leads to decreased *Cd36* expression and downregulation of *DNL* genes. Overexpression of miR-411-5p in the liver of high-fructose-fed mice alleviates lipid deposition in an *EIF4G2-FOXO3*-dependent manner [97]. Thus, microRNAs have the potential to regulate hepatic lipid metabolism and uptake in mice. Further studies of their relevance in humans are, however, needed.

6.2. miRNA regulation of thyroid hormone signaling

β-oxidation decreases in patients with MASLD and is tightly regulated by thyroid hormone action. *THRβ*, the major thyroid hormone receptor of the liver, translates thyroid hormone signaling into a gene expression response via acting as a transcription factor. However, other genes are important for thyroid hormone import into hepatocytes (*SLC10A1*, *SLC16A2*) or catalyzing the activation from inactive T4 to active T3 form (*DIO1*). We demonstrate by target-gene seed prediction that miR-34a-5p has the potential to regulate these vital genes of thyroid hormone transport and metabolism (*THRB*, *DIO1*, *SLC10A1,* and *SLC16A2*) [92]. miR-34a-5p is upregulated in the liver and circulation of patients with obesity-associated MASH compared to age-matched obese patients without MASH [92,98]. Also, its expression is negatively correlated with the expression of thyroid hormone transporter genes *SLC10A1* and *SLC16A2*. Overexpression of miR-34a-5p in HepG2 cells results in downregulation of *DIO1*, *THRB*, *THRA*, and *SLC10A1*, which cannot be rescued by T3 treatment in the presence of miR-34a-5p. This represents another layer, in addition to *THRB* hypermethylation, that may blunt thyroid hormone signaling in MASH patients, potentially interfering with resmetirom efficiency [92].

6.3. miRNA regulation of insulin signaling

T2D, insulin resistance, and partial insulin resistance are features of MASLD progression, and miRNAs are shown to interfere with insulin signaling in various ways. *PTPN1* is a negative regulator of insulin signaling through dephosphorylation of the insulin receptor (*INSR*) or *IRS* proteins [99]. Overexpression of miR-206 in murine livers suppresses *DNL* gene expression (*Fasn*, *Scd1*, *Srebf1*, *Gpat*) and gluconeogenesis gene expression (*G6pc*, *Pepck*) while simultaneously increasing *Insr* phosphorylation. Furthermore, hepatic lipid content decreases after miR-206 overexpression [100]. Employing *in vivo* mouse models and *in vitro* human cell line models (HepG2 and Huh7) for dietary MASLD induction confirms consistent downregulation of miR-206 across species, and additionally, downregulation of miR-206 leads to increased lipid accumulation by mediated *ROCK1*-silencing [101]. Furthermore, miR-222 is observed to be upregulated in the liver of mice subjected to HFD and patients with MASLD or MASH [102]. miR-222 targets *Irs1* and therefore leads to blunted insulin signaling as assessed by phosphorylation of Akt [103]. A second member of the *PTPN* family, *PTPN11* (alias *SHP2*), is a negative regulator of insulin signaling. It participates in insulin receptor endocytosis but also plays a pivotal role in macrophage infiltration in obesity-related inflammation-driven insulin resistance [104]. miR-514a-3p is reported to target *PTPN11* in placental cells but lacks evidence of a similar mechanism in hepatocytes [105]. Interestingly, the *Ptpn11* promoter is hypermethylated in murine livers after steatosis induction by HFD, and DNA methylation patterns cycle with diet restriction and HFD refeeding [106]. Additionally, *IRS2* is described by us to be regulated by hypermethylation of the promoter and promoter-proximal intron 1 in patients with T2D compared to obese patients without T2D, in addition to let-7e-5p-mediated *IRS2* downregulation [64]. Similar findings in mice overexpressing the complete let-7 miRNA cluster are observed. Besides growth retardation, the animals suffer from glucose intolerance and insulin resistance in muscles and liver, strengthening the role of let-7 in diabetes. Applying a let-7 anti-sense oligonucleotide (ASO) restores insulin sensitivity and upregulates hepatic and muscle *Insr* and *Irs2* expression [107].

Aside from involvement in thyroid hormone signaling, miR-34a appears to play a role in fatty acid-induced insulin resistance. Treatment of AML-12 cells with miR-34a mimics or palmitate-induced miR-34a-5p expression results in similar cellular responses by decreasing glucose consumption, decreasing pAkt levels, and reducing glycogen synthesis. Palmitate effects are ameliorated by inhibiting miR-34a with an ASO [108]. Similar outcomes are obtained from *in vivo* mouse models. The authors identify *Eno3* as a direct miR-34a target that mediates insulin resistance [109]. Global overexpression of miR-18a in muscle, adipose tissue, and liver results in improved insulin sensitivity and increased pAKT. miR-18a targets *PTEN*, a potent negative regulator of insulin signaling. Interestingly, these effects are more dominant in muscle and adipose tissue than in the liver. Circulating miR-18a levels are downregulated in patients with T2D [110]. A negative feedback loop in *Foxo1*$^{-/-}$ mice results in upregulation of miR-205-5p and gain of function of this miRNA in turn increases pAKT levels, resulting in improvements in glucose tolerance. Overexpression of miR-205-5p leads to downregulation of *Foxo1* [111]. *ORP8* is a positive modulator of AKT signaling. Upregulation of miR-143 in obese patients with T2D leads to decreased *ORP8* expression and therefore blunts *INSR*-mediated AKT signaling [112].

However, miRNAs might not always be the cause of dysregulated insulin signaling. Liver-specific *Insr* knockout mice as a model for impaired insulin signaling show aberrant miRNA expression profiles. Especially, miR-205 and miR-883b are regulated downstream of insulin signaling, therefore representing a consequential response to impaired insulin signaling rather than implying a causative role in interfering with insulin signaling [113]

6.4. miRNA regulation of amino acid metabolism

Aside from lipid metabolism and insulin signaling, miRNAs are also involved and dysregulated in other metabolic pathways connected to MASLD progression, such as amino acid metabolism and ammonia detoxification via the urea cycle. Although less attention has been devoted to these pathways, their importance in liver function cannot be neglected. Notably, the most frequently assessed metabolic parameters from blood to estimate liver function are aminotransferases such as aspartate aminotransferase (*AST*, alias *GOT*) and alanine aminotransferase (*ALT*, alias *GPT*). Additionally, hyperammonemia is a phenotype presented in patients with advanced MASLD, which can be traced to a dysregulated urea cycle in the liver of these patients. For instance, overexpression of miR-26a in the liver of mice increases plasma AST and ALT levels, whereas inhibition of miR-26a utilizing an ASO reduces AST and ALT levels. However, miR-26a does not target AST and ALT directly. The upregulation of aminotransferases after miR-26a overexpression is a consequence of liver injury induction connected with induced apoptotic and cell arrest processes [114].

Modulating miRNA expression serves as a powerful tool to treat MASLD given their pleiotropic functions in various pathways (Table 1). Notably, MASLD is a multifactorial disease where multiple genes and pathways are affected. Since miRNAs possess multiple target genes, application of a single miRNA has the potential to rescue completely dysregulated pathways in MASLD, either by enhancing endogenous miRNA expression (miRNA mimics) or inhibiting endogenous miRNAs (miRNA-ASOs, antagomirs).

7. Extracellular vesicles and interorgan communication in MASLD

7.1. Principles of EV-mediated epigenetic communication

Epigenetic mechanisms do not exclusively exert local effects on gene expression within a cell or organ. Non-coding RNAs are packaged in extracellular vesicles (EVs) and are secreted from one organ into the blood circulation to modulate gene expression in distal target organs, where EVs are assimilated, and their cargo is released [115]. Based on the cargo of an EV or the presence of specific membrane proteins, EVs can be traced back to their originating organ, and their target organ specificity can be determined. In this way, the liver can serve from both an efferent position by secreting EVs and an afferent position by receiving EVs from other tissues. The liver is a hub for circulating EV clearance but also a major contributor to circulating EVs through secretion [116]. During MASLD, the content of EVs and EV membrane composition changes, thereby fostering disease progression [117,118]. Since EV concentration is high in blood, EV content could be assessed as noninvasive biomarkers or diagnostic tools. Additionally, utilizing EVs as a delivery system for miRNAs has gained increasing interest in recent decades.

7.2. Liver-derived EVs as biomarkers and mediators of disease

Analysis of the proteome and miRNA content of EVs isolated from the blood of mice fed a choline-deficient diet shows increased levels of miR-122, a liver-specific miRNA, which are significantly correlated with parameters for MASLD progression (ALT, collagenase 1, α-SMA expression in the liver). The authors conclude that EVs must be liver-derived and that assessing miR-122 levels in EVs could serve as a surrogate to estimate liver damage or MASLD progression state [119]. Whereas miR-122 concentrations increase in EVs of MASLD-induced mice, hepatic expression levels decrease, supporting the claim that hepatic miR-122 depletion is a consequence of increased miR-122 secretion from the liver. Similar results are reported independently by another group, where free-circulating miR-122 in serum (not in EVs) increases with steatosis degree and is increased in MASH patients compared with MASLD patients. Circulating miR-122 levels are associated with hepatocyte ballooning. Furthermore, a link between circulating miR-122 and obstructive lung disease (OLD) is found. OLD is a disease with increased prevalence in MASLD patients, and MASLD patients are reported to have reduced lung function [120–122]. Researchers found that previously described elevated circulating miR-122 levels in MASLD patients might be causative. Induced liver injury results in an increased release of miR-122 from the liver, which enters alveolar macrophages and activates TLR7 signaling. This miR-122 uptake results in

Table 1. miRNA dysregulated in MASLD/MASH and their involved pathways.

miRNA		Expression in MASLD/MASH [liver]	Levels in circulation MASLD/MASH	In vitro MASLD models [human]	Target genes	Affected metabolic function	Reference
miR-20a-5p	Human	Unknown	Decreased levels	Downregulated	*CD36*	Increase fatty acid uptake into hepatocytes	[97]
	Mouse models	Downregulated	Unknown		*Cd36*	Increase fatty acid uptake into hepatocytes	[97]
miR-411-5p	Human	Unknown	Unknown	Unknown			
	Mouse models	Downregulated	Unknown		*Cd36*	Increase fatty acid uptake into hepatocytes	[98]
miR-34a-5p	Human	Upregulated	Increased	Upregulated	*DIO1, THRB, THRA, SLC10A1*	Thyroid hormone uptake and signaling	[93]
	Mouse models	Upregulated	Unknown		*Eno3*	Insulin signaling	[110]
miR-206	Human	Unknown	Unknown	Downregulated	*ROCK1*	Insulin signaling and de novo lipogenesis	[102]
	Mouse models	Downregulated	Unknown	Downregulated	*Rock1, Fasn, Scd1, Srebf1, Gpat, G6pc, Pepck*	Insulin signaling and de novo lipogenesis	[101,102]
miR-222	Human	Upregulated	Unknown	Unknown			
	Mouse models	Upregulated	Unknown		*Irs1*	Insulin signaling and lipid accumulation	[104]
let-7e-5p	Human	Upregulated	Unknown	Upregulated	*IRS2*	Insulin signaling	[64]
	Mouse models	Upregulated	Unknown		*Irs2, Insr*	Insulin sensitivity and glucose metabolism	[108]
miR-18a	Human	Unknown	Decreased levels	Unknown			
	Mouse models	Downregulated	Unknown		*Pten*	Insulin signaling	[111]
miR-205-5p	Human	Unknown	Unknown	Unknown			
	Mouse models	Downregulated	Unknown		*Foxo1*	Insulin signaling	[112]
miR-143	Human	Upregulated	Unknown	Upregulated	*ORP8*	Insulin signaling	[113]
	Mouse models	Unknown	Unknown				
miR-26a	Human	Unknown	Unknown	Unknown			
	Mouse models	Upregulated	Unknown		Unknown	Apoptosis and liver injury	[115]

Legend: CD36 = cluster of differentiation 36/fatty acid translocase, DIO1 = deiodinase 1, THRB = thyroid hormone receptor beta, THRA = thyroid hormone receptor alpha, SLC10A1 = solute carrier family 10 member 1, Eno3 = enolase 3, Rock1 = Rho kinase 1, Fasn = fatty acid synthase, Scd1 = stearoyl-CoA desaturase 1, Srebf1 = sterol regulatory element binding transcription factor 1, Gpat = glycerol-3-phosphate acyltransferase, G6pc = glucose-6-phosphatase alpha, Pepck = phosphoenolpyruvate carboxykinase, IRS1/2 = insulin receptor substrate 1/2, Insr = insulin receptor, Pten = phosphatase and tensin homolog, Foxo1 = forkhead-box protein O1, ORP8 = oxysterol binding protein like 8.

increased lung inflammation and reduced lung function [123]. Additionally, miR-192 and miR-375 follow similar characteristics as miR-122 and are upregulated in the circulation of MASLD patients. These miRNAs are involved in glucose homeostasis and inflammation [124]. However, whether these hepatic-derived miRNAs exert systemic functions in inducing inflammation or regulating glucose homeostasis in foreign tissues requires investigation. miR-542-3p, miR-574-3p, and miR-200a-3p are also significantly enriched in EVs from a serum of patients with MASLD [125].

7.3. Hepatocyte-derived EVs in metabolic interorgan crosstalk

Isolated EVs from primary mouse hepatocyte (PMH) cultures of DIO mice display differential expression of 15 miRNAs compared to those from chow diet-fed mice. Among these, miR-504-3p, miR-3070–2-3p, miR-5622-3p, miR-7218-5p, miR-7219-5p, and miR-7654-3p are significantly downregulated in DIO mice EVs. Target genes of these miRNAs are predicted to be involved in glucose metabolism. Treatment of MIN6, a pancreatic β-cell line, with PMH-derived EVs from DIO mice leads to the downregulation of these six miRNAs in MIN6 cells and upregulation of predicted target genes, among them *Cd74*, a proliferative gene, and inducer of hyperplasia in β-cells. The same expression pattern was observed *in vivo*. The authors link the downregulation of miR-7218-5p in PMH-derived EVs from DIO mice to increased expression of *Cd74* in DIO mice β-islet cells, which in turn induces hyperplasia and insulin resistance [126]. Additionally, hepatocyte-derived EVs in DIO mice contribute to adipocyte remodeling during lipid overload. HFD feeding increases the hepatic EV production, which is enriched with miR-23b-3p, miR-103-3p, miR-29b-3p, miR-210-3p, and let-7e-5p. These secreted hepatic EVs are absorbed by adipocytes, and EV-derived miRNAs increase lipogenic genes and decrease lipid oxidation in adipocytes, forcing lipid deposition in adipose tissue [127]. miR-122 is reported among the top upregulated miRNAs in EVs isolated from the serum of DIO mice, linking the origin from the exosomes to the liver. Furthermore, transfection of chow-derived EVs isolated from the serum with a cocktail of obesogenic miRNAs (miR-122, miR-192, miR-27a-3p, miR-27b-3p), which are then applied via tail vein injection back into the animals, results in hyperglycemia, insulin resistance, elevated triglyceride, and free-fatty acid levels in serum. Further, those obesogenic miRNAs packaged within EVs caused adipose tissue inflammation and hepatic steatosis in chow-fed animals [128]. In another study, adipose tissue-residing macrophages showed elevated secretion of EVs enriched for miR-155 and miR-34a that activated hepatic stellate cells, driving liver fibrosis. Thus, the authors termed these miRNAs fibrogenic [129].

7.4. EVs in MASLD-associated cancer progression

miRNA-derived EVs appear to play a crucial role in MASLD-mediated tumor progression. A leading cause of death in patients with colorectal cancer is liver metastasis. Researchers show that MASLD-induced mice secrete EVs enriched for miR-25, miR-92, and miR-103 from fatty acid-accumulated hepatocytes. These EV-derived miRNAs mediate *LATS2* repression within liver metastases. *LATS2* is a key repressor for the proto-oncogenic *YAP* gene, which regulates cell proliferation and apoptosis. Through the increased secretion of miR-25, miR-92, and miR-103 from MASLD-induced hepatocytes, liver metastases show increased infiltration and survivability, promoting a beneficial tumor microenvironment [130].

7.5. EVs from other organs targeting the liver

Specifically, muscle-derived miRNAs, termed myoMirs (miR-133a, miR-133b, miR-206, and miR-205), are upregulated in blood circulation of post-exercise animals and have been shown to improve glucose and lipid metabolism in the liver by targeting *Foxo1*. Interestingly, this effect persists when EVs are isolated from the serum of post-exercise animals and transfected into sedentary animals that did not undergo exercise intervention [131]. However, to date, there are no data on whether myoMirs are downregulated in the circulation of patients with MASLD and whether treatment of insulin-resistant or MASLD-induced mice with myoMirs wrapped in muscle-derived EVs targeting the liver improves insulin sensitivity or liver metabolism. EVs secreted from M2 bone marrow-derived macrophages (BMDM) containing miR-690 are shown to regulate systemic insulin sensitivity by targeting liver, adipose, and muscle tissue. HFD-fed animals treated with M2 exosomes from lean mice show improved insulin sensitivity, glucose tolerance, and higher levels of phosphorylated Akt within the liver [132].

MASLD is a disorder not solely restricted to the liver, and other organs such as adipose tissue contribute to lipid accumulation. Several miRNAs acting in the liver are secreted by other organs and vice versa (Figure 5). However, research on EVs in metabolic diseases, especially with focus on MASLD, is still in its early stages, and more investigations are needed to fully reveal the therapeutic potential. Nevertheless, these examples provide evidence that local changes in epigenetics of one organ can have systemic effects on the whole organism and can affect lipid accumulation, glucose metabolism, and insulin sensitivity.

8. Epigenetic therapeutic strategies for MASLD

8.1. Principles of epigenome editing technologies

As highlighted previously, changes in DNA methylation, histone modifications, and miRNA expression contribute to MASLD development by affecting various pathways. Current technologies employ different approaches to modulate aberrant gene expression by altering epigenome landmarks or by modulating endogenous miRNA expression (Figure 6). miRNAs are chemically modified to increase miRNA stability. Whereas miRNA-mimics generally increase the endogenous miRNA pool, miRNA ASOs or antagomirs bind selective miRNAs and induce miRNA degradation (Figure 6(a)). Advances in CRISPR technology have made epigenome editing increasingly accessible. These CRISPR technologies commonly employ dead-Cas9 (dCas9) fusion proteins, in which catalytically inactive

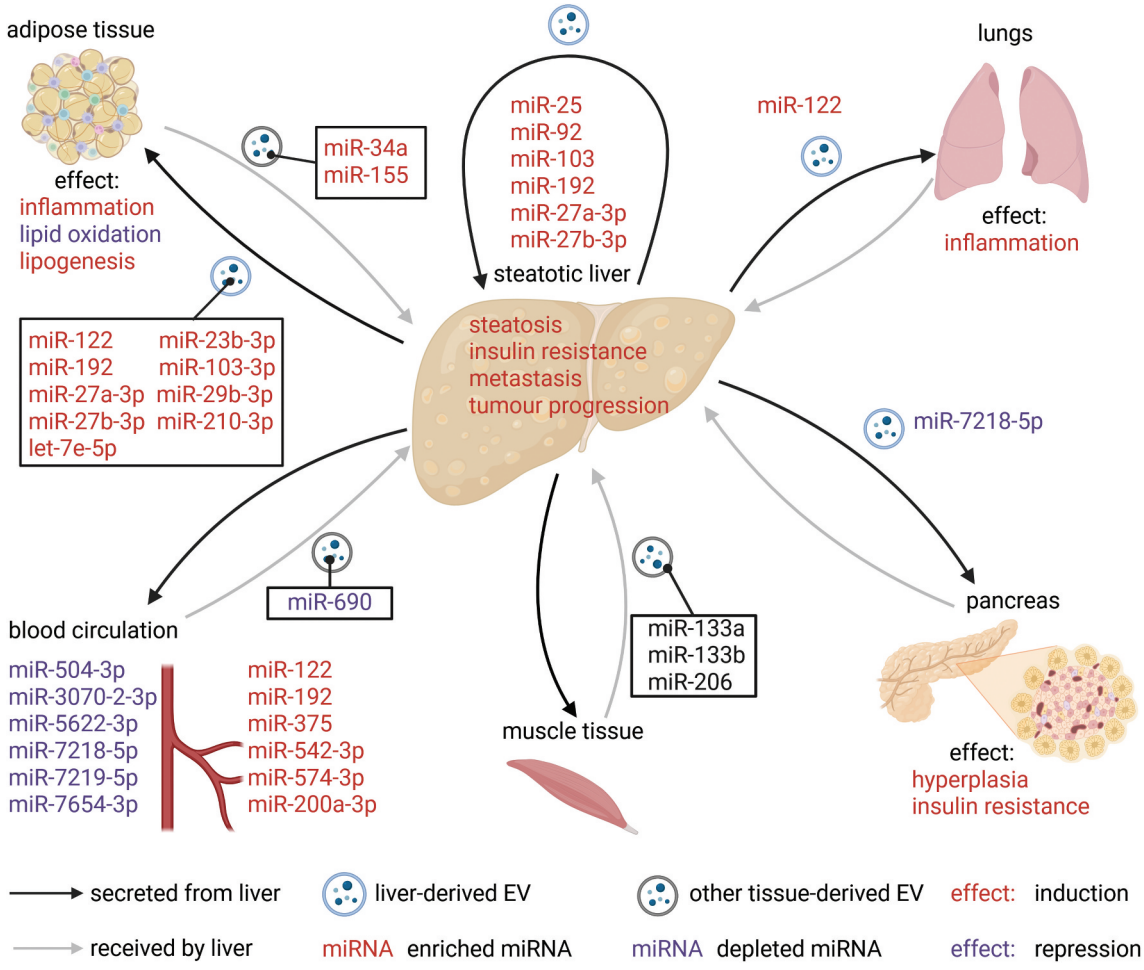

Figure 5. Schematic overview of miRNAs secreted from the liver in extracellular vesicles or received by the liver from other organs.
Red labeled miRNAs display enrichment in MASLD, whereas blue miRNAs are downregulated in MASLD. Especially, adipose tissue and liver are reported to display strong communication. Created in BioRender. Britsemmer, J. (2026) https://BioRender.com/edgeeel.

dCas9–either through truncated Cas9 or mutated Cas9 versions–is fused via a linker to effector enzymes [133]. Effector enzymes function as either DNA methylation or histone mark erasers (functions of TET enzymes, histone modifiers) or DNA methylation introducers (function of DNMTs). The dCas9-fusion system is applied together with a locus-specific guided RNA (gRNA), which utilizes the dCas9-loaded gRNA complex as a transporter of effector enzymes to the locus to introduce DNA methylation or histone mark changes. These dCas-fusion proteins made locus-specific modulation of DNA-methylation possible; however, large off-target effects exist [134]. Additionally, gRNA-independent systems exist. Zinc-finger proteins (ZFPs) can be employed to guide effector proteins to DNA. Individual ZFPs recognize 3–4 nucleotide sequences, and arrays of multiple ZFPs can be designed to bind highly specific motifs on DNA. These ZFP arrays, fused to effector proteins, enable precise gene-specific epigenome editing or silencing without the need for gRNAs [135,136] (Figure 6(b)).

In general, information for these systems is encoded on DNA vectors delivered into the system with viral vehicles (AAVs), which are also responsible for organ-specific delivery. Virus-like particles (VLPs) are able to deliver dCas9-effector systems in the form of ribonucleoproteins for a transient expression profile [137]. Recent approaches also apply to organ-specific lipid nanoparticles (LNPs), which can carry various cargos (DNA (~10kb), mRNA, miRNA, or protein). However, LNPs deliver cargo into the cytoplasm of cells, whereas AAVs deliver to the nucleus (Figure 6(c) and d)). Therefore, LNPs have low efficiency for DNA-nucleus delivery. Different AAV vehicles and epigenome editing systems have been discussed previously [138–140].

8.2. *Targeting DNA methylation: PCSK9 as proof-of-concept*

Loss-of-function mutations in the *PCSK9* gene, which is involved in cholesterol homeostasis, are associated with a protective phenotype in patients with MASLD, and hepatic *PCSK9* expression correlates with severity of liver steatosis. *PCSK9* inhibitors are approved for hypercholesterolemia treatment and are under discussion for MASLD treatment [141,142]. However, these compounds are not tissue-specific and require long-term treatment [143]. Recently, an improved ZFP-based epigenetic silencing system is tested in mice, combining *DNMT3A*, *DNMT3L*, and a krüppel-associated box (KRAB) domain fused to preselected ZFP arrays to introduce DNA

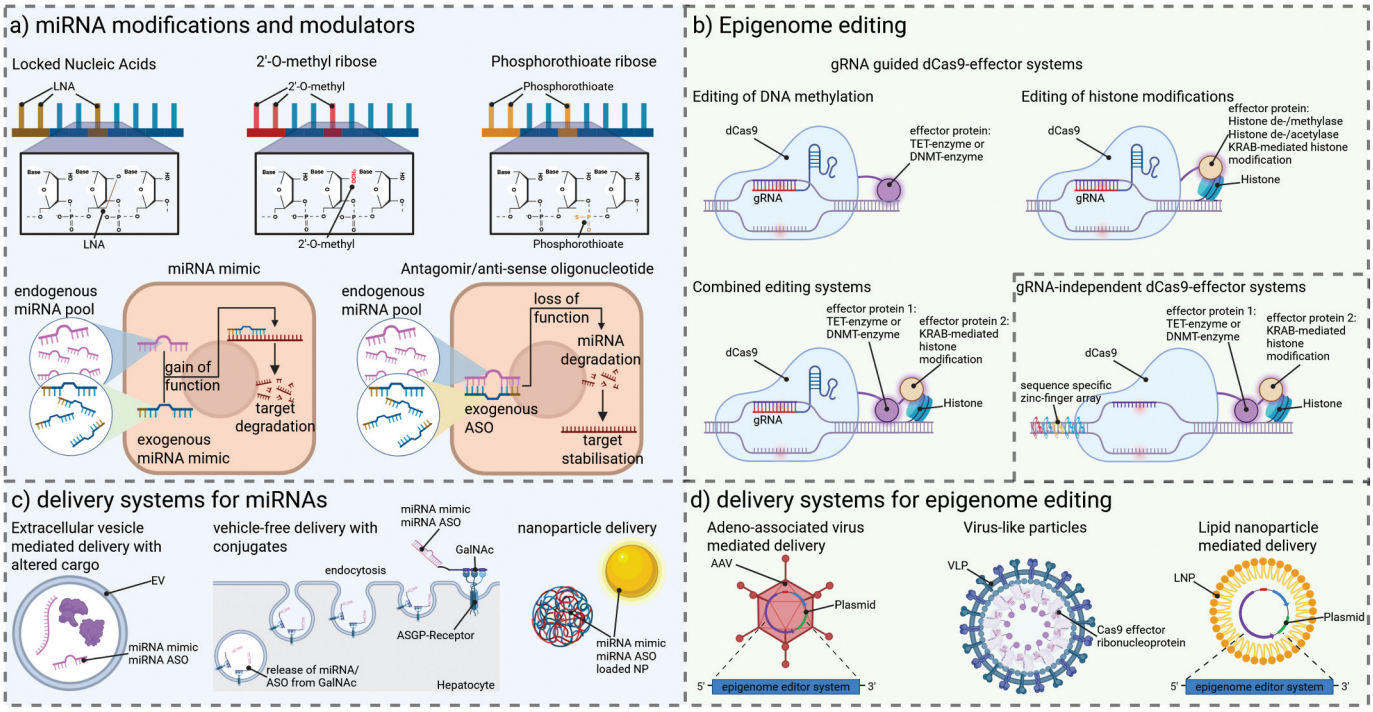

Figure 6. Schematic overview of miRNA modification and epigenome editing systems and their primary delivery systems.

Delivery systems listed are not exclusively used for miRNA/ASO or CRISPR-based epigenome editing systems and could be applied to both. (a) miRNA mimics or ASO modifications that increase stability and mode of action of miRNA mimics or miRNA ASOs. (b) Epigenome editing systems that employ a dead Cas9 (dCas9) fused to effector proteins. As a locus-specific guiding mechanism, they either rely on a guided RNA (gRNA) or gRNA-independent guidance by zinc-finger protein (ZFP) arrays. (c) Delivery mechanisms for small, chemically modified RNA molecules (miRNA mimics or miRNA ASOs). (d) Delivery systems for epigenome editing systems. Whereas AAV and LNP-based delivery systems utilize DNA plasmids for delivery of epigenome editing systems, VLPs can also deliver ribonucleoproteins for transient effects.

Abbreviations: LNA = locked nucleic acids, 2′O-methyl = 2′O-methyl ribose, EV = extracellular vesicle, ASO = antisense oligonucleotide, ASGP-Receptor = asialoglycoprotein receptor, NP = nanoparticle, gRNA = guide RNA, dCas9 = dead Cas9, TET = ten-eleven translocation methylcytosine dioxygenase, DNMT = DNA methyltransferase, KRAB = Krüppel-associated box, AAV = adeno-associated virus, VLP = virus-like particles, LNP = lipid nanoparticles.

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methylation and KRAB-mediated silencing of *Pcsk9* in murine liver. This system reduces plasma *Pcsk9* levels by up to 75% and increases hepatic DNA methylation at the *Pcsk9* locus by more than 50%. These effects persist for over 300 days after a single administration, with minimal off-target gene expression changes. Moreover, *Pcsk9* silencing and methylation are maintained after partial hepatectomy, suggesting heritable epigenetic changes through mitosis during liver regeneration after a single treatment. Since a single dosage is sufficient to reduce *Pcsk9* levels, these systems are termed hit-and-run epigenome editors. The system is delivered via liver-targeted lipid nanoparticles in vivo [144]. An alternative dCas9-based system fused with *DNMT3A*, *DNMT3B*, and KRAB repressor targeting *PCSK9* with gRNAs is tested in primary human hepatocytes and *in vivo* in mice and cynomolgus macaques. In primary human hepatocytes isolated from chimeric humanized liver PXB-mice, the system shows specific hypermethylation of the *PCSK9* promoter with minor genome-wide off-target effects. In mice, administration of a single LNP-delivered *PCSK9* epigenetic inhibitor reduces circulating *PCSK9* levels by 98% for up to 1 year and also significantly reduces serum cholesterol levels. Also, the introduced hypermethylation at the *PCSK9* locus remains stable for 1 year. Similar to the previous study, in a partial hepatectomy mouse model treated with a single dose of PCSK9 epigenetic inhibitor prior to surgery, hypermethylation of the PCSK9 locus remains stable 55 days post-surgery, demonstrating that introduced methylation changes by the *PCSK9* epigenetic inhibitor are inherited to daughter cells in liver regeneration. Comparable durability and stability of DNA methylation at the *PCSK9* locus are confirmed in cynomolgus macaques [145]. Whether these systems are similarly effective in a MASLD context or dietary mouse models requires further elucidation. In conclusion, because these epigenetic-based inhibitors of gene expression indicate long-lasting effects after a single-dose application, they have future potential for effective MASLD treatment. Nevertheless, safety and efficiency need to be tested more rigorously.

8.3. Targeting hepatokines: FGF21 epigenetic activation

Hepatokines such as *FGF21*, which regulate insulin sensitivity, lipid metabolism, and glucose homeostasis, are potential therapeutic targets in MASLD [146]. Epigenetic regulation of the *Fgf21* promoter by DNA methylation in proximity to *Ppara* binding motifs is implicated by us and others in mouse models [147,148]. A dCas9-Tet1 system is tested in *in vitro* cell culture and mice. Transfection of Hepa1-6 cells with the epigenetic activator reduces DNA methylation in the *Fgf21* promoter region. Introduced DNA methylation changes persist only over two passages in cell culture (6 days) and are completely lost after 42 days. However, hydrodynamic tail vein

injection of the dCas9-Tet1 system for *Fgf21* activation results in no significant changes in DNA methylation in wild-type mice; only when injected into *Ppara*$^{-/-}$ mice, a significant reduction in *Fgf21* DNA methylation is observed due to *Ppara*-dependent *Fgf21* methylation. In *Ppara*$^{-/-}$ mice, *Fgf21* epigenetic activation enhances *Fgf21* serum levels [149]. This approach demonstrates the potential for transient epigenetic activation, though stability and clinical applicability require further optimization.

8.4. Emerging delivery systems for epigenome editors

A recent approach for transient epigenome editing employed virus-like particles (VLPs) to deliver dCas9 epigenome-editor ribonucleoproteins. This system is delivered *in vitro* into HepG2 cells (among others) and achieves robust silencing of *CD55*, a gene involved in immune response in the liver [150,151]. However, safety profiles and efficiency *in vivo* require confirmation.

Epigenome editing harbors future potential as treatment for MASLD because by combining multiple epigenome editors (DNMTs with KRAB repressors), a higher efficiency effect duration can be achieved. Furthermore, single-dose applications can potentially introduce heritable epigenetic changes that might interfere with MASLD progression or development. Employing different delivery systems such as viral vectors or LNPs provides further tools for liver-specific delivery of epigenetic editors, though to date no epigenetic editor systems have entered clinical trials for MASLD treatment.

8.5. miRNA-based therapeutic strategies

8.5.1. Principles of miRNA therapeutics

In the context of modulating miRNA expression, either modified miRNA mimics or miRNA inhibitors (ASOs) are delivered to the liver. Generally, miRNA modifiers are optimized for stability by utilizing locked nucleic acids (LNAs) or other chemical modulation instead of the regular RNA backbone to prevent RNA endonuclease degradation (Figure 6(a)) [152]. For liver-specific delivery of miRNA ASOs or mimics, conjugations with GalNAc molecules are utilized [153]. Alternatively, endogenous EVs are isolated from circulation, transfected with ASOs or mimics and reinjected into the organism for organ-specific delivery [154]. Organ-specific lipid nanoparticles (LNPs) are alternative options as delivery vehicles [155]. Non-coding RNA-based, especially short non-coding RNA (such as miRNA) treatment therapies for MASLD are promising given their multiple involvement in MASLD progression as outlined above. Because endogenous miRNA expression is often dysregulated in MASLD, restoration of physiological miRNA levels using antisense oligonucleotides (ASOs) or miRNA mimics could be future options for MASLD therapy.

8.5.2. miR-22 inhibition: clinical translation

Currently, a miR-22 ASO is in clinical phase I trials (RES-010) for obesity and MASLD/MASH treatment (euclinicaltrials.eu, 2024-514871-17-00). RES-010 is a locked nucleic acid (LNA) ASO that does not utilize any liver-specific conjugation or vehicle but is applied systemically. Additionally, a liver-specific version of RES-010 exists which is a miR-22 ASO conjugated with GalNAc (RES-020). miR-22-3p regulates glycogen metabolism and genetic mouse models for miR-22 deficiency (miR-22$^{-/-}$) are resistant to weight gain under HFD feeding compared to wild-type mice. miR-22$^{-/-}$ mice have higher brown adipose tissue (BAT) activity as measured by heat dissipation in infrared imaging and *UCP1* marker expression. Furthermore, miR-22 is upregulated especially in adipose tissue of individuals with obesity compared to normal-weight individuals [156]. Concomitantly, when non-human primates are fed a fast-food diet and treated with RES-010, white adipose tissue shows signs of browning [156], indicating that RES-010 could have anti-obesity effects.

8.5.3. Targeting mitochondrial function: MCJ inhibition

One approach to reduce lipid accumulation in hepatocytes is stimulation of β-oxidation. Methylation-controlled J protein (*MCJ*) is a transmembrane protein in mitochondria that acts as a negative regulator of complex I. *MCJ* knockout mice show decreased lipid accumulation in the liver, lower AST serum levels, and decreased liver fibrosis. *MCJ* expression is upregulated in the liver of human individuals with MASLD. Mice fed a MASH-inducing methionine/choline-deficient diet (MCD) and treated with MCJ-ASOs conjugated to GalNAc show decreased hepatic lipid content and fibrosis, which is achieved by enhanced hepatic β-oxidation [157]. Whether MCJ-ASO conjugates could also be used to treat human MASH remains to be tested.

8.5.4. EV-mediated miRNA delivery

As outlined in the previous chapter, miRNA delivery utilizing isolated EVs from circulation and reinjection after cargo manipulation is possible. Age is considered one risk factor for developing MASLD since insulin sensitivity decreases and fat accumulation increases [158,159]. When miRNA expression from isolated EVs of young and old mice is compared, miR-30c is identified to be involved in age-dependent liver fibrosis as miR-30c-5p is increased in EVs of young mice. Subsequently, isolated EVs are transfected using Exo-Fect with miRNA-LNA 30c-5p mimic or mimic control and reinjected into aged mice via tail vein injection, which leads to reduced serum ALT and AST levels in aged miR-30c-5p receiving mice. Additionally, fibrosis scores and αSMA expression–a marker for fibrosis expressed by HSCs–are significantly reduced, and hepatic *FOXO3* protein levels show reduced levels compared to aged mice without miR-30c-5p loading [160]. Whether a similar approach is viable in humans, where EVs could be isolated from patients followed by modulation of miRNA cargo and reinjection into the patient to hijack the patient-derived delivery system, requires critical future investigation. Nevertheless, miRNAs harbor compelling potential as potential treatment strategies for MASLD progression by introducing transient modulation of miRNA expression. However, with the advent of incretin analogues, many clinical trials of miRNA-based drugs have been discontinued [161]. The current application of miRNA-based treatments for Huntington's disease might revive the miRNA-therapeutics field for MASH and broaden our knowledge about the advantages and drawbacks of miRNA therapies in metabolic diseases [162].

9. Future perspectives

9.1. Integration of epigenetic mechanisms in MASLD pathogenesis

The complex relationship between dietary intake, hepatic metabolism, and epigenetic regulation represents a fundamental mechanism linking nutrition to gene expression and MASLD pathogenesis. The liver's central role in generating and regulating epigenetic substrates through one-carbon metabolism and acetyl-CoA metabolism positions it as a critical nexus between environmental exposures and genomic regulation. Western dietary patterns, characterized by excessive fat and insufficient micronutrients, profoundly disrupt these metabolic pathways, leading to altered availability of methyl and acetyl donors. The consequent epigenetic dysregulation contributes to the development and progression of MASLD, MASH, and hepatocellular carcinoma. Importantly, the bidirectional nature of this relationship whereby metabolic dysfunction alters epigenetics and epigenetic changes exacerbate metabolic disease creates self-perpetuating cycles that underlie chronic liver pathology. Epigenetic mechanisms are evidently crucial for establishing and maintaining liver zonation, with disruptions in these regulatory networks contributing to the spatial metabolic dysregulation observed in MASLD. The accumulation of lipids preferentially in pericentral zones early in disease progression, coupled with zone-specific alterations in DNA methylation, histone modifications, and miRNA expression, highlights the spatial dimension of epigenetic dysregulation in MASLD.

9.2. Current state of epigenetic therapeutics for MASLD

Recent advances in epigenome editing technologies, particularly hit-and-run systems combining DNMTs with KRAB repressors or TET enzymes with transcriptional activators, demonstrate remarkable potential for durable gene expression modulation. The success of PCSK9 epigenetic silencing systems in achieving sustained effects lasting over one year following single administration represents a paradigm shift in therapeutic strategies. These systems offer several advantages over conventional pharmacological approaches: (1) single-dose administration with long-lasting effects, (2) heritability through cell division during liver regeneration, (3) minimal off-target effects when properly designed, and (4) potential for tissue-specific delivery via LNPs or AAVs. miRNA-based therapeutics offer complementary advantages through their ability to simultaneously modulate multiple targets within dysregulated pathways. The pleiotropic nature of miRNAs is particularly well suited to addressing the multifactorial pathogenesis of MASLD. GalNAc-conjugated ASOs and mimics have demonstrated excellent liver-specific delivery and favorable safety profiles, as evidenced by ongoing clinical trials such as RES-010 for miR-22 inhibition. Furthermore, the recent success of miRNA-based therapies in other diseases, such as Huntington's disease, has reinvigorated interest in this therapeutic modality. Despite promising preclinical data, several significant obstacles must be overcome before epigenetic therapies for MASLD achieve widespread clinical application. First, the heterogeneity of MASLD patient populations complicates therapeutic development. Patients vary considerably in disease stage, metabolic profiles, genetic backgrounds, and environmental exposures, necessitating stratification approaches to identify responders. Second, long-term safety concerns remain for permanent or semi-permanent epigenome editing. While current studies demonstrate minimal off-target effects, comprehensive assessment of unintended consequences across the genome and over extended timeframes is essential. Additionally, delivery systems require further optimization. While LNPs and AAVs show promise for liver-specific delivery, achieving optimal biodistribution, minimizing immunogenicity, and ensuring efficient cellular uptake across diverse hepatocyte populations within zonated liver architecture remain challenges. The potential for immune responses to viral vectors or repeated administration of therapeutic compounds must be carefully evaluated. Identifying optimal therapeutic targets requires moreover a comprehensive understanding of which epigenetic changes are causative versus consequential in MASLD progression. Many observed epigenetic alterations may represent adaptive responses rather than disease drivers, necessitating careful target validation. Lastly, the bidirectional relationship between metabolic dysfunction and epigenetic dysregulation suggests that epigenetic monotherapies may have limited efficacy without addressing underlying metabolic disturbances. Combination approaches integrating epigenetic therapeutics with lifestyle interventions or metabolic modulators (such as incretin analogues) may prove more effective. The recent discontinuation of several miRNA-based clinical trials following the success of GLP-1 receptor agonists underscores the competitive landscape and the need to demonstrate added value beyond existing therapies.

Future Perspective Epigenetic dysregulation represents a fundamental mechanism in MASLD pathogenesis, spanning from alterations in substrate availability through one-carbon metabolism to zone-specific changes in DNA methylation, histone modifications, and miRNA expression. The reversibility and druggability of epigenetic marks position them as attractive therapeutic targets. Advances in epigenome editing technologies and miRNA-based therapeutics provide powerful tools to modulate these processes with increasing precision and durability. However, successful translation requires addressing substantial challenges Comprehensive longitudinal genome-wide studies assessing DNA methylation, histone modification, and miRNA expression patterns across MASLD stages in human liver, with particular focus on transcription factor-binding site methylation, are still urgently needed. Such studies should employ spatial transcriptomics and epigenomics to capture zone-specific alterations and should integrate multiomics data to identify causal relationships between epigenetic changes and metabolic outcomes. Second, mechanistic studies elucidating how dietary factors and metabolic intermediates influence epigenetic enzyme activity and substrate availability will provide insights into disease pathogenesis and potential preventive strategies. Understanding how Western dietary patterns deplete SAM pools and alter histone acetylation may

identify nutritional interventions to complement therapeutic approaches.

Furthermore, the investigation of interorgan communication via extracellular vesicles in MASLD is in its infancy. Understanding how liver-derived EVs influence metabolic homeostasis in adipose tissue, muscle, pancreas, and even lung may reveal systemic therapeutic opportunities. Conversely, understanding how EVs from other organs influence hepatic metabolism could identify novel intervention points. The potential to utilize patient-derived EVs as personalized delivery vehicles for miRNA therapeutics still warrants thorough investigation. Thus, developing predictive biomarkers to identify patients most likely to benefit from specific epigenetic interventions is essential for personalized medical approaches. The integration of epigenetic signatures with clinical parameters, metabolomics, and genetic variants may enable patient stratification for clinical trials but is hindered by limited access to human liver biopsies. Circulating miRNAs and EV contents on the other hand show promise as noninvasive biomarkers for MASLD staging and therapeutic monitoring but also require further validation studies in large human cohorts. Lastly, investigating combination therapies integrating epigenetic modulators with established treatments such as resmetirom or semaglutide may reveal synergistic anti-MASH effects in the future. For example, combining THRβ agonists with therapies targeting THRB or DIO1 methylation might enhance therapeutic responses in resmetirom non-responders. Similarly, combining miRNA-based insulin sensitizers with GLP-1 analogues might provide complementary metabolic benefits.

The translational pathway for epigenetic therapeutics in MASLD should proceed systematically. Preclinical studies should prioritize validation in dietary models that recapitulate human MASLD pathophysiology more closely than genetic models. Studies should assess not only hepatic endpoints but also systemic metabolic parameters and extrahepatic manifestations. In addition, long-term safety studies in large animal models are essential before clinical translation. Subsequently, early-phase clinical trials should focus on well-defined patient populations with specific epigenetic signatures predictive of response. The endpoints should include not only standard biochemical and histological measures but also epigenetic biomarkers demonstrating target engagement. Adaptive trial designs allowing for dose optimization and patient selection based on interim analyses may accelerate the development of epigenetic MASH therapeutics. However, successful translation requires addressing substantial challenges related to patient heterogeneity, target validation, delivery optimization, long-term safety, and demonstrating clinical value beyond existing therapies. Lastly, regulatory frameworks for epigenetic therapies, particularly those involving permanent (epi-)genome modifications, require careful consideration. While durable gene expression changes offer therapeutic advantages, they also raise questions about reversibility and long-term consequences. Therefore, balanced risk-benefit assessments considering the serious morbidity and mortality associated with MASLD progression will guide approval decisions.

The liver's unique dual role as both producer and consumer of epigenetic substrates, combined with its regenerative capacity enabling heritable transmission of therapeutic epigenetic modifications, positions MASLD as an ideal indication for pioneering epigenetic therapeutic approaches. With continued research elucidating the complex interplay between nutrition, metabolism, and epigenetics, and with ongoing technological advances in precise epigenome editing and targeted delivery, epigenetic therapeutics hold substantial promise for addressing the growing global burden of MASLD.

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