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NAFLD: Basic Pathogenetic Mechanisms and Diagnosis

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Abstract: Nonalcoholic fatty liver disease (NAFLD), now termed metabolic-associated fatty liver disease (MAFLD), stands as one of the most prevalent liver diseases globally, closely intertwined with the escalating obesity epidemic. Despite its increasing prevalence, there exists a conspicuous absence of pharmacological treatments tailored specifically for NAFLD. This gap in therapeutic options can be attributed to the multifaceted nature of NAFLD, characterized by an incomplete understanding of its underlying mechanisms, a dearth of accurate and affordable imaging tools, and the inadequacy of non-invasive biomarkers for effective diagnosis and monitoring.

Moreover, this review elucidates the existing diagnostic modalities for NAFLD, emphasizing the burgeoning significance of non-coding RNAs as promising diagnostic biomarkers. The imperative need for non-invasive biomarkers, coupled with accurate and cost-effective diagnostic tools, cannot be overstated, as they are pivotal in detecting early signs of NAFLD progression. These advancements hold the promise of expediting clinical trials and validating emerging therapeutic treatments, thus paving the way for improved management strategies for NAFLD patients.

Keywords: non-alcoholic fatty liver disease (NAFLD); metabolic-associated fatty liver disease (MAFLD); non-alcoholic steatohepatitis (NASH); metabolic-associated steatohepatitis (MASH); liver; biomarkers.

Introduction:

Nonalcoholic fatty liver disease (NAFLD) stands as one of the foremost causes of liver diseases globally, with its prevalence on a relentless upward trajectory [1]. The economic burden imposed by NAFLD is staggering, surpassing €35 billion annually in four major European countries and exceeding $100 billion in the United States alone [2]. NAFLD, a spectrum of liver disorders occurring in the absence of significant alcohol consumption, has recently undergone a nomenclature change to metabolic-associated fatty liver disease (MAFLD) to better reflect its nature as a distinct metabolic disorder [3]. However, for clarity within this review, the term NAFLD will be utilized.

NAFLD encompasses hepatic steatosis characterized by more than 5% of liver weight comprised of fat, with the potential to progress to nonalcoholic steatohepatitis (NASH), marked by inflammation, cellular damage, and heightened severity [4]. Notably, NAFLD constitutes a leading cause of hepatocellular carcinoma (HCC) and necessitates liver transplantation in severe cases [4]. Furthermore, its ramifications extend beyond hepatic confines, encompassing cardiovascular complications and linking with other metabolic disorders such as obesity and type 2 diabetes (T2D) [5,6].

The prevalence of NAFLD has surged in tandem with the obesity epidemic, afflicting approximately 24% of the general populace [6]. Its prevalence is particularly pronounced among individuals with obesity and T2D, affecting up to 70% of overweight individuals and over 90% of those classified as morbidly obese [7]. Alarmingly, NAFLD can also manifest in lean individuals, with ethnic disparities further complicating its prevalence and presentation [9-12].

Children and adolescents haven't been spared from this epidemic, as evidenced by the escalating incidence of NAFLD in this demographic [13]. Despite its burgeoning prevalence and significant healthcare burden, NAFLD remains devoid of specific pharmacological therapies. This therapeutic

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lacuna can be attributed to the multifaceted nature of NAFLD, characterized by a limited understanding of its pathogenic mechanisms and the absence of precise non-invasive biomarkers for diagnosis and monitoring.

This review aims to elucidate the intricate mechanisms underpinning NAFLD pathogenesis, evaluate current diagnostic modalities, explore sirtuins (SIRTs) as potential therapeutic targets, and underscore the emerging role of non-coding RNAs as diagnostic biomarkers for NAFLD. By addressing these key facets, this review seeks to contribute to the ongoing discourse surrounding NAFLD management and spur advancements in diagnostic and therapeutic strategies.

Pathogenesis of NAFLD

2.1. Liver Cells and NAFLD

The liver assumes a pivotal role in lipid metabolism, serving as a hub for lipid uptake, synthesis, oxidation, and distribution to peripheral tissues. Within the liver, hepatocytes constitute the predominant cell population, accounting for approximately 78% of the total cell population [14]. Complementing hepatocytes, non-parenchymal cells such as liver sinusoidal endothelial cells (LSECs), Kupffer cells (KCs), hepatic stellate cells (HSCs), and hepatic natural killer (NK) cells collectively contribute to the intricate landscape of hepatic function [14,15].

While hepatocytes are primarily implicated in lipid metabolism, KCs emerge as key orchestrators of liver inflammation [4,5]. As resident macrophages comprising roughly 30% of sinusoidal cells [16] and representing 80% to 90% of macrophages in the human body [7,8], KCs wield substantial influence over the pathogenesis of NAFLD. Upon encountering liver injury, KCs undergo activation, culminating in the release of proinflammatory cytokines and chemokines that exacerbate NAFLD progression [8]. Notably, the balance between proinflammatory M1 and anti-inflammatory M2 phenotypes of KCs intricately regulates liver inflammation [9].

The liver serves as a nexus for the interaction between various substances, including nutrients and gut-derived bacterial products, coursing through the portal circulation, which are effectively managed by KCs [2]. Moreover, KCs assume a pivotal role in the production of an array of inflammatory mediators, encompassing tumor necrosis factor-alpha (TNF-α), interleukin (IL)-1β, IL-6, IL-12, IL-18, and various chemokines [8].

HSCs, typically quiescent under physiological conditions, undergo activation in response to inflammation and hepatocyte injury induced by lipotoxicity, assuming a myofibroblast-like phenotype and augmenting collagen secretion, thereby driving fibrosis [11,12]. Although the precise contribution of LSEC lipotoxicity to NAFLD progression remains elusive, emerging evidence suggests that LSEC lipotoxicity precipitates reductions in nitric oxide levels coupled with escalated reactive oxygen species (ROS) production, thereby instigating oxidative stress and fostering the progression to nonalcoholic steatohepatitis (NASH) [3].

Despite considerable advancements in our understanding, the nuanced interplay between parenchymal and non-parenchymal liver cells in NAFLD pathogenesis and the intricate signaling cascades governing disease progression continue to evolve, warranting further exploration [8,12]

2.2. Mechanisms for NAFLD Pathogenesis

The prevailing model elucidating the inflammation development and progression of NAFLD is the "multiple hits" model, which posits the involvement of various stressors [4,5] (Figure 1). Despite advancements in understanding hepatic steatosis development, elucidating the pathogenesis of nonalcoholic steatohepatitis (NASH) remains incomplete. Influential factors in NASH progression encompass lipotoxicity, endoplasmic reticulum (ER) stress, mitochondrial dysfunction, oxidative stress, gut-derived endotoxins, and alterations in the gut microbiota composition [12,15]. Lipid overload can incite lipotoxicity, fostering inflammation, oxidative stress, and fibrosis (Figure 1). The adoption of a Western lifestyle, characterized by high-calorie diets and sedentary behavior, stands as a pivotal factor in NAFLD development. The inundation of free fatty acids (FFAs) can prompt

uncoupling of respiration from adenosine triphosphate (ATP) production, potentially engendering excessive reactive oxygen species (ROS) production and exacerbating NASH [8] (Figure 1).

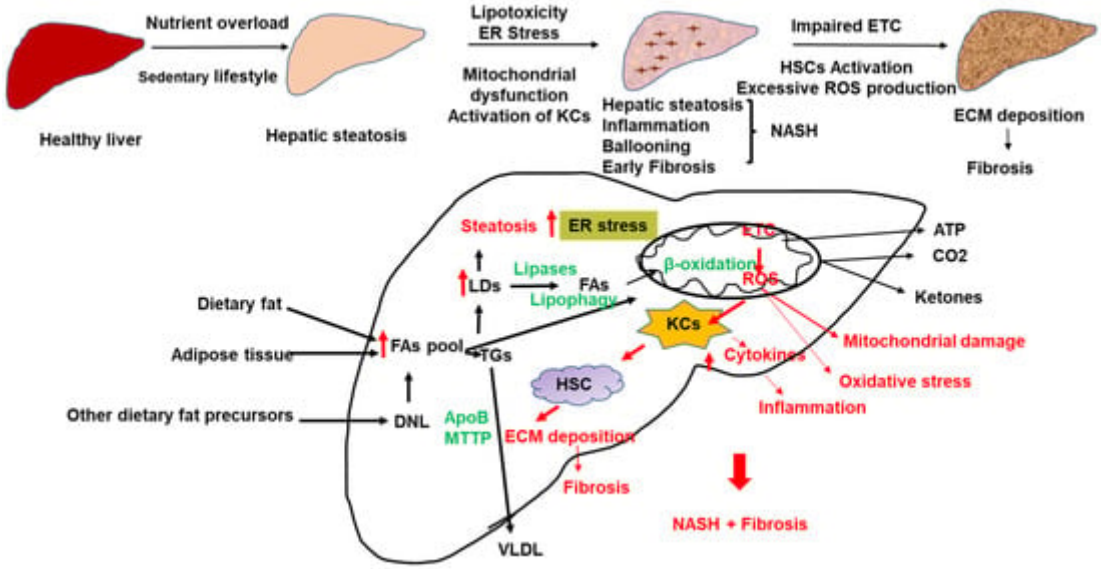

Figure 1. Pathogenic Pathways Involved in NAFLD

Top Panel: A schematic representation of the progression of NAFLD and the factors involved. Hepatic steatosis arises from nutrient overload and sedentary lifestyles. Multiple factors contribute to inflammation and NASH, ultimately leading to fibrosis.

Bottom Panel: Mechanisms for the development and progression of NAFLD. The pool of fatty acids (FAs) in the liver originates from dietary fat, adipose tissue lipolysis, or de novo lipogenesis (DNL) from carbohydrates or other dietary precursors. Within the liver, FAs undergo esterification into triglycerides (TG) and assembly into very-low-density lipoprotein (VLDL) for secretion into circulation, oxidation in the mitochondria (β-oxidation), or storage in lipid droplets (LDs) (<5% of liver weight). LDs undergo lipid hydrolysis (via lipolysis and lipophagy) during fasting to provide FAs for β-oxidation. In NAFLD, chronic nutrient overload and insulin resistance lead to an imbalance where the influx of FAs to the liver exceeds their disposal via VLDL secretion or β-oxidation. This lipotoxicity results in impaired LDs lipolysis and increased lipid accumulation in LDs, precipitating hepatic steatosis (>5% of liver weight). Hepatic steatosis triggers endoplasmic reticulum (ER) stress, oxidative stress, and activation of Kupffer cells (KCs) to produce inflammatory cytokines, fostering inflammation. Additionally, lipotoxicity induces mitochondrial dysfunction and impairs electron transport chain (ETC) function, leading to reactive oxygen species (ROS) production. ROS, in turn, exacerbates mitochondrial damage, perpetuating NASH. Inflammatory cytokines and ROS activate hepatic stellate cells (HSCs) to produce excessive extracellular matrix, driving progressive fibrosis.

The accumulation of fat in the liver and the development of NAFLD entail an imbalance between FA delivery to the liver (from the diet, de novo lipogenesis (DNL), and adipose tissue lipolysis), lipid synthesis and oxidation, and triglyceride (TG) export out of the liver in the form of very-low-density lipoproteins (VLDLs). Both VLDL secretion and β-oxidation are initially elevated in NAFLD to compensate for increased FA influx to the liver; however, sustained FA influx leads to lipotoxicity, liver injury, and NASH. NASH patients exhibit lower VLDL secretion and reduced FA oxidation compared to individuals with fatty liver.

2.2.1. Dietary Fatty Acids

Dietary fatty acids (FAs) are absorbed from the small intestine, packaged into chylomicrons, and released into the bloodstream, where the majority is stored in adipose tissue, with a portion taken up by the liver. In the postprandial state, FAs in the liver originate from chylomicron-derived spillover FAs and remnants. Conversely, in the fasted state, FAs primarily stem from adipose tissue lipolysis. It has

been estimated that in patients with NAFLD, approximately 15% of liver FAs originate from the diet, 59% from circulation, and 26% from de novo lipogenesis (DNL). Furthermore, the composition of dietary FAs can influence hepatic fat accumulation.

2.2.2. De Novo Lipogenesis

Insulin resistance drives de novo lipogenesis (DNL) in NAFLD. DNL is a tightly regulated metabolic pathway in which cells convert excess carbohydrates, typically glucose, into FAs. Following glycolysis and the tricarboxylic acid (TCA) cycle, dietary glucose produces citrate in the mitochondria. Citrate is then transported to the cytosol, where it serves as a substrate for acetyl-CoA synthesis via ATP-citrate lyase. Acetyl-CoA is subsequently converted to malonyl-CoA by acetyl-CoA carboxylases (ACC), with ACC1 predominating in hepatic DNL regulation. ACC1 activity is modulated by phosphorylation via AMP-activated protein kinase (AMPK), and it is inhibited by malonyl-CoA and palmitoyl-CoA while activated by citrate. Malonyl-CoA also inhibits carnitine palmitoyl-CoA transferase 1 (CPT1), thereby impeding long-chain fatty acyl CoA import into mitochondria for β-oxidation. Fatty acid synthase (FASN) converts malonyl-CoA into palmitate, the initial product of DNL, which then undergoes elongation and desaturation reactions to yield complex FAs. Transcription factors such as sterol regulatory element-binding protein-1 (SREBP-1), carbohydrate responsive element-binding protein (ChREBP), and liver X receptors (LXRs) govern the expression of enzymes directly involved in DNL. Besides glucose, amino acids, and short-chain FAs like acetate and fructose also contribute to DNL.

2.2.3. Fatty Acid Uptake As mentioned above, 59% of FAs are derived from the circulation in NAFLD. The release of FAs from adipose tissue occurs under the control of adipose triglyceride lipase (ATGL), hormone-sensitive lipase, and monoglyceride lipase. Insulin resistance in obesity and NAFLD increases adipose tissue lipolysis and the release of FAs in the circulation. The liver takes up FAs from the circulation through both passive diffusion and active transport. Different proteins take part in FA uptake in the liver, including the FA translocase CD36, the FA transport proteins (FATPs), and the FA binding proteins (FABPs). CD36 is closely associated with the development of NAFLD. CD36 expression is increased in animal models, and in humans with NAFLD. CD36 knockout mice have normal rates of FA uptake compared to controls. However, upregulation of CD36 increases FA uptake in the liver, suggesting a role for the protein in pathogenic conditions. Liver fat content in morbidly obese patients is associated with increased liver CD36 mRNA and protein levels. The translocation of CD36 to the plasma membrane of the hepatocytes in NAFLD patients might be a key factor in the pathophysiology of hepatic steatosis. The uptake of FAs by the liver drives hepatic steatosis and, when excessive, might cause lipotoxicity and contribute to the progression of NAFLD. In addition to its role in FA uptake, CD36 might play other intracellular roles in lipid processing, such as VLDL secretion. FATPs also play a role in the uptake of FAs in the liver. FATP2 and FATP5 are the two major FATPs present in the liver. In mice, deletion of FATP2 or FATP5 decreased FA uptake in the liver. Overexpression of FATP2 increases FA uptake in human hepatoma cells. The level of FATP5 correlated inversely with histological features of MASH, including ballooning and fibrosis. Studies have shown that FATP5 expression is elevated in patients with less severe steatohepatitis but is reduced during advanced NASH. Nine different FABPs have been identified with different tissue distributions. FABP1 is the highly expressed FABP in the liver and mediates the transport, storage, and use of FAs and their acyl-CoA derivatives; FABP1 may exert a protective effect against lipotoxicity by facilitating FAs oxidation or their incorporation into TGs. Interestingly, FABP1 protein levels are upregulated in obese patients with steatosis, but it decreases in NASH with a further decrease in advanced fibrosis. Other studies have shown no relationship between FABP1 expression and steatohepatitis histology. The pool of FAs from the different pathways is then directed to LDs for storage as TGs, incorporated into lipoproteins and secreted into the circulation, used in β-oxidation, or used for posttranslational modifications.

2.2.4. Triglyceride Synthesis The glycerol-3-phosphate acyltransferase (GPAT) is the rate-limiting enzyme in the de novo pathway of TG synthesis. The glycerol-3-phosphate (G3P) pathway provides over 90% of the total TG synthesis. GPAT converts G3P and long-chain acyl-CoA to lysophosphatidic

acid (LPA). In the endoplasmic reticulum, the acylglycer-ol-3-phosphate acyltransferases (AGPAT) acylates LPA to form phosphatidic acid (PA). PA is dephosphorylated by phosphatidate phosphohydrolase (PAP, Lipin) to form diacylglycerol (DG). The DG acyltransferase (DGAT) catalyzes the conversion of DG to TG. Inhibition of DGAT2 in obese mice improved hepatic steatosis but aggravated liver damage and fibrosis, supporting the hypothesis that TGs have a protective role in the liver.

2.2.5. Lipoprotein Secretion

Lipoproteins comprise a lipid core rich in triglycerides (TGs) and cholesteryl esters, enveloped by a monolayer composed of phospholipids, free cholesterol, and apolipoproteins. Very-low-density lipoproteins (VLDL) are TG-rich lipoproteins primarily secreted by the liver, facilitating the transport of fatty acids to peripheral organs such as adipose tissue, muscle, and the heart. Mammals express two forms of apolipoprotein B (apoB): a long form (apoB100) predominantly synthesized in the human liver and a short form (apoB48) in the intestine and rodent liver. Apobec1, an mRNA editing enzyme, converts cytidine at position 6666 of full-length apoB mRNA to uracil, generating a premature stop codon in rodents' intestine and liver. This results in the translation of a truncated apoB48 protein, comprising the N-terminal 48% of apoB100. VLDL assembly occurs in two steps involving apoB and microsomal triglyceride transfer protein (MTTP). Initially, apoB is lipidated co-translationally by MTTP in the endoplasmic reticulum (ER), forming a small primordial particle. Further lipidation occurs through fusion with lipid droplets (LDs) in the ER, culminating in the formation of mature VLDL particles. VLDL secretion is intricately linked to ER stress and NAFLD progression. While moderate fatty acid exposure enhances apoB100 secretion, prolonged exposure induces ER stress, leading to apoB degradation and reduced secretion. Transport of VLDL from the ER to the Golgi involves specialized vesicles termed VLDL transport vesicles, comprising components like transmembrane 6 superfamily 2 (TM6SF2), surfeit 4 (SURF4), secretion-associated Ras-related GTPase 1B (SAR1B), and meningioma-expressed antigen 6 (Mea6). Clinical and epidemiological evidence implicates TM6SF2 in NAFLD development, as liver-specific deletion in mice leads to steatosis and reduced VLDL TGs. Moreover, TM6SF2 deficiency in mice promotes hepatic steatosis, fibrosis, and hepatocellular carcinoma (HCC).

Defective VLDL assembly and secretion are pivotal contributors to NAFLD pathogenesis. Genetic defects in apoB (hypobetalipoproteinemia) and MTTP (abetalipoproteinemia) impair VLDL secretion and precipitate hepatic steatosis. Rare mutations in apoB and MTTP are associated with progressive liver disease. Liver-specific deletion of MTTP induces hepatic steatosis and complete inhibition of VLDL and apoB secretion. Insulin suppresses hepatic lipid export by promoting apoB100 degradation and MTTP synthesis inhibition. In NAFLD, hepatic insulin resistance augments de novo lipogenesis (DNL) without reducing VLDL production [10]. While VLDL secretion increases in NAFLD patients, liver TG content directly correlates with VLDL-TG secretion rates [11]. However, once hepatic fat content exceeds 10%, VLDL TG secretion plateaus [12]. Notably, apoB synthesis rates are lower in NASH patients compared to lean or obese controls without NASH [82]. Thus, the liver's ability to balance lipid storage and VLDL secretion critically influences NAFLD outcomes

3. Current NAFLD Diagnosis Methods and Tools.

Liver fibrosis, reversible in its initial stages, stands as the most potent predictor of mortality in individuals with metabolic-associated steatohepatitis (MASH). Hence, accurate fibrosis staging and distinguishing NASH from early fibrosis are pivotal in identifying patients at risk of disease progression. A gamut of diagnostic methods, including both traditional and innovative tools such as imaging and biomarkers, are employed to diagnose and grade NAFLD, each with its merits and limitations.

Blood Transaminases: Liver function tests, notably blood transaminases, are widely conducted, yet their reliability in predicting NAFLD progression remains uncertain. Abnormal and normal liver enzyme levels have been observed in NAFLD patients, with decreased alanine aminotransferase (ALT) detected in advanced liver diseases. Various biomarker panels, including the Hepatic Steatosis

Index (HSI), Fatty Liver Index (FLI), Steatotest, and Liver Fat Score (LFS), have been utilized to assess liver fat.

Non-invasive Scoring Systems: Scoring systems like Fibrosis-4 (FIB-4), NAFLD Fibrosis Score (NFS), Hepamet Fibrosis Score (HFS), and Platelet Ratio Index (APRI) aid in detecting NAFLD progression risk but exhibit modest sensitivity in diagnosing early NASH and fibrosis stages. However, there's a notable discordance between these scoring systems when applied to the same patient [15].

Liver Biopsy: Despite its invasive nature, expense, sampling errors, and associated risks like bleeding and, albeit rare, death, liver biopsy remains the gold standard for NASH diagnosis. Histologically, NASH manifests as hepatic steatosis, ballooning, inflammation, with or without fibrosis [16,17]. Although liver biopsy distinguishes between NAFL and NASH, its limitations underscore the necessity for minimally invasive diagnostic alternatives.

Histological Scoring Systems: Commonly employed histological scoring systems include the NAFLD Activity Score (NAS) and the steatosis-activity-fibrosis (SAF) score. NAS, comprising steatosis, lobular inflammation, and hepatocellular ballooning scores, and SAF, integrating ballooning and lobular inflammation scores, demonstrate high concordance in diagnosing definite NASH [12].

In light of the preventive potential of early NASH detection to forestall fibrosis, ongoing efforts are directed towards developing minimally invasive imaging tools and biomarkers for NAFLD evaluation, progression risk assessment, and treatment validation in clinical settings.

Biomarkers for NAFLD

Cytokeratin 18 (CK18) Fragment Levels: Plasma CK18 fragment levels are widely utilized biomarkers for hepatocyte injury in NAFLD. However, their moderate accuracy and variability limit their diagnostic utility. Combining CK18 with other biomarkers like adiponectin, resistin, and IL-6 could enhance sensitivity and specificity [13,14]. Inflammation-related circulating markers, including cytokines, chemokines, shed receptors, and circulating exosomes, have also been proposed as NAFLD scoring tools, albeit requiring validation studies [15,16].

Imaging Techniques:

1. Ultrasound (US): Ultrasound serves as the frontline imaging modality for suspected NAFLD, showcasing a typical hyperechogenic liver appearance. However, its efficacy is limited to detecting moderate-to-severe steatosis (>20%) and can be influenced by severe fibrosis [17]. Novel approaches like computed-assisted US hepatic/renal ratio (H/R) and hepatic attenuation rate offer potential for early steatosis assessment [18].
2. Magnetic Resonance Spectroscopy (MRS): MRS stands out as the most accurate non-invasive method for quantifying liver fat, relying on proton signal separation to differentiate fat and water fractions. Magnetic resonance imaging proton density-derived fat fraction (MRI-PDFF) serves as a validated tool for liver fat evaluation, with a 30% relative reduction in liver fat content associated with histologic NASH improvement. However, limitations include patient discomfort, cost, and limited availability.
3. Transient Elastography (TE): TE, utilizing Fibroscan with an M probe, assesses liver fibrosis, while the controlled attenuation parameter (CAP) concurrently evaluates steatosis. The XL probe enhances assessment accuracy in obese individuals, yet limitations persist, particularly in predicting significant liver fibrosis in morbidly obese subjects [12,13]. Fibrotouch liver elastography emerges as a cost-effective, straightforward alternative for assessing fibrosis across all patients, regardless of obesity.
4. Magnetic Resonance Elastography (MRE): MRE estimates liver stiffness, offering accurate evaluation independent of BMI. However, its adoption is hindered by cost, availability, and examination time constraints.

Emerging Biomarkers: Non-coding RNA (ncRNA)

Overview of ncRNA: Non-coding RNAs (ncRNAs) encompass a diverse group of RNA transcripts that do not encode proteins. They include microRNAs (miRNAs) and long noncoding RNAs (lncRNAs), which play crucial roles in regulating cell physiology and functions through epigenetic gene silencing and post-transcriptional mRNA stability modulation. Abnormal expression of ncRNAs has been linked to various pathologies, including NAFLD, with exosome miRNAs recently gaining attention in NAFLD research.

Circulating miRNAs: Circulating miRNAs, such as miR-122, miR-34, miR-192, and miR-375, exhibit dysregulated expression in NAFLD, positively correlating with disease severity. Notably, miR-122, the most abundant liver-specific miRNA, shows significant upregulation in NAFLD patients' serum, suggesting its potential as a biomarker for NAFLD and its progression. Experimental evidence indicates that miR-122 inhibition suppresses lipid production and lipogenic gene expression, implicating its role in NAFLD pathogenesis [17]. Other miRNAs like miR-34c, miR-378, and miR-421 also modulate NAFLD by regulating pathways involving SIRT1, PPARα, NF-κB-TNFα axis, and oxidative stress.

Mitochondrial circRNAs (circRNAs): Mitochondrial circRNAs, like the steatohepatitis-associated circRNA ATP5B Regulator (SCAR), are downregulated in fibroblasts from NASH patients. SCAR exhibits a protective role by inhibiting mitochondrial ROS production and fibroblast activation, suggesting its potential as a therapeutic target [13].

Long Noncoding RNAs (lncRNAs): lncRNAs are implicated in oxidative stress-related liver diseases, including NAFLD progression [3,4]. For instance, lncRNA-AK044604 downregulation by thyroid-stimulating hormone (TSH) leads to increased mitochondrial stress via CypD acetylation, indicating its involvement in NAFLD pathogenesis [12]. lncRNAs may act as miRNA sponges, adding complexity to regulatory networks and contributing to disease pathogenesis [9].

Conclusions and Perspective

Understanding NAFLD Pathophysiology: Significant progress has been made in elucidating the pathophysiology of hepatic steatosis and non-alcoholic steatohepatitis (NASH). However, the transition from NASH to fibrosis, a critical determinant of mortality in NAFLD patients, remains poorly understood. This gap in knowledge underscores the urgency of further research to unravel the mechanisms driving NAFLD progression.

Role of Liver Biopsy: Liver biopsy remains the gold standard for assessing liver health in NAFLD patients. However, its invasive nature, sampling limitations, and potential errors underscore the need for alternative diagnostic tools.

Emerging Biomarkers and Tools: Identification of accessible, non-imaging tools and accurate biomarkers is crucial for advancing NAFLD management and validating emerging treatments in clinical trials. Non-invasive, inexpensive methods for accurately staging NAFLD progression are urgently needed to improve patient care.

Unmet Needs: Despite recent advancements, there is still an unmet need for reliable biomarkers and cost-effective, non-invasive tools to accurately stage NAFLD progression. Addressing these gaps will facilitate early diagnosis, risk stratification, and treatment monitoring in NAFLD patients.

In conclusion, while progress has been made in understanding NAFLD pathophysiology, significant challenges remain in translating this knowledge into effective treatments. Continued research efforts aimed at elucidating the mechanisms driving NAFLD progression and developing novel diagnostic and therapeutic strategies are essential to improve patient outcomes in this growing epidemic.

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