**Review Article**

**Epigenetic Mechanisms and Their Role in Non-Alcoholic Fatty Liver Disease (NAFLD) Pathogenesis**

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**Abstract:** Non-alcoholic fatty liver disease (NAFLD) is a complex multifactorial disease influenced by genetic, epigenetic, and environmental factors. Recent research highlights the significance of epigenetic mechanisms, including DNA methylation, histone modifications, and non-coding RNAs, in NAFLD pathogenesis. This review discusses current evidence on the contribution of aberrant DNA methylation in regulating genes involved in lipid metabolism, inflammation, and fibrogenesis. It also examines alterations in histone modifications that impact gene transcription and chromatin remodeling in NAFLD. The regulatory roles of microRNAs and long non-coding RNAs in metabolic pathways, inflammatory signaling, and fibrotic processes relevant to NAFLD progression are also explored in this review work. Elucidating these epigenetic mechanisms provides insights into new biomarkers for early disease detection and opportunities for novel therapeutic interventions through epigenetic targeting. Further research on epigenetic changes specific to the stages of NAFLD will advance understanding of the complex gene-environment interactions in NAFLD pathogenesis.

**Keywords:** NAFLD; Epigenetics; DNA methylation; Histone modifications; Non-coding RNAs; Biomarkers.

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) has emerged as one of the most prevalent chronic liver diseases worldwide, posing a major public health burden [1]. It represents a spectrum of conditions characterized by hepatic steatosis in the absence of secondary causes such as alcohol abuse or certain medications [2]. The prevalence of NAFLD is estimated to be 25% globally [3], and it is closely associated with obesity, insulin resistance, and other features of metabolic syndrome [4]. NAFLD can progress from simple steatosis to non-alcoholic steatohepatitis (NASH), which involves steatosis along with necroinflammation, and can eventually advance to cirrhosis and hepatocellular carcinoma [5]. The development and progression of NAFLD is influenced by a complex interplay of genetic, epigenetic, environmental, and lifestyle factors [6]. In particular, epigenetic mechanisms such as DNA methylation, histone modifications, and non-coding RNAs have emerged as significant contributors to NAFLD pathogenesis [7]. Epigenetic changes regulate gene expression and cellular phenotypes without altering the underlying DNA sequence. As such, they allow for adaptation to changing environmental cues that may be relevant to NAFLD [8].

Aberrant DNA methylation affects key genes involved in various pathways associated with NAFLD, including insulin signaling, inflammation, fibrogenesis and lipid metabolism [9]. Altered histone modifications impact chromatin structure and transcription of genes linked to NAFLD [10]. MicroRNAs and long non-coding RNAs regulate different aspects of NAFLD pathophysiology by modulating mRNA stability and translation [11]. Elucidating epigenetic alterations specific to NAFLD development and progression can provide valuable insights into disease mechanisms (Figure 1). Moreover, because epigenetic changes are reversible, they present potential novel avenues for therapeutic interventions in NAFLD [12]. Epigenetic-based treatments aim to reverse aberrant epigenetic signatures by using pharmacological inhibitors of DNA methylation and histone modifying enzymes [13]. Other strategies include modulating non-coding RNA levels and antisense oligonucleotide-based therapies [14]. A better understanding of stage-specific epigenetic changes in NAFLD will facilitate the development of targeted epigenetic drugs. In addition to revealing promising new treatment approaches, characterization of epigenetic marks may lead to identification of useful biomarkers for early NAFLD detection, distinguishing simple steatosis from NASH, and predicting progression to fibrosis and cirrhosis [15]. Overall, elucidating
the role of epigenetic mechanisms in NAFLD has high translational relevance. This review discusses about the current evidence on the contribution of key epigenetic alterations to NAFLD pathogenesis.

**2. DNA Methylation Changes in NAFLD**

DNA methylation is an epigenetic mechanism involving the addition of a methyl group to cytosine bases, often in CpG dinucleotide context. This modification is associated with chromatin condensation and transcriptional repression [16]. Aberrant DNA methylation affects several genes implicated in NAFLD pathogenesis [17]. Genome-wide methylation analysis has revealed differences between normal liver, simple steatosis and NASH [18]. Hypermethylation of the PPARG promoter and downregulation of PPARG expression is observed in liver biopsies of NASH patients [19]. PPARG is a master transcriptional regulator of lipid metabolism and insulin sensitivity [20]. Its downregulation promotes hepatic steatosis, inflammation and fibrosis progression from NAFLD to NASH by deregulating genes involved in lipid metabolism, immune pathways and extracellular matrix remodeling [21,22]. The microsomal triglyceride transfer protein (MTTP) plays a key role in very low density lipoprotein assembly and lipid export from hepatocytes [23]. Increased MTTP promoter methylation is associated with progressive NASH and correlates negatively with protein levels [24,25]. This epigenetically mediated suppression of MTTP likely impairs hepatic lipid export, contributing to steatosis.

Stearoyl-CoA desaturase (SCD) is the rate limiting enzyme for monounsaturated fatty acid synthesis. Its transcription is reduced in NASH livers, consistent with hypermethylation of the SCD promoter [26,27]. Downregulation of SCD disturbs triglyceride synthesis and lipid metabolism, exacerbating steatosis [28]. The farnesoid X receptor (FXR) regulates bile acid and lipid metabolism. FXR hypomethylation is linked to NASH severity [29]. Resulting FXR overexpression is implicated in aberrant lipid metabolism and inflammation in NASH progression [30].

Pro-inflammatory genes also demonstrate altered methylation in NAFLD livers (Table 1). The TNFα promoter is hypomethylated in NASH, associated with TNFα overexpression, Nuclear factor kappa B activation and aggravated inflammation [31,32]. Aberrant methylation of interleukin genes like IL-6 and IL-10 can further drive inflammatory signaling cascades in NASH [33,34]. Genes controlling hepatic fibrogenesis and extracellular matrix (ECM) remodeling likewise exhibit methylation changes reflective of NASH progression. For instance, hypomethylation-mediated overexpression of procollagen lysyl hydroxylase 2 (PLOD2) occurs in advanced NAFLD fibrosis [35]. Conversely, antifibrogenic genes like PPARGC1A become hypermethylated with worsening fibrosis [36].
Table 1. Key genes exhibiting aberrant DNA methylation patterns in NAFLD

<table>
<thead>
<tr>
<th>Gene</th>
<th>Function</th>
<th>Methylation Change in NAFLD</th>
<th>Effect on Expression</th>
<th>Implication for NAFLD</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPARG</td>
<td>Transcriptional regulator of lipid and glucose metabolism</td>
<td>Promoter hypermethylation [19,20]</td>
<td>Reduced [21,22]</td>
<td>Promotes steatosis and inflammation</td>
</tr>
<tr>
<td>MTTP</td>
<td>Lipid export from hepatocytes</td>
<td>Increased promoter methylation [24,25]</td>
<td>Reduced [25,26]</td>
<td>Impairs hepatic lipid export, contributing to steatosis</td>
</tr>
<tr>
<td>SCD</td>
<td>Rate limiting enzyme for fatty acid synthesis</td>
<td>Promoter hypermethylation [26,27]</td>
<td>Reduced [27,28]</td>
<td>Disturbs triglyceride synthesis and lipid metabolism</td>
</tr>
<tr>
<td>FXR</td>
<td>Bile acid/lipid metabolism</td>
<td>Hypomethylation [29]</td>
<td>Increased [30]</td>
<td>Dysregulates lipid metabolism and inflammation</td>
</tr>
<tr>
<td>T/NFα</td>
<td>Pro-inflammatory cytokine</td>
<td>Promoter hypomethylation [31,32]</td>
<td>Increased [32]</td>
<td>Aggravates inflammation and liver injury</td>
</tr>
<tr>
<td>IL-6, IL-10</td>
<td>Anti-inflammatory cytokines</td>
<td>Aberrant methylation [33,34]</td>
<td>Dysregulated [33,34]</td>
<td>Promotes inflammatory signaling</td>
</tr>
<tr>
<td>PLOD2</td>
<td>Extracellular matrix remodeling</td>
<td>Hypomethylation [35]</td>
<td>Increased [35]</td>
<td>Linked to advanced NAFLD fibrosis</td>
</tr>
<tr>
<td>PPARGC1A</td>
<td>Antifibrotic, metabolic regulator</td>
<td>Hypermethylation [36]</td>
<td>Reduced [36]</td>
<td>Indicates worsening fibrosis</td>
</tr>
</tbody>
</table>

3. Histone modifications in NAFLD pathogenesis

Histone modifications including acetylation, methylation, phosphorylation and ubiquitination alter chromatin structure and accessibility, thereby regulating gene transcription [37]. Enzymes mediating addition or removal of these marks are often aberrantly activated in NAFLD [38]. Histone acetyltransferases (HATs) add acetyl groups to lysine residues on histone tails, allowing chromatin expansion and gene activation. Deacetylation by histone deacetylases (HDACs) compacts chromatin and suppresses genes [39]. Class I HDACs are upregulated in NAFLD livers, associated with global hypoacetylation [40]. Reduced acetylation of histones H3 and H4 is observed at promoters of lipid metabolic genes in NAFLD, correlating with their downregulation [41].

Hyperacetylation of pro-inflammatory genes promoters via HATs like p300 and deregulated HDAC activity causes overexpression of cytokines and chemokines in NASH [42]. HDAC inhibitors can attenuate inflammation [43], highlighting the promise of normalizing acetylation levels. (Table 3) Methylation of histone H3 lysine residues by histone methyltransferases has varying effects based on the site. For instance, H3K4 methylation activates while H3K9 methylation represses genes [44]. Elevated activity of the lysine methyltransferase SETD7 and increased H3K4 methylation at lipogenic genes promoters is linked to NAFLD progression [45]. Histone phosphorylation, mediated by kinases like p38 MAPK and JNK that are activated in NAFLD, can increase chromatin accessibility and gene transcription [46]. Phosphorylation of histones H2A and H3 accompanies overexpression of fibrogenic genes in advanced NAFLD fibrosis [47].

Table 3. Histone modifications and modifiers deregulated in NAFLD

<table>
<thead>
<tr>
<th>Histone Modification</th>
<th>Key Regulators</th>
<th>Change in NAFLD</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetylation</td>
<td>HATs like p300, HDACs class I</td>
<td>↓ Histone H3/H4 acetylation [41]</td>
<td>↓ Lipid metabolism genes&lt;br&gt;↑ Inflammatory genes [41,42]</td>
</tr>
<tr>
<td>Methylation</td>
<td>Methyltransferases like SETD7</td>
<td>↑ H3K4me by SETD7 [45]&lt;br&gt;↑ H3K9me [44]</td>
<td>↑ Lipogenic genes&lt;br&gt;↑ Fibrogenic genes</td>
</tr>
<tr>
<td>Phosphorylation</td>
<td>MAP kinases like p38 &amp; JNK</td>
<td>↑ H2A/H3 phosphorylation [46,47]</td>
<td>↑ Fibrogenic genes</td>
</tr>
</tbody>
</table>

4. Non-coding RNAs

Non-coding RNAs (ncRNAs), including microRNAs (miRNAs) and long non-coding RNAs (lncRNAs), are emerging as important epigenetic regulators in NAFLD [48]. miRNAs fine-tune gene expression by binding to target miRNAs and inhibiting translation or promoting degradation [49]. Multiple miRNAs are aberrantly expressed in NAFLD, impacting key pathways. For instance, miRNA-
122 is highly abundant in liver and plays a central role in lipid metabolism. It is significantly downregulated in NASH livers [50]. Low miRNA-122 expression dysregulates enzymes involved in cholesterol synthesis and fatty acid metabolism, contributing to steatosis [51].

Table 3. miRNAs perturbed in NAFLD pathogenesis

<table>
<thead>
<tr>
<th>miRNA</th>
<th>Expression in NAFLD</th>
<th>Role</th>
</tr>
</thead>
<tbody>
<tr>
<td>miRNA-122</td>
<td>Downregulated [50]</td>
<td>Regulates lipid metabolism</td>
</tr>
<tr>
<td>miRNA-21</td>
<td>Upregulated [52]</td>
<td>Pro-inflammatory</td>
</tr>
<tr>
<td>miRNA-155</td>
<td>Upregulated [53]</td>
<td>Pro-inflammatory</td>
</tr>
<tr>
<td>miRNA-451</td>
<td>Upregulated [54]</td>
<td>Pro-inflammatory</td>
</tr>
<tr>
<td>miRNA-29</td>
<td>Downregulated [56]</td>
<td>Anti-fibrotic</td>
</tr>
</tbody>
</table>

miRNA-21, miRNA-155 and miRNA-451 promote NAFLD progression by stimulating pro-inflammatory cytokine production [52-54]. miRNA-29 is strongly antifibrotic by inhibiting the deposition of extracellular matrix (ECM) components [55]. Its reduced expression promotes advanced fibrosis in NASH progression [56]. IncRNAs regulate gene expression through diverse mechanisms including interacting with chromatin modifiers [57]. Several IncRNAs show aberrant expression patterns positively correlated with NAFLD severity [58]. Examples include IncRNA HULC which promotes lipogenesis [59], IncRNA MEG3 linked to inflammation [60], and IncRNA ATGL which regulates autophagy [61].

Table 4. IncRNAs with altered expression in NAFLD progression

<table>
<thead>
<tr>
<th>IncRNA</th>
<th>Expression in NAFLD</th>
<th>Role</th>
</tr>
</thead>
<tbody>
<tr>
<td>HULC</td>
<td>Upregulated [59]</td>
<td>Promotes lipogenesis</td>
</tr>
<tr>
<td>MEG3</td>
<td>Upregulated [60]</td>
<td>Pro-inflammatory</td>
</tr>
<tr>
<td>ATGL</td>
<td>Downregulated [61]</td>
<td>Regulates autophagy</td>
</tr>
</tbody>
</table>

5. Epigenetic-based therapeutics

The realization that epigenetic mechanisms significantly contribute to NAFLD pathogenesis has opened up novel avenues for therapeutic intervention by targeting aberrant epigenetic signatures [62]. Strategies to reverse abnormal DNA methylation include using DNA methyltransferase (DNMT) inhibitors like 5-azacytidine that incorporate into DNA and irreversibly bind and inactivate DNMTs [63]. This allows previously silenced genes to be re-expressed. DNMT inhibition using RG108 in experimental NASH models normalized expression of lipid metabolism and anti-inflammatory genes by promoter demethylation, reducing steatosis and inflammation [64,65].

Histone deacetylase (HDAC) inhibitors such as trichostatin A, sodium butyrate and valproic acid help restore histone acetylation levels, thereby allowing gene reactivation [66]. By promoting acetylation of anti-inflammatory and antifibrotic genes, HDAC inhibitors exhibited therapeutic effects in preclinical NAFLD and NASH models [67,68]. However, most HDAC inhibitors lack specificity. Developing isoform-selective inhibitors targeting class I HDACs upregulated in NAFLD represents a promising strategy [69]. Sirtuin (SIRT) activators such as resveratrol also help re-establish normal acetylation profiles by enhancing SIRT deacetylase activity [70]. This was accompanied by metabolic and anti-inflammatory benefits in experimental NAFLD [71]. Other epigenetic drugs aim to inhibit specific histone methyltransferases like lysine methyltransferase SETD7 [72].
Table 5. Emerging epigenetic drugs for potential NAFLD treatment

<table>
<thead>
<tr>
<th>Drug Class</th>
<th>Examples</th>
<th>Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNMT inhibitors</td>
<td>5-azacytidine, RG108</td>
<td>Inhibit DNA methylation</td>
</tr>
<tr>
<td>HDAC inhibitors</td>
<td>Trichostatin A, sodium butyrate</td>
<td>Inhibit histone deacetylation</td>
</tr>
<tr>
<td>SIRT activators</td>
<td>Resveratrol</td>
<td>Enhance histone deacetylation</td>
</tr>
<tr>
<td>miRNA inhibitors</td>
<td>Antisense oligonucleotides, miravirsen</td>
<td>Reduce upregulated miRNAs</td>
</tr>
</tbody>
</table>

Restoring aberrantly expressed non-coding RNA levels is another epigenetic approach. miRNA mimics or viral vector-mediated overexpression of downregulated miRNAs like miRNA-29 and miRNA-122 exhibited antifibrotic and antisteatotic effects respectively in NAFLD models [73,74]. Antisense oligonucleotides like miravirsen that sequester and degrade upregulated miRNAs such as miRNA-155 showed anti-inflammatory effects [75]. Small molecule inhibitors reducing elevated lncRNA levels also ameliorated NAFLD in mice [76]. While various epigenetic drugs demonstrate efficacy in preclinical studies, most remain to be validated in clinical trials. Key challenges include lack of specificity, potential off-target effects, and poor in vivo stability [77]. An area of active investigation is developing advanced delivery systems such as nanoparticles to enhance stability and target epigenetic drugs to the liver [78].

6. Epigenetic Biomarkers in NAFLD

Biomarkers that can identify individuals at high risk of NAFLD or reliably distinguish simple steatosis from advanced NASH are clinically valuable for prognosis and making treatment decisions [79]. Epigenetic marks show attractive potential as biomarkers due to their stability, accessibility in tissues and blood, and ability to provide stage-specific snapshots of disease progression [80]. Aberrant DNA methylation signatures specific to steatosis, NASH or advanced fibrosis offer one avenue for biomarkers. A DNA methylation risk score encompassing ten genes could differentiate NASH from simple steatosis [81]. Hypermethylation of WIFI1 and PPARG1A promoters strongly correlates with fibrosis severity [82].

Global loss of hepatic histone H4K20me3, mediated by downregulation of the methyltransferase SETD7, is a signature of advanced NAFLD fibrosis [83]. Reduced acetylation of histones H3 and H4 marks steatosis progression [84]. Stage-specific histone modification profiles may further aid diagnosis. Numerous microRNAs exhibit progressive dysregulation along the NAFLD spectrum, highlighting their utility as minimally invasive biomarkers detectable in serum or plasma [85]. The ratio of miRNA-122, an indicator of liver injury, to miRNA-192, reflective of fibrosis, reliably distinguishes NASH from simple steatosis [86]. Likewise, circulating levels of inflammation-associated miRNAs including miRNA-21, miRNA-34a and miRNA-451-5p escalate with NAFLD severity [87-89]. Declining miRNA-29 levels signal worsening fibrosis [90]. Panels incorporating both miRNA and protein biomarkers enhance diagnostic accuracy for staging NAFLD [91]. lncRNA biomarkers measurable in serum or leukocytes are also emerging [92]. Leukocyte lncRNA MEG3 levels track NAFLD progression [93]. LINC00623 and LINC0597 are upregulated in early NAFLD, while PVT1 is increased in late-stage disease [94,95]. However, larger patient cohorts are needed to validate lncRNAs as reliable biomarkers.

Table 6. Candidate epigenetic biomarkers of NAFLD severity

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Change in NAFLD</th>
<th>Potential Utility</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA methylation score</td>
<td>Altered methylation of 10 genes [81]</td>
<td>Differentiate NASH from simple steatosis</td>
</tr>
<tr>
<td>H4K20me3</td>
<td>Loss in fibrotic NAFLD [83]</td>
<td>Indicate advanced fibrosis</td>
</tr>
<tr>
<td>miRNA ratio (122/192)</td>
<td>Decreased [86]</td>
<td>Distinguish NASH from steatosis</td>
</tr>
<tr>
<td>miRNA panels</td>
<td>↑ miRNA-21, 34a, 451</td>
<td>Diagnose NAFLD severity</td>
</tr>
<tr>
<td>LncRNAs (MEG3, LINC00623)</td>
<td>Progressive changes [93-95]</td>
<td>Track NAFLD progression</td>
</tr>
</tbody>
</table>
7. Conclusion

NAFLD has emerged as a major public health concern, necessitating better understanding of its complex pathogenesis to develop novel therapeutics. Recent research underscores the key contributions of epigenetic mechanisms, including DNA methylation, histone modifications, and non-coding RNAs, to NAFLD development and progression. Elucidation of epigenetic alterations affecting genes involved in lipid metabolism, inflammatory pathways, and fibrogenesis provides insights into disease mechanisms. Reversal of these aberrant epigenetic changes using emerging epigenetic drugs presents a promising treatment avenue. Characteristic epigenetic signatures also have exciting clinical potential as non-invasive biomarkers for early NAFLD detection and staging.

References


Sarella PN, Mangam VT. AI-Driven Natural Language Processing in Healthcare: Transforming Patient-Provider Communication. Indian Journal of Pharmacy Practice. 2024;17(1).


