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MICROBIOTA-miRNA INTERACTIONS IN NAFLD: MECHANISMS AND MOLECULAR INSIGHTS

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ABSTRACT

Non-alcoholic fatty liver disease (NAFLD) is a multifactorial disorder characterized by hepatic steatosis, inflammation, and fibrosis, with a growing body of evidence highlighting the critical roles of gut microbiota and microRNAs (miRNAs) in its pathogenesis. The gut-liver axis serves as a key regulatory network, where microbiota-derived metabolites, including short-chain fatty acids (SCFAs), bile acids, and lipopolysaccharides (LPS), influence hepatic lipid metabolism and immune responses. Concurrently, miRNAs regulate gene expression in the liver and intestines, modulating lipid homeostasis, inflammation, and fibrosis. Notably, miR-122, miR-34a, and miR-21 play significant roles in disease progression. Recent studies suggest a bidirectional relationship between microbiota and miRNAs, where microbial metabolites alter miRNA expression, and host-derived miRNAs shape microbiota composition and gut-liver signaling. Dysbiosis-induced changes in miRNA profiles contribute to increased intestinal permeability, endotoxemia, and chronic hepatic inflammation, exacerbating NAFLD progression. Understanding these intricate molecular interactions is essential for elucidating NAFLD pathophysiology. This review explores the complex interplay between gut microbiota and miRNAs in NAFLD, providing insights into the mechanisms underlying disease development and progression. A deeper understanding of these relationships may enhance our comprehension of NAFLD as a systemic disorder involving the gut-liver axis.

Key words: NAFLD, miRNAs, microbiotas, fibrosis.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) has emerged as the most prevalent chronic liver disorder worldwide, affecting approximately 25% of the global population. It encompasses a spectrum of liver conditions ranging from simple

hepatic steatosis to non-alcoholic steatohepatitis (NASH), which can progress to fibrosis, cirrhosis, and hepatocellular carcinoma. NAFLD is closely linked to metabolic disorders such as obesity, insulin resistance, and dyslipidemia, making it a major public health concern. Despite extensive research, the exact molecular mechanisms underlying its pathogenesis remain incompletely understood.

The gut microbiota, a diverse community of microorganisms residing in the gastrointestinal tract, has gained significant attention as a key player in liver health and disease. Through the gut-liver axis, microbiota influence hepatic metabolism, immune responses, and inflammation via microbial metabolites, endotoxins, and bile acid modulation. Dysbiosis, an imbalance in microbial composition, has been implicated in NAFLD progression by altering lipid metabolism, increasing intestinal permeability, and promoting chronic inflammation.

MicroRNAs (miRNAs), small non-coding RNA molecules that regulate gene expression at the post-transcriptional level, have also been identified as critical regulators of NAFLD pathophysiology. Several miRNAs, including miR-122, miR-34a, and miR-155, are involved in lipid metabolism, fibrosis, and inflammatory pathways in the liver. Changes in miRNA expression have been associated with disease severity, making them potential biomarkers and therapeutic targets for NAFLD.

Recent evidence suggests a complex interplay between microbiota and miRNAs in NAFLD development and progression. Gut microbiota can regulate miRNA expression through microbial metabolites and inflammatory signaling, while miRNAs can modulate intestinal barrier function, immune responses, and microbial composition. Understanding this bidirectional relationship may provide new insights into the molecular mechanisms of NAFLD and open avenues for novel therapeutic strategies targeting both microbiota and miRNA pathways.

In this review, we will explore the individual roles of microbiota and miRNAs in NAFLD, examine their mutual interactions, and discuss potential therapeutic implications.

NAFLD: Pathogenesis and Key Molecular Mechanisms

NAFLD is a complex metabolic disorder characterized by excessive fat accumulation in the liver in the absence of significant alcohol consumption. Its pathogenesis involves multiple interconnected mechanisms, including dysregulated lipid metabolism, chronic inflammation, oxidative stress, and fibrosis, which drive disease progression from simple steatosis to non-alcoholic steatohepatitis (NASH) and advanced liver damage.

1. Dysregulated Lipid Metabolism and Insulin Resistance

One of the key drivers of NAFLD is an imbalance in hepatic lipid metabolism. Excessive free fatty acids (FFAs) derived from adipose tissue, dietary intake, and de novo lipogenesis contribute to triglyceride accumulation in hepatocytes. Normally, hepatic lipid homeostasis is maintained by a balance between fatty acid uptake, oxidation, and export via very low-density lipoproteins (VLDL). However, in NAFLD:

- Increased lipolysis in adipose tissue due to insulin resistance leads to an excess influx of FFAs into the liver.
- De novo lipogenesis, driven by sterol regulatory element-binding protein 1c (SREBP-1c) and carbohydrate-responsive element-binding protein (ChREBP), is upregulated.
- Impaired mitochondrial β-oxidation results in lipid accumulation and oxidative stress.

2. Chronic Inflammation and Immune Dysregulation

As NAFLD progresses, the accumulation of lipotoxic intermediates triggers hepatocellular stress, activating inflammatory pathways:

- Kupffer cells, the resident liver macrophages, become activated and release pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interleukin-1β (IL-1β).
- The activation of nuclear factor kappa B (NF-κB) and the inflammasome pathway exacerbates immune responses, leading to hepatocyte apoptosis and further inflammation.
- Dysregulated gut microbiota (dysbiosis) contributes to systemic inflammation through increased intestinal permeability and translocation of bacterial endotoxins (e.g., lipopolysaccharides), which stimulate toll-like receptor 4 (TLR4) signaling in hepatocytes.

3. Oxidative Stress and Mitochondrial Dysfunction

Mitochondrial dysfunction plays a crucial role in NAFLD progression by promoting oxidative stress and hepatocellular injury:

- Impaired fatty acid oxidation leads to excessive production of reactive oxygen species (ROS).
- ROS induce lipid peroxidation, damaging cell membranes and activating stress-related pathways such as c-Jun N-terminal kinase (JNK) signaling.
- Oxidative stress also triggers endoplasmic reticulum (ER) stress, which contributes to hepatocyte apoptosis and further aggravates liver inflammation.

4. Fibrosis and Disease Progression

The transition from NAFLD to NASH is marked by the activation of hepatic stellate cells (HSCs), which are responsible for liver fibrosis:

- Chronic liver injury and inflammation activate HSCs, leading to excessive extracellular matrix (ECM) deposition and fibrotic tissue formation.
- Transforming growth factor-beta (TGF-β) and platelet-derived growth factor (PDGF) are key profibrotic mediators driving HSC activation.
- Advanced fibrosis increases the risk of cirrhosis and hepatocellular carcinoma (HCC), significantly impacting liver function.

Current Understanding of Disease Progression

NAFLD progression is highly heterogeneous, with some patients remaining in the simple steatosis stage while others advance to NASH and fibrosis. Several factors influence disease trajectory, including:

- **Genetic predisposition** (e.g., PNPLA3, TM6SF2, and MBOAT7 polymorphisms)
- **Epigenetic modifications**, including miRNA dysregulation
- **Gut microbiota composition and intestinal permeability**
- **Lifestyle factors** such as diet, physical activity, and metabolic health

Given the multifactorial nature of NAFLD, an integrated approach that considers liver metabolism, inflammation, fibrosis, and gut-liver interactions is essential for understanding its pathophysiology and developing targeted therapies.

Microbiota in NAFLD

The gut microbiota, consisting of trillions of microorganisms including bacteria, archaea, fungi, and viruses, plays a critical role in host metabolism, immune regulation, and liver function. Emerging evidence suggests that alterations in gut microbiota composition, known as dysbiosis, contribute to NAFLD progression by disrupting gut-liver communication and modulating key metabolic and inflammatory pathways.

1. Gut-Liver Axis and Dysbiosis in NAFLD

The gut and liver are closely linked through the **gut-liver axis**, which includes the portal vein, bile acids, and immune signaling pathways. Under normal conditions, the gut barrier prevents harmful bacterial products from reaching the liver, while the liver regulates gut homeostasis through bile acids and immune responses. However, in NAFLD, this balance is disrupted:

- **Increased Intestinal Permeability:** NAFLD patients often exhibit a weakened intestinal barrier due to reduced expression of tight junction proteins (e.g., zonulin, occludin). This allows bacterial endotoxins such as **lipopolysaccharides (LPS)** to translocate into the portal circulation, triggering hepatic inflammation via Toll-like receptor 4 (TLR4) signaling.
- **Dysbiosis:** An imbalance in gut microbial composition is commonly observed in NAFLD. Specific changes include:
    - Increased abundance of **Proteobacteria** (e.g., Escherichia, Enterobacter) and **Firmicutes** (e.g., Lactobacillus, Clostridium), which are associated with inflammation and lipogenesis.
    - Reduced levels of **Bacteroidetes** and beneficial bacteria such as **Akkermansia muciniphila** and **Faecalibacterium prausnitzii**, which help maintain gut barrier integrity and produce anti-inflammatory metabolites.

2. Microbiota-Derived Metabolites and Their Role in NAFLD

Gut microbiota influence liver function through various metabolites, including short-chain fatty acids (SCFAs), lipopolysaccharides (LPS), bile acids, and microbial ethanol. These compounds contribute to NAFLD progression by modulating lipid metabolism, insulin sensitivity, and inflammation.

a) Short-Chain Fatty Acids (SCFAs) – Protective or Harmful?

SCFAs such as acetate, propionate, and butyrate are produced by gut bacteria through the fermentation of dietary fiber. Their role in NAFLD is complex:

- **Protective Effects:** Butyrate and propionate improve insulin sensitivity, reduce inflammation, and enhance gut barrier function by strengthening tight junctions. They also activate G-protein-coupled receptors (GPCRs) to regulate lipid metabolism and energy homeostasis.
- **Harmful Effects:** Excess acetate can promote de novo lipogenesis in the liver, contributing to hepatic fat accumulation.

b) Lipopolysaccharides (LPS) – Key Triggers of Inflammation

LPS, a component of Gram-negative bacterial cell walls, plays a major role in NAFLD-associated inflammation:

- LPS translocation into the portal vein activates **TLR4 signaling** in Kupffer cells and hepatocytes, leading to the production of pro-inflammatory cytokines (e.g., TNF-α, IL-6).
- Chronic LPS exposure induces **low-grade systemic inflammation**, insulin resistance, and oxidative stress, accelerating NAFLD progression.

c) Bile Acids and Their Impact on NAFLD

Bile acids (BAs) are synthesized in the liver and metabolized by gut microbiota, regulating lipid digestion, metabolism, and inflammation:

- **Primary BAs** (cholic acid and chenodeoxycholic acid) are converted into **secondary BAs** (deoxycholic acid and lithocholic acid) by gut bacteria.
- Disrupted BA metabolism alters **farnesoid X receptor (FXR) and Takeda G-protein receptor 5 (TGR5) signaling**, leading to increased lipogenesis, reduced insulin sensitivity, and enhanced inflammation in NAFLD.

d) Microbial Ethanol and Its Contribution to Liver Injury

Certain gut bacteria (e.g., Escherichia coli and Klebsiella) produce endogenous ethanol, which can exacerbate liver damage:

- Ethanol metabolism generates **reactive oxygen species (ROS)** and promotes oxidative stress, contributing to hepatocyte injury.
- Increased ethanol production correlates with gut dysbiosis and worsens NAFLD-associated inflammation.

miRNA and NAFLD

MicroRNAs (miRNAs) are small, non-coding RNAs that regulate gene expression at the post-transcriptional level by binding to target mRNAs and inhibiting their translation or promoting their degradation. In NAFLD, miRNAs play a crucial role in lipid metabolism, inflammation, and fibrosis, influencing disease progression and serving as potential diagnostic biomarkers and therapeutic targets.

1. miRNAs in Lipid Metabolism

Hepatic lipid homeostasis is tightly regulated by miRNAs, which control fatty acid synthesis, oxidation, and lipoprotein metabolism. Several key miRNAs contribute to lipid accumulation in NAFLD:

- **miR-122:** The most abundant liver-specific miRNA, miR-122, regulates cholesterol and fatty acid metabolism. It enhances lipid storage by targeting enzymes involved in fatty acid oxidation and reducing lipoprotein secretion. Downregulation of miR-122 in NAFLD promotes hepatic lipid accumulation and inflammation.
- **miR-34a:** Upregulated in NAFLD, miR-34a inhibits SIRT1 (sirtuin 1), leading to increased lipogenesis and insulin resistance.
- **miR-33a/b:** These miRNAs regulate cholesterol homeostasis by targeting ATP-binding cassette transporters (ABCA1, ABCG1) and inhibiting fatty acid oxidation.

2. miRNAs in Inflammation

Chronic inflammation is a key driver of NAFLD progression, and several miRNAs modulate immune responses:

- **miR-155:** A pro-inflammatory miRNA that activates macrophages and Kupffer cells by targeting suppressor of cytokine signaling 1 (SOCS1), leading to increased TNF-α and IL-6 production.
- **miR-146a:** Acts as an anti-inflammatory regulator by inhibiting NF-κB signaling, reducing pro-inflammatory cytokine secretion.
- **miR-21:** Elevated in NAFLD, miR-21 promotes inflammation and fibrosis by inhibiting PTEN (phosphatase and tensin homolog), thereby activating the PI3K/Akt pathway.

3. miRNAs in Fibrosis

The progression from simple steatosis to fibrosis involves hepatic stellate cell (HSC) activation and excessive extracellular matrix (ECM) deposition. Fibrosis-related miRNAs include:

- **miR-29 family (miR-29a/b/c):** These miRNAs suppress collagen synthesis by targeting key fibrogenic genes (COL1A1, COL3A1). Their downregulation in NAFLD promotes liver fibrosis.
- **miR-200 family:** Regulates epithelial-mesenchymal transition (EMT) and HSC activation, contributing to fibrosis progression.
- **miR-21:** Apart from its role in inflammation, miR-21 also promotes fibrosis by enhancing TGF-β/SMAD3 signaling, leading to HSC activation.

miRNAs play essential roles in NAFLD pathogenesis by regulating lipid metabolism, inflammation, and fibrosis. miR-122, in particular, is a key regulator of hepatic metabolism and a promising therapeutic target. Understanding the molecular mechanisms of miRNA involvement in NAFLD may pave the way for novel miRNA-based diagnostic and therapeutic strategies.

Interactions Between Microbiota and miRNA in NAFLD

The bidirectional relationship between gut microbiota and miRNAs plays a crucial role in NAFLD pathogenesis. Gut microbiota influence hepatic and intestinal miRNA expression through microbial metabolites and inflammation,

while miRNAs modulate microbiota composition and gut-liver signaling. Understanding these interactions provides new insights into disease mechanisms and potential therapeutic targets.

1. How Microbiota Regulate miRNA Expression

Gut microbiota affect miRNA expression in the liver and intestines through various mechanisms, including microbial metabolites, inflammation, and epigenetic modifications.

a) Microbial Metabolites and miRNA Regulation

Microbiota-derived metabolites such as short-chain fatty acids (SCFAs), bile acids, and lipopolysaccharides (LPS) influence miRNA expression, thereby affecting lipid metabolism, inflammation, and fibrosis in NAFLD:

- **SCFAs (Butyrate, Propionate, Acetate):** SCFAs produced by gut bacteria modulate histone acetylation and methylation, affecting miRNA transcription. Butyrate, for example, increases **miR-122** expression, improving lipid metabolism, while propionate regulates **miR-34a**, reducing inflammation.
- **Bile Acids:** Microbiota-mediated bile acid metabolism influences FXR and TGR5 signaling, which in turn regulate miRNA expression. FXR activation modulates **miR-144** and **miR-192**, affecting lipid metabolism and insulin sensitivity.
- **LPS and Endotoxins:** Dysbiosis-induced LPS production activates TLR4/NF-κB signaling, altering miRNA profiles. Increased **miR-155** expression promotes inflammation, while downregulated **miR-29a** enhances fibrosis.

b) Microbiota-Induced Inflammation and miRNA Modulation

Chronic inflammation in NAFLD is closely linked to dysbiosis and miRNA dysregulation:

- **TLR Signaling Activation:** LPS-TLR4 activation leads to upregulation of pro-inflammatory miRNAs such as **miR-21** and **miR-146a**, promoting hepatic inflammation and fibrosis.
- **Cytokine-Induced miRNA Changes:** TNF-α and IL-6 influence miRNA expression, exacerbating lipid accumulation and immune dysregulation.

2. How miRNAs Influence Microbiota Composition and Gut-Liver Signaling

miRNAs not only respond to microbial signals but also shape gut microbiota composition and function, influencing intestinal permeability, immune responses, and bile acid metabolism.

a) miRNAs Regulate Gut Barrier Function

The integrity of the intestinal barrier is crucial in preventing bacterial translocation and inflammation in NAFLD. miRNAs modulate tight junction proteins and mucosal immunity:

- **miR-122 and miR-21:** Regulate epithelial integrity by targeting tight junction proteins (e.g., occludin, ZO-1). Downregulation of miR-122 weakens the gut barrier, allowing LPS leakage into the portal circulation.
- **miR-223:** Maintains intestinal homeostasis by suppressing inflammatory responses and controlling gut microbiota composition.

b) miRNAs Shape Microbiota Composition

Host-derived miRNAs can be transported into the gut lumen via exosomes, directly influencing bacterial populations:

- Studies show that fecal miRNAs, such as **miR-146a** and **miR-155**, selectively regulate the growth of commensal and pathogenic bacteria.
- miR-515 and miR-328 affect microbial gene expression, altering bile acid metabolism and SCFA production.

c) miRNA-Mediated Regulation of Bile Acid Metabolism

Bile acids act as key signaling molecules in the gut-liver axis, influencing both microbiota composition and metabolic pathways:

- **miR-194 and miR-192** regulate bile acid transporters and FXR signaling, indirectly shaping the microbiome.
- miRNA-mediated changes in bile acid pools affect the growth of bile-acid-metabolizing bacteria, reinforcing a feedback loop between microbiota and liver metabolism.

Conclusion

Non-alcoholic fatty liver disease (NAFLD) is a complex disorder driven by metabolic dysregulation, chronic inflammation, and fibrosis, with gut microbiota and microRNAs (miRNAs) playing central roles in its pathogenesis. The gut-liver axis serves as a critical communication network, where microbiota-derived metabolites influence hepatic lipid metabolism and immune responses, while miRNAs regulate gene expression in both the liver and intestines.

Dysbiosis, characterized by an imbalance in microbial composition, contributes to NAFLD progression through mechanisms such as increased intestinal permeability, lipopolysaccharide (LPS)-induced inflammation, and alterations in bile acid metabolism. At the same time, miRNAs, particularly **miR-122**, **miR-34a**, **miR-21**, and **miR-29**, modulate key pathways in lipid homeostasis, inflammation, and fibrosis. Moreover, recent evidence suggests a bidirectional relationship between microbiota and miRNAs, where microbial metabolites shape miRNA expression, and host miRNAs influence microbiota composition and gut-liver signaling.

A deeper understanding of these interactions offers promising avenues for therapeutic interventions in NAFLD. Strategies such as **probiotics, prebiotics, fecal microbiota transplantation (FMT), and miRNA-based therapies** could potentially restore gut-liver homeostasis and mitigate disease progression. Future research should focus on uncovering specific microbiota-miRNA networks and developing personalized treatment approaches targeting these molecular pathways.

By integrating insights from microbiota and miRNA research, we can move closer to effective diagnostic tools and therapeutic strategies for NAFLD, ultimately improving patient outcomes and reducing the burden of this increasingly prevalent liver disease.

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