Association of m6A and ncRNAs with Liver Diseases

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Noncoding RNAs (ncRNAs), including circular RNAs (circRNAs) and N6-methyladenosine (m6A), have been shown to play a critical role in the development of various diseases including obesity and metabolic disorder-associated fatty liver disease (MAFLD). Obesity is a chronic disease caused by excessive fat accumulation in the body, which has recently become more prevalent and is the foremost risk factor for MAFLD. Causes of obesity may involve the interaction of genetic, behavioral, and social factors. m6A RNA methylation might add a novel inspiration for understanding the development of obesity and MAFLD with post-transcriptional regulation of gene expression. In particular, circRNAs, microRNAs (miRNAs), and m6A might be implicated in the progression of MAFLD. Interestingly, m6A modification can modulate the translation, degradation, and other functions of ncRNAs. miRNAs/circRNAs can also modulate m6A modifications by affecting writers, erasers, and readers. In turn, ncRNAs could modulate the expression of m6A regulators in different ways. However, there is limited evidence on how these ncRNAs and m6A interact to affect the promotion of liver diseases. It seems that m6A can occur in DNA, RNA, and proteins that may be associated with several biological properties.

N6-methyladenosine noncoding RNA

1. Introduction

Circular RNAs (circRNAs) are a class of single-strand closed RNA molecules [1]. They are generated by the reverse splicing process, which may be involved in the development of many diseases, including various liver diseases. Most circRNAs are non-coding RNAs (ncRNAs), while part of cytoplasmic circRNAs with coding capability can be translated into peptides, which may contribute to several important biological and/or physiological processes since many circRNAs originate from exons and reside in the cytoplasm [2]. Therefore, innovative research for circRNA-based therapeutic and/or diagnostic strategies has been conducted [3]. circRNAs are also observed in eukaryotic cells [4], whose expression pattern might enable them to support the clinical diagnosis as biological markers in many types of disease [5]. In particular, circRNAs are highly stable and easily detected in the circulation, which has been shown to be valuable as non-coding RNAs represent a promising non-invasive approach for predicting a non-alcoholic fatty liver disease (NAFLD) [6].

Generally, ncRNAs may lack the capability of translating into protein/peptide; however, ncRNAs can work as crucial transcripts to regulate the expression of other genes [7]. These ncRNAs may be typically divided into several groups, including microRNAs (miRNAs), long ncRNAs (lncRNAs), and circRNAs [8]. In addition, the n6-methyladenosine (m6A) modification in those ncRNAs has been increasingly described to have a dense

association with the pathological machinery of various diseases [9]. Several crucial regulators derived from m6A modification could affect the profound function of ncRNAs to take part in the initiation and/or progression of related diseases. Remarkably, ncRNAs can mediate downstream signaling to impact the expression of readers, writers, and/or erasers for the additional modification of m6A functions [9]. m6A RNA modification may highly regulate hepatic function and the development of liver diseases, providing some directions to understand the mechanism of malicious activities in the development of liver diseases [10] (**Figure 1**).

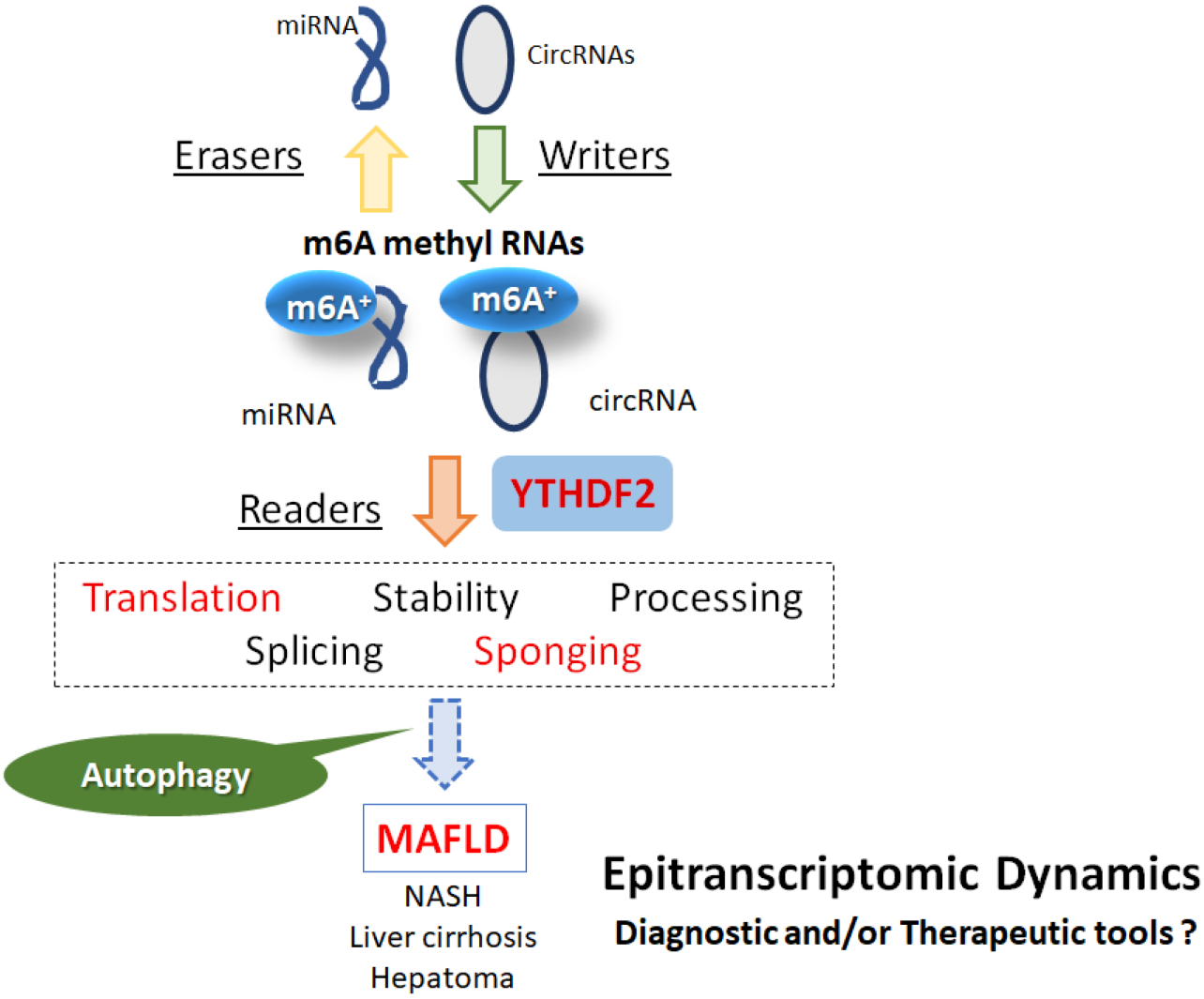

Figure 1. Schematic diagram for the association of noncoding RNAs and m6A methyl RNAs (m6A+) with biological function and liver diseases. In the beginning, m6A modification may be controlled by methyltransferase writers and demethylase erasers. m6A+ binding proteins, including YT521-B homology (YTH) domain-containing-2 (YTHDF2), are called readers, which have been suggested in several RNA activities including translation as well as sponging for the plausible alteration of autophagy. Consequently, m6A methylation in noncoding RNAs could be an important tool for diagnostics and/or therapeutics in several diseases including MAFLD.

m6A, the most prevalent modification associated with eukaryotic RNAs, can affect several steps of RNA metabolism, including translation, splicing, and the decay/stability of RNAs, which may be the epitranscriptomic

modification that occurs on the N6 position of adenosine [11]. It is the most abundant modification in eukaryotic RNAs, accounting for about 0.4% of all adenosines in RNAs, which may be involved in the functional alteration of RNAs [12]. Interestingly, m6A modification may be closely associated with the initiation and/or development of obesity and non-alcoholic fatty liver disease (NAFLD), which may progress to end-stage liver disease [13]. Obesity is the main risk factor for NAFLD. The feature of the disease is hepatic steatosis with the accumulation of surplus fat in the liver and metabolic liver dysfunction. Therefore, it has been suggested that NAFLD should be retitled as metabolic disorder-associated fatty liver disease (MAFLD). Here, researchers use the term MAFLD instead of NAFLD. MAFLD is often assumed to be asymptomatic. However, many MAFLD patients complain of exhaustion, which may disturb their quality of life (QOL). Currently, there are no specific pharmacotherapies for MAFLD. Mostly, the treatment may include lifestyle adjustments and medicines for improving fat metabolism and balancing oxidation. Therefore, further research and development of novel therapeutic tactics are required [13]. In addition, obesity is a chronic metabolic disease that is closely related to type 2 diabetes mellitus, cardiovascular diseases, and osteoarthritis [14]. The prevalence of obesity is increasing rapidly, which is considered to be a global public health burden [14]. However, the precise mechanism and role of m6A-modified ncRNAs in MAFLD are not well understood. m6A modification may play a role in the formation of specific and complexed microenvironments in the liver [15]. In fact, recent studies have shown that nucleotide methylation may be directly associated with the inflammatory grade, lipid synthesis, and/or oxidative stress, playing a crucial role in the progression of MAFLD [15]. Therefore, m6A modification may be a key player in the pathogenesis of MAFLD, which may provide new mechanistic insight into MAFLD [16]. Recent studies have explored the roles of m6A RNA methylation in the pathogenesis of liver diseases, providing new insights for studying the molecular mechanism of liver diseases [17]. Epigenetic modification in RNA has become the hotspot of the field, offering the potential of m6A as a treatment option for several liver diseases.

2. Association of m6A and ncRNAs with Various Liver Diseases

M6A may be one of the highly prevalent modifications in various mRNAs and/or ncRNAs that affect the epigenetic effects, which could play a critical role in cellular physiology and/or pathology. The effects of m6A are determined by reader (recognition), writer (methyltransferase), and eraser (demethylase) molecules [18] (**Figure 1**). Examples of writers are special methyltransferases, including Wilms tumor 1 associated protein (WTAP), methyltransferase-like 3 (METTL3), Vir-like m6A methyltransferase-associated (VIRMA), and methyltransferase-like 14 (METTL14). The AlkB homolog 5 (ALKBH5) and fat mass and obesity-associated protein (FTO) are known examples of erasers, which are special demethylases that can reverse m6A methylation. m6A modifications are regularly recognized by m6A-binding reader molecules, including YT521-B homology (YTH) domain-containing-1 and -2 (YTHDC-1 and -2) and YTH domain family proteins 1-3 (YTHDF1-3). In addition, the heterogeneous nuclear ribonucleoprotein (HNRNP) family, including HNRNPC and heterogeneous nuclear ribonucleoprotein A2/B1 (HNRNPA2B1), may have also a YTH domain for binding the m6A site of m6A modified RNAs [19]. It has been suggested that eukaryotic initiation factor 3 (eIF3) may also initiate the translation in a cap-independent manner by binding to the m6A sites in the 5′-UTR of mRNAs, while insulin-like growth factor 2 mRNA-binding protein 1/2/3

(IGF2BP1/2/3) can increase the stability of the target RNAs [19]. m6A modification may play an essential role in the proliferation and/or differentiation of hepatocytes, which are essential to the liver response to injury and regeneration. For example, m6A content might be decreased in patients with type 2 diabetes, and mRNA expression levels of FTO, METTL3, METTL14, and WTAP seem to be enhanced [20]. Remarkably, m6A content may be negatively associated with the expression level of METTL3, METTL14, and FTO mRNA, which could play an important role in the proliferation and/or differentiation of hepatocytes. High glucose can also upregulate the protein expression of FTO in HepG2 cells [20]. In addition, the FTO could decrease the concentration of m6A in RNA transcripts, thereby regulating the expression of related transcripts [21]. It has been demonstrated that m6A modification has an impact on the regulation of carboxylesterase 2 (CES2), a serine esterase responsible for the hydrolysis of endogenous substrates, including triglycerides and diacylglycerides, affecting lipid metabolism [22]. Furthermore, the phosphorylation of p70 ribosomal subunit 6 kinase (S6K) and/or mammalian target of rapamycin (mTOR) may be reduced by the knockout or knockdown of WTAP [23].

Circular RNAs (circRNAs) can also play a part in physiological and/or pathological processes through a similar pathway as a new type of molecule with intriguing functions [24], which may represent a favorable non-invasive approach for predicting MAFLD [6]. In addition, proteins encoded by circRNAs have been confirmed to be related to multiple pathophysiological processes, including immunity [25]. m6A modifications can regulate the metabolism of circRNAs. It has been shown that circRNAs containing m6A residues can be translated into protein by non-cap-dependent structures [26]. m6A-modified circRNA might be recognized by the YTHDF2 [27], in which the open reading framework (ORF) may be verified for the translational capability of the circRNA. Several effects of the peptide/protein from circRNA translation have been identified [28]. FTO demethylase could diminish the rate of circRNA translation, but METTL3 methyltransferase can boost the translation rate [29].

Remarkably, these proteins may also be significant players in the development of MAFLD. In particular, m6A methyltransferase is known to exert regulatory functions in liver-related diseases [30]. The liver is a vital metabolic and digestive organ in the pathophysiological processes. Recent studies have suggested that m6A RNA modification can regulate hepatic function and the development of liver diseases [10][31]. For example, METTL14 can modulate the expression of miR-34a-5p, impairing mitochondrial homeostasis in MAFLD [31]. In addition, many of these epigenetic factors are amenable to dietary or lifestyle interventions. For example, several interventions may include diets low in carbohydrates, free sugars, fructose, and lipids, in addition to healthy eating patterns and probiotics [32]. At diverse levels, the nutritional and metabolic status of individuals may interact with epigenetics, which are chemical modifications that affect gene expression without altering DNA sequences [33]. Therefore, it is important to understand how researchers can control epigenetic characteristics through both lifestyle habits and some clinical interventions. Misbalanced epigenetic regulation can result in various metabolic diseases and/or aging [33]. Epigenetic modifications are potentially reversible and can provide new therapeutic avenues for treating these diseases using epigenetic modulators [34]. Many studies have shown that m6A modification is closely related to the pathology of MAFLD [35]. Recently, studies have found that the regulation of immune cells by m6A modification may also play a role in MAFLD.

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