Review

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RNA-binding proteins in metabolic-associated fatty liver disease (MAFLD): From mechanism to therapy

Jiawei Xu[1,§], Xingyu Liu[1,§], Shuqin Wu[1], Deju Zhang[2], Xiao Liu[3], Panpan Xia[4], Jitao Ling[4], Kai Zheng[5], Minxuan Xu[4], Yunfeng Shen[4], Jing Zhang[1,6,*], Peng Yu[1,4,*]

[1] The Second Clinical Medical College / The Second Affiliated Hospital of Nanchang University, Nanchang, Jiangxi, China;
[2] Food and Nutritional Sciences, School of Biological Sciences, The University of Hong Kong, Hong Kong, China;
[3] Department of Cardiology, The Second Affiliated Hospital of Sun Yat-Sen University, Guangzhou, Guangdong, China;
[4] Department of Endocrinology and Metabolism, The Second Affiliated Hospital of Nanchang University, Nanchang, Jiangxi, China;
[5] Medical Care Strategic Customer Department, China Merchants Bank Shenzhen Branch, Shenzhen, Guangdong, Guangdong, China;
[6] Department of Anesthesiology, The Second Affiliated Hospital of Nanchang University, Nanchang, China.

SUMMARY Metabolic-associated fatty liver disease (MAFLD) is the most common chronic liver disease globally and seriously increases the public health burden, affecting approximately one quarter of the world population. Recently, RNA binding proteins (RBPs)-related pathogenesis of MAFLD has received increasing attention. RBPs, vividly called the gate keepers of MAFLD, play an important role in the development of MAFLD through transcription regulation, alternative splicing, alternative polyadenylation, stability and subcellular localization. In this review, we describe the mechanisms of different RBPs in the occurrence and development of MAFLD, as well as list some drugs that can improve MAFLD by targeting RBPs. Considering the important role of RBPs in the development of MAFLD, elucidating the RNA regulatory networks involved in RBPs will facilitate the design of new drugs and biomarkers discovery.

Keywords metabolic-associated fatty liver disease (MAFLD), non-alcoholic fatty liver disease (NAFLD), RNA-binding protein (RBP), non-coding RNA, RNA-RBP interaction

1. Introduction

In view of many shortcomings in the term of non-alcoholic fatty liver disease (NAFLD), the international expert panel in 2020 proposed to uniformly rename it as metabolism related fatty liver disease (MAFLD), and further give MAFLD a new and detailed definition (*1*). The diagnostic methods for MAFLD are updated as follows: patients have liver steatosis and metabolic dysfunction at the same time. Patients with metabolic dysfunction meet one of the following three standards, including overweight/obesity, Type 2 diabetes or metabolic disorder (*1,2*). The global prevalence rate of MAFLD is up to 25%, and it is now listed as the most common liver disease and a major threat to human health (*3*). The metabolic defects of MAFLD may also lead to its progression to nonalcoholic steatohepatitis (NASH), liver fibrosis and cirrhosis (*4*). Furthermore, it may eventually evolve into hepatocellular carcinoma (HCC) and liver failure (*5*). In addition, the metabolic syndrome of MAFLD is very similar to obesity, which can lead to multiple complications through different extrahepatic pathways, such as diabetes, cardiovascular disease and hypertension (*6*). The high prevalence, increased risk of death and coexistence of multiple complications all indicate that MAFLD-related liver damage and its complications will pose a significant health and economic burden to patients, their families and society. Therefore, it is necessary to explore effective therapeutic approaches for the treatment of MAFLD.

RNA binding proteins (RBPs) are involved in coordinating (ribonucleic acid) RNA processing and post transcriptional gene regulation (PTGR) as well as the maturation, localization, stabilization and translation of coding and non-coding RNAs (*7*). The loss of RBP function or functional mutation will destroy homeostasis, leading to various diseases, particularly metabolic diseases, which also involves MAFLD (*8,9*). For instance, studies have shown that in cytoplasm rich liver tissues, an RBP called Human antigen R (HuR) interacts with mRNAs that are involved in lipid transport/metabolism, cholesterol metabolism or endoplasmic reticulum (ER) stress response pathway (*10*). HuR dysfunction will cause a series of liver lipid metabolism

disorders, which gradually evolves into MAFLD. A MAFLD mouse model suggests that Quaking I-5 (QKI 5) mediated by Sirtuin 1 (SIRT1) significantly affected the synthesis of triglyceride (TG) in the liver of the MAFLD mouse model. Compared with the control group, the expression level of QKI 5 in MAFLD mice decreased (*11*). Therefore, we think it would be better to clarify the pathogenesis of MAFLD through discussing the roles of RBPs in the development of MAFLD.

Although numerous studies have elaborated the role and mechanism of RBPs in liver diseases, especially in non-alcoholic fatty liver diseases, there is a lack of knowledge summary on this subject at present. Our article reviews the mechanism and complications of different RBPs in MAFLD and some drugs that can improve MAFLD by regulating RBPs, aiming to provide some reference for the pharmacological targeted MAFLD treatment strategies that are based on RBPs regulatory network.

2. RBPs

2.1. Structure and Function

PTGR is essential for the maintenance of cell homeostasis and the coordination of the maturation, transport, stabilization, and degradation of coding and non-coding RNAs (*7*). RBP is a key regulator of PTGR that can interact with multiple RNAs and individual transcripts and has the potential to interfere with a large number of regulatory networks and maintain the integrity of cells (*12*). In RBP, there is an RNA-binding domain (RBD), a functional unit responsible for RNA binding existing in the coding sequence (intron and exon domains), 5' untranslated regions (5'UTR) and 3' untranslated regions (3' UTR) of RNA (*13*). RBPs can be divided into conventional and non-conventional RBPs two groups based on their RBD. Of these, the RBDs of conventional RBPs consist of an RNA recognition motif (RRM), cold-shock domain (CSD), K homology (KH) domain, arginine-glycine-glycine (RGG) motif and zinc-finger domains (*14*), while non-conventional RBPs bind with RNAs through intrinsically disordered regions lacking RBDs (*15*) (Figure 1). Normally multiple RBDs coexist in one RBP and the arrangement of these RBD modules can coordinate and enhance specific binding with RNA (*16*). As many RBDs are small and have few residues as effective parts, they often achieve specific binding with RNA in the form of Hydrogen Bonds, Van der Waals Interactions, Hydrophobic, and p Interactions and Stacking (*17*). The adaptor between RBDs can mediate RNA contacts, by which whether RBDs work independently or cooperatively can be determined (*18*). Some RBDs can also act as the mediator for Protein-Protein Interaction (*19*). RNA Recognition Motif, present in about 1% of all human proteins, is the most extensively seen and studied RBD (*20*). RBD also includes K Homology, Zinc Finger, and Pumilio Homology Domain, which all perform their own duties in RNA metabolism.

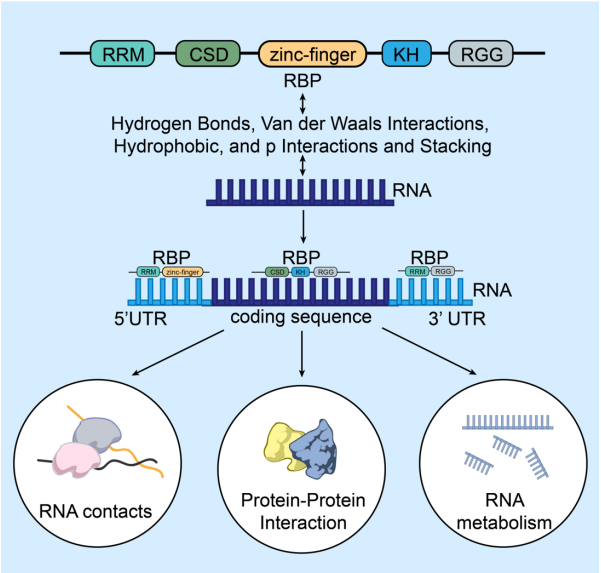

Figure 1. The structure and function of RNA binding proteins. Based on numerous studies, RBP can bind to RNA and the binding of RBP to RNA is via the RBDs in RBP. Depending on the presence or absence of RBD, RBP can be divided into conventional RBPs and non- conventional RBPs. And the RBDs of conventional RBP consist of RRM, CSD, KH, RGG motif and zinc-finger domains, while non-conventional RBP does not have RBDs. RBDs bind with RNA by the forces of Hydrogen Bonds, Van der Waals Interactions, Hydrophobic, and p Interactions and Stacking. With multiple RBDs coexisting of a single RBP, the modules are arranged to coordinate and enhance specific binding to the coding sequence, 5'UTR and 3'UTR of RNA. Eventually, RBP binds to RNA and performs the function of RNA contacts, protein-protein interaction and RNA metabolism.

2.2. The Molecular mechanisms of RBPs in PTGR

The abundance of RBDs allows RBPs to bind to other biomolecules in a variety of ways, which gives RBPs an important role in PTGR. Of them, the molecular mechanisms of RBPs involved in PTGR can be summarized into 3 levels (Figure 2): RBP-RNA, RBP-RBP and RBP-protein-RNA.

2.2.1. RBP-RNA

As a regulator of biological cytology, RBPs are involved in various aspects of RNA regulation, including transcription regulation (*21*), AS (alternative splicing) (*22*), alternative polyadenylation (*23*), stability (*24*), and subcellular localization (*25*). Disturbing levels of RBPs in specific microenvironments or mutations in coding genes can lead to serious diseases. Several serious maladies can be caused when disorder of the RBPs levels in specific microenvironments or mutation of coding genes occur. For example, as an important splicing factor required by AS, serine/arginine splicing factors have a strictly regulated process of phosphorylation, which

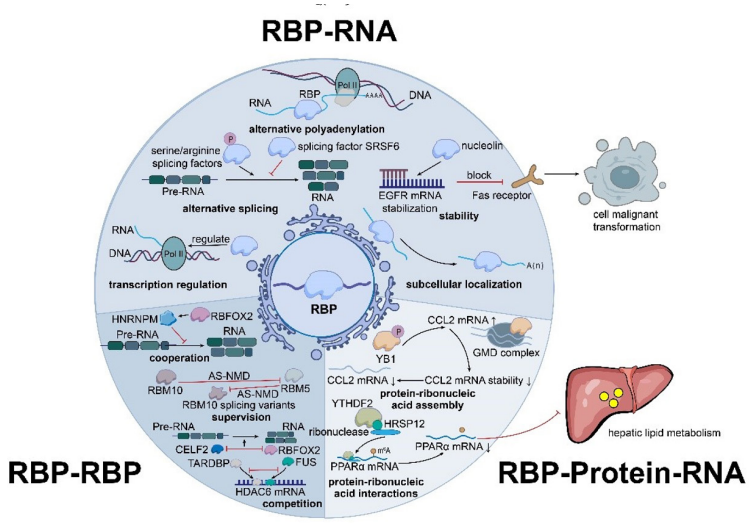

Figure 2. The mechanisms of RNA binding proteins functions. According to many studies, RBPs can bind to various biomolecules to produce different functions. The mechanisms involved in these functions can be summarized as: RBP-RNA, RBP-RBP, and RBP-Protein-RNA. In the RBP-RNA mechanism, RBPs regulate biological cytology and various aspects of RNA through transcription regulation, alternative splicing, alternative polyadenylation, stability and subcellular localization. Meanwhile, RBPs have a cooperation, supervision and competition function with each other at the RBP-RBP level. In the meantime, RBPs also affect hepatic lipid metabolism by regulating and modifying protein-ribonucleic acid assembly and protein-ribonucleic acid interactions. In general, RBP can produce important cellular physiological functions through the three mechanisms described above, and interference with their normal functioning may contribute to the development of disease.

can influence the AS modes of many pre-mRNAs (*26*). Another case in point is that a lower expression of liver splicing factor serine and arginine rich splicing factor 6 in the mice models of MAFLD and NASH can contribute to disorder of RNA splicing, thus worsening MAFLD (*27*). Nucleotin is a multifunctional RBP, relocation on the cell membrane and overexpression of which have been verified to bring about cancers of different tissue origins (*28*). This can be exemplified by a highly expressed nucleolin in actively dividing cancer cells, which can promote the stability of epidermal growth factor receptor mRNA and block Fas receptors that induce apoptosis, contributing to cell malignant transformation (*29*).

2.2.2 RBP-RBP

In addition, the functions of cooperation, supervision, and competition are also represented among RBPs (*30*). Adenosine methylation at the N (6) position (m6A) is a dynamic and abundant epitranscriptomics marker, which can regulate key aspects of the metabolism of eukaryotic RNAs in many biological processes. This modification process involves the collaborative coupling of a series of protein complexes that are defined by researchers as Writer, Eraser, and Reader to correspond to the transmethylation catalase, demethylase, and reading protein of m6A that modifies RNA, respectively (*31*). For example, RNA methyltransferase-like 3 (METTL3) and METTL14 are components of the multi-subunit m6A writer complexes, the enzyme activities of which are significantly higher than those of METTL3/METTL14 alone (*32*). For instance, splicing inhibition mediated by RBP Heterogeneous ribonucleoprotein M is stimulated by RBP FOX-1 homologue 2 (RBFOX2), indicating there is extensive synergy between the two RBPs (*33*). Splicing is one of the methods widely used by RBPs to monitor the expression of other RBPs. RBFOX2 regulates other RBPs by controlling Alternative Splicing Coupled Nonsense-mediated Decay (AS-NMD). While RBP can always regulate itself, RBFOX2 serves as a global controller to dominate self-regulation of RBP (*34*). AS-NMD can also be found in the mutual supervision of RBPs. For example, while AS-NMD is used for the inhibition of RBM10 (RNA binding motif protein 10) to RBM5 (RNA binding motif protein 5), RBM5 also controls the expression of RBM10 splicing variants in turn through AS-NMD to reduce the action of cancer promotion (*35,36*). In RNA processing, there is a competition pattern between RBPs. Genome-wide analysis has revealed the antagonistic effects of splicing regulation between CUGBP (CUG-binding protein 1) Elav-like family member 2 (CELF2) and RBFOX2. These RBPs bind to overlapping sites of several mRNA precursors, with opposite consequences for the processing of exons (*37*). In addition to splicing, other processing steps are also affected by RBPs competition. On the mRNA of recombinant protein histone deacetylase 6, TAR DNA binding protein competes with FUS for overlapping binding sites to regulate its processing and nuclear export (*38*).

2.2.3. RBP-Protein-RNA

RBPs are involved in the formation of spliceosome and other ribonucleoprotein complexes, and in the regulation and modification of the interactions between proteins and ribonucleic acids to maintain accurate RNA translation and splicing (*12*). The Glucocorticoid-GR system acts as a transcriptional activator or inhibitor in the degradation of subsets of mRNAs despite its independence of translations, which is known as GR-mediated mRNA Decay (GMD). At this time, the Glucocorticoid-GR system is deemed as an assembly of RBP (*39*). Y-box binding protein 1 (YB1) is the most common cold shock protein involved in the evolution of many inflammatory diseases. Based on one research finding, the rapid degradation of chemokine (C-C motif) ligand 2 (CCL2) mRNA is mediated by GMD during

the dephosphorylation of YB1. The dephosphorylation of YB1 accelerates the combination of YB1 and GMD complexes. Then, the YB1-GMD complex is guided to CCL2 mRNA through the interaction between YB1 and CCL2 mRNA, thus triggering GMD, which can affect the stability of mRNA and boost the reduction of CCL2 mRNA. Based on this, the evolution of atherosclerosis is influenced by YB1 through inflammation regulation [51]. Gem Nuclear Organelle Associated Protein 5 (GEMIN5), an RBP protein, plays a key role in the formation of Survival Motor Neuron protein complexes and formation of small nuclear ribonucleic proteins (snRNPs). The mutation of GEMIN5 can damage the assembly of the snRNP complexes in neurons of induced pluripotent stem cells of patients, resulting in growth retardation, hypotonia, and cerebellar ataxia (*40*). As an important reading protein for the modification of RNA m6A, YTHN6-Methyladenosine RNA Binding Protein 2 (YTHDF2, YTH Domain Family Protein 2) contains a YTH domain that can specifically recognize the RNA modified by binding m6A and mediate the degradation of RNA. It has been proved that HRSP12 (adapter protein) can be used to connect YTHDF2 with ribonuclease P/MRP, resulting in rapid degradation of YTHDF2 binding RNA (*41*). YTHDF2 can also regulate somatic cell reprogramming by recruiting CCR4-NOT deadenylation complexes (*42*). The circadian clock impairs the hepatic lipid metabolism by cutting down the Peroxisome proliferator-activated receptor α (PPARα) mRNA mediated by YTHDF2, which is why people with irregular schedules are often susceptible to MAFLD (*43*). Similarly, fat mass and obesity-associated (FTO) protein is the first identified RNA demethylase that can erase m6A inside mRNA. A study shows that the down-regulation of FTO can reduce the modification of m6A in PPARα, and then regulate its transcriptional level in an m6A-YTHDF2 dependent manner (*44*).

The level of disorder of RBPs may lead to cellular stress, weaker cell adaption, or cell death as they are involved in a variety of important cellular functions, triggering MAFLD, diabetes, cancer, neurodegenerative diseases, and other kinds of diseases (*45*). Recently, increasingly extensive attention has been paid to the regulation of RBPs to MAFLD and its complications. This review will summarize the mechanisms of different RBPs in MAFLD and its complications, and some drugs that can improve MAFLD by adjusting RBPs, by which the design of new drugs for MAFLD and the disclosing of biomarkers can be advanced.

3. RBPs Implicated in The Occurrence and Development of MAFLD

MAFLD, a reflection of metabolic dysfunction in the liver, is described as a series of liver diseases with a prevalence of 70–80% in obese and diabetic patients, which can manifest initially as insulin resistance and changes in gut flora due to imbalances in energy intake and expenditure (*46,47*). The insulin resistance and changes in gut flora can lead to fatty deposits in the liver. During the accumulation of aberrant fat, there can be more serious hepatic insulin resistance and intracellular damage, which will further exacerbate inflammation, fibrosis, and carcinogenesis (*48*). A small percentage of patients progress from simple steatosis to liver inflammation and fibrosis [nonalcoholic fatty liver disease (NASH)]. During liver damage and repair, dysregulated hepatocytes can promote hepatic stellate cells (HSCs) activation through paracrine signaling, ultimately developing hepatic fibrosis and cirrhosis. Non-coding RNAs particularly play a functional role in MAFLD. Alterations in alternative RNA splicing are associated with inflammation, metabolic disorders and cancer, which are all important markers in the natural course of MAFLD. MicroRNAs (miRNAs), non-coding RNAs, and relevant RBPs are engaged in various core cellular processes (*2,48*). For instance, Zhao *et al*. revealed that the expression of the long noncoding RNA (lncRNA) brown fat-enriched lncRNA 1(Blnc1) in the liver is greatly increased in obesity and MAFLD mice, which is needed for inducing hepatic lipogenic genes. Specific inactivation of Blnc1 can erase insulin resistance and hepatic steatosis that are induced by high-fat diet, thus avoiding occurrence of MAFLD (*49*). And another study reported that hepatic Irs2 mRNA was decreased in MAFLD patients and its downregulation may be associated with insulin resistance (*50*).

Translation-regulated RNA-binding proteins (TTR-RBPs) are important proteins that regulate gene expression patterns. TTR-RBPs can control gene expression by cooperating or competing with specific miRNAs at the post-transcriptional level, affecting pre-mRNA splicing, mRNA transfer to the cytoplasm, turnover, storage, translation and so on (*51,52*). The overwhelming majority of TTR-RBPs can regulate numerous post-transcriptional processes, as HuR and nuclear factor 90 regulate mRNA stability and translation, while only a few TTR-RBPs can regulate a process specifically such as mRNA splicing *via* Tristetraprolin (TTP) and KH-type regulatory protein splicing (*53-55*). Therefore, the present study will introduce some specific RBPs in detail to expound the importance of RBPs in the occurrence and development of MAFLD (Table 1).

3.1. Insulin Resistance and Liver Fat Deposition

Insulin resistance is a systemic disease that affects many organs and insulin regulatory pathways. Fat deposition is negatively associated with insulin resistance. Insulin resistance and Liver Fat Deposition can be early manifestations of MAFLD. By searching relevant articles, we found that the RBPs associated with insulin resistance and hepatic fat deposition included DDX1, QKI 5, TTP, *etc* (*53-55*). We summarized

and demonstrated the first three RBPs with clearer mechanisms in insulin resistance and liver fat deposition (Figure 3).

3.1.1. Tristetraprolin

TTP (also known as zinc finger protein 36) is an mRNA-binding protein that can suppress more than 20 cytokines, including Tumor Necrosis Factor α (TNF-α), interleukin 6 (IL-6), IL-1, and Monocyte Chemoattractant Protein-1 (*56*). Under certain circumstances, inflammation can improve insulin sensitivity. For instance, downregulation of the TNF-α pathway can promote insulin sensitivity (*57,58*). TTP has been reported to control the TNF-α level through binding to the AU-rich element region of TNF-α mRNA (*59*). TTP can also be upregulated by TNF-α treatment (*60*). Therefore, we theorize that TTP and TNF-α reciprocally regulate each other and play a crucial role in inflammation and metabolic disturbance. Recent reports suggest a potentially novel role for TTP in the regulation of metabolism, especially in hepatic glucose and lipid metabolism (*61*). TTP has an antagonistic effect with HuR and their relationship has been extensively studied in the field of metabolic syndrome (*62,63*). In healthy humans, hepatic TTP at a base level can maintain systemic insulin sensitivity. Caracciolo *et al*. demonstrated that in the liver of obese mice, enhancement of TTP expression levels occurred in Kupffer cells but not in hepatocytes, which was associated with increased liver inflammation and protection against insulin resistance (*64*). According to an analysis of secreted hepatic factors by Sawicki *et al*., fibroblast growth factor 21 (FGF21), an important hormone in liver, is posttranscriptionally repressed by TTP and further modulates insulin responsiveness. Loss of TTP also amplifies FGF21 expression (*65*). In summary, lowering hepatic TTP levels, which has significant functional impact on hepatic and systemic insulin sensitivity, may open up a new avenue for treating various metabolic syndromes.

3.1.2 Heterogeneous nuclear ribonucleoprotein A1

Heterogeneous nuclear ribonucleoproteins (hnRNPs) are a big family of over 20 RNA-binding proteins found in mammalian cells (*66*). Human heterogeneous nuclear ribonucleoprotein A1 (HnRNPA1) is a highly enriched hnRNP that is generally used to stabilize mRNA and regulate mRNA gene expression (*67*). Recent evidence suggests that hnRNPA1 plays a key role in regulating lipid and glucose metabolism. HnRNPA1 has been shown to be related to the formation of pyruvate kinase isoform 2 mRNA (*68*). In the adipose tissues of obese patients, hnRNPA1 expression is significantly decreased. Moreover, hnRNPA1 is also associated with insulin receptor alternative splicing in people with weight loss (*69*). According to the experiment conducted by Zhao *et al*., hnRNPA1 knockout mice exhibited decreased glycogen storage, severe insulin resistance and hepatic steatosis (*70*). They further suggested that this was because hnRNPA1 can interact with glycogen synthase 1(gys1) mRNA, thereby promoting glycogen synthesis and maintaining the sensitivity of insulin (*70*). Another study elaborated that lncRNA suppressor of hepatic gluconeogenesis and lipogenesis (lncSHGL) can recruit hnRNPA1, and co-regulate calmodulin (CaM) protein at the post-transcriptional level, which perform an important part in inhibiting hepatic gluconeogenesis and adipogenesis. The lncSHGL/hnRNPA1/CaM pathway also takes part in the regulation of phosphatidyl inositol 3-kinase (PI3K)/Akt pathway activity, which affects the production of hepatic glucose (*71*). Besides, a study by Gui *et al*. also revealed that hnRNPA1 regulates lipid metabolism by interacting with H19 and increasing the translation of fatty acid oxidation-related genes carnitine palmitoyl transferase 1B (CPT1b) and peroxisome proliferators-activated receptor γ coactivator 1 alpha (PGC1α), thereby improving insulin resistance (*72,73*).

3.1.3. Serine rich splicing factor 10

Serine rich splicing factor 10 (SRSF10), which belongs to the SR-like protein family of splicing factors, can regulate RNA processing (*74*). For the past few years, SRSF10 has been demonstrated to be associated with adipocyte differentiation and lipogenesis, and its expression level is decreased in liver and muscle of obese populations (*60*). LPIN1, a key regulator of lipid metabolism, is positively correlated with insulin resistance in adipose tissue and liver (*74,75*). Research shows that SRSF10 can selectively down-regulate the alternative splicing of LPIN1 and produce the LPIN1 β isoform associated with increased expression of adipogenesis genes, thereby stimulating lipogenesis and causing hepatic steatosis (*76*). Besides, insulin can regulate the expression of SRSF10. SRSF10 in liver is increased by the overexpression of constitutively active Forkhead Box 01(Fox01), which is resistant to nuclear exclusion by insulin (*77*). Furthermore, the activation of Cdc2-like kinase family proteins (CLK) by insulin can alter the phosphorylation and activity of SRSF10 (*78*). In addition, several studies also found a class III histone deacetylase in the liver, mainly called SIRT1, which can regulate multiple lipid metabolism pathways such as liver lipogenesis, fatty acid beta-oxidation, lipoprotein uptake and secretion (*11*). The interaction between SRSF10 and SIRT1 has been extensively studied. At both transcriptional and post-transcriptional levels, SRSF10 is upregulated by SIRT1, which is attributed to the increased stability of SRSF10 mRNA. Some studies also speculated that SIRT1 also physically interacted with SRSF10 though altering the acetylation status of SRSF10 and preventing its proteasomal degradation, thereby stabilizing and increasing the expression level of

Table 1. RBPs in the occurrence and development of MAFLD

RBPs	Expression	Target	Expression	Mechanism	*Ref.*
TTP	↑	TNF-α mRNA, Linc-SCRG1	↑	increase liver inflammation and protect against insulin resistance	(*60,127*)
HnRNPA1	↓	gys1 mRNA, CaM, H19	↓	decrease glycogen storage, and induce hepatic gluconeogenesis and adipogenesis	(*70*)
SRSF10	↓	LPIN1	-	stimulate lipogenesis and induce hepatic steatosis	(*79*)
HuR	↓	Insig1 mRNA, C/EBPβ mRNA, APOA4 mRNA,PTEN mRNA, HAMP mRNA	↓	influence liver homeostasis and hepatic iron deposition	(*87,89*)
CPEB1	↓	IL-6, PTEN, STAT3	↑	interfere with glucose metabolism and cause insulin resistance	(*120*)
CPEB4	↑	PFKFB3	↑	regulate UPR	(*97*)
EIF4E	↑	CD36 mRNA	↑	increase liver inflammation	(*100*)
IGF2BP2	↑	IGF1 mRNA	↓	increase hepatic iron deposition and free cholesterol	(*148*)
FTO	↑	TSC1 mRNA	↓	increase ROS release and mitochondrial dysfunction	(*149*)
HnRNPU	↓	TrkB, Blnc1	↑	promote liver inflammation and stress-induced cell death	(*109*)
AEG-1	↑	PPARα NF-κB	↓ ↑	drive hepatic inflammation and fibrosis	(*112*)
LIN28	↑	miR-200c	↓	cause liver fibrosis	(*117*)
CUGBP1	↑	IFN-γ mRNA	↓	promote HSCs activation	(*118*)
RBMS4	↑	Prx1 mRNA	↑	promote HSCs activation	(*121*)

RBPs, RNA binding proteins; MAFLD, metabolic-associated fatty liver disease; TTP, Tristetraprolin; TNF-α, tumor necrosis factor α; HnRNPA1, Human heterogeneous nuclear ribonucleoprotein A1; gys1, glycogen synthase 1; CaM, calmodulin; SRSF10, Serine rich splicing factor 10; HuR, Human antigen R; Insig1, insulin-induced gene 1; C/EBPβ, CCAAT enhancer-binding protein beta; APOA4, Apolipoprotein A-IV; PTEN, phosphatase and tensin homolog; HAMP, Heparin affin regulatory peptide; CPEB1, Cytoplasmic polyadenylation element binding protein 1; PFKFB3, 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3; EIF4E, Eukaryotic initiation factor 4E; CD36, Recombinant Cluster Of Differentiation 36; IGF2BP2, insulin-like growth factor 2 mRNA binding protein; FTO, fat mass and obesity-associated protein; TSC1, tuberous sclerosis 1; hnRNPU, a nuclear matrix protein; TrkB, Tyrosine Kinase receptor B; Blnc1, brown fat-enriched lncRNA 1; AEG-1, astrocyte elevated gene-1; PPARα, Peroxisome proliferator-activated receptor α; NF-κB, nuclear factor kappa-B; CUGBP1, CUG-binding protein 1; IFN-γ, interferon γ; RBMS4, an RNA binding protein; Prx1, Peroxiredoxin 1; ROS, reactive oxygen species; HSCs, hepatic stellate cells.

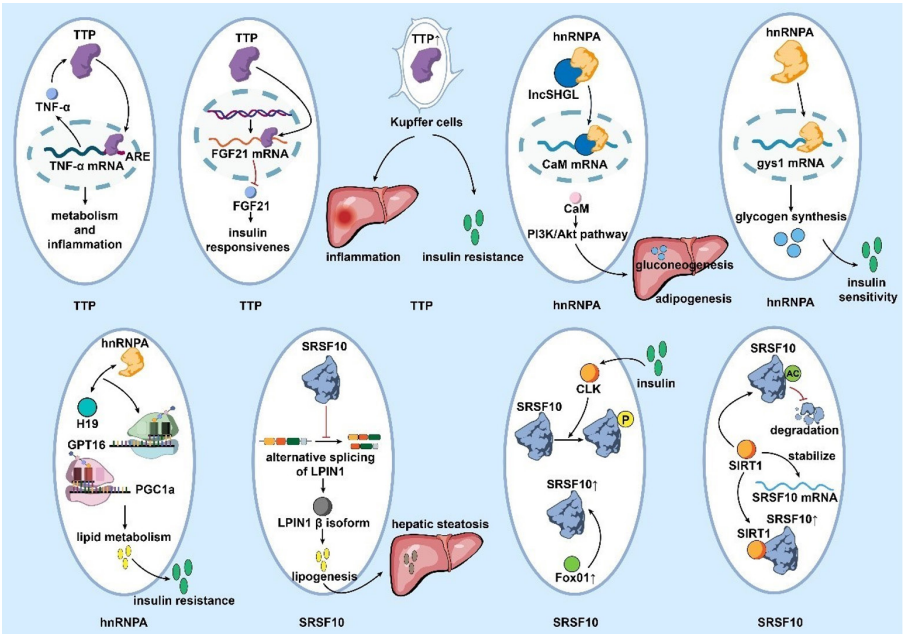

Figure 3. RNA-RBPS interaction in insulin resistance and liver fat deposition. Here, we summarize and show the main mechanisms of RNA-RBPS on insulin resistance and liver fat deposition. TTP can control the TNF-α level by binding to the AU-rich element (ARE) region of TNF-α mRNA and be upregulated by TNF-α treatment therefore playing a role in inflammation and metabolic disturbance. Post-transcriptional repression of FGF21 mRNA by TTP, which in turn regulates insulin responsiveness. Increased levels of TTP expression in Kupffer cells heighten liver inflammation and insulin resistance. lncSHGL regulates CaM levels at the post-transcriptional level and the lncSHGL / hnRNPA1 / CaM pathway is also involved in regulating the activity of the PI3K/Akt pathway to influence hepatic gluconeogenesis and adipogenesis. hnRNPA1 can interact with gys1 mRNA, thereby promoting glycogen synthesis and maintaining the sensitivity of insulin. hnRNPA1 interacts with H19 increasing the translation of fatty acid oxidation-related genes CPT1b and PGC1α, thereby improving insulin resistance. SRSF10 selectively down-regulates alternative splicing of LPIN1 to produce the LPIN1 β isoform, thereby stimulating adipogenesis and leading to hepatic steatosis. Activation of CLK by insulin can alter the phosphorylation and activity of SRSF10, and SRSF10 can be increased in the liver by overexpression of Fox01, which is resistant to nuclear rejection by insulin. SIRT1 upregulates SRSF10 by increasing the mRNA stability of SRSF10. SIRT1 also interacts with SRSF10 and alters the acetylation state of SRSF10 and prevents its proteasomal degradation, thereby increasing the expression level of SRSF10 protein.

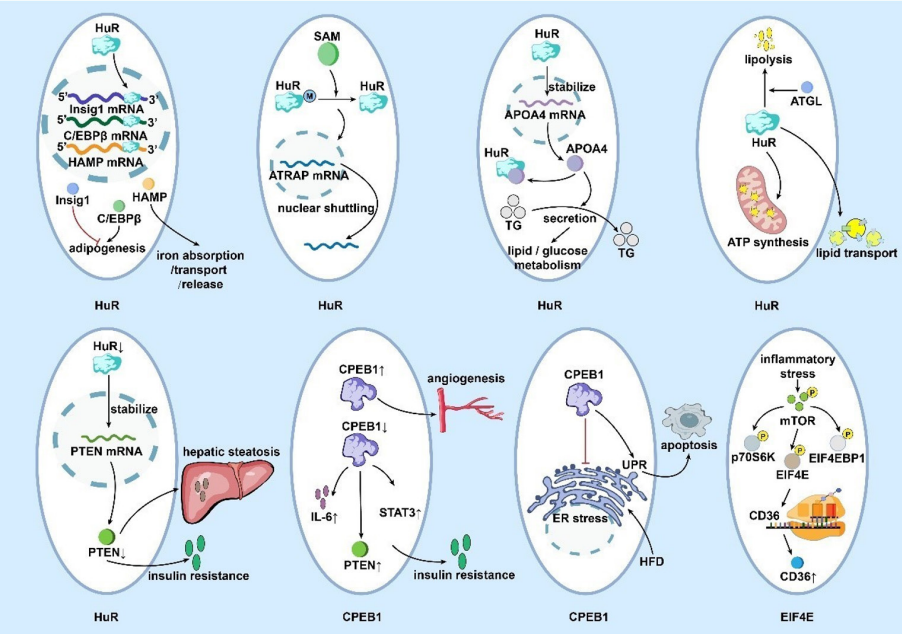

Figure 4. RNA-RBPS interaction in MAFLD. Here, we summarize and show the main mechanisms on RNA-RBPS in MAFLD. HuR can inhibit lipid formation by binding and stabilizing Insig1 mRNAs and C/EBPβ mRNAs and regulate iron absorption, transport and release by binding to the 3'UTR of HAMP mRNA. The low SAM levels induced by MAFLD lead to the demethylation of HuR, resulting in downstream nuclear shuttling of ATRAP mRNA. HuR also interacts directly with APOA4 and stabilizes its mRNA expression, regulating lipid and glucose metabolism by promoting the secretion of TG. ATGL mediates the regulation of lipolysis by HuR, which regulates lipid transport and ATP synthesis to prevent MAFLD. HuR also regulates glucolipid metabolism by increasing the stability of PTEN mRNA. HuR deletion decreases PTEN expression, which exacerbates hepatic steatosis in mice, but also reduces insulin resistance. Elevated levels of CPEB1 induce pathological angiogenesis in chronic liver disease, while decreased levels of CPEB1 lead to upregulation of IL-6, PTEN and STAT3, resulting in insulin resistance. CPEB4 affects the transduction of UPR to pro-apoptotic signaling in hepatocytes, and the translational regulation of CPEB4 contributes to the attenuation of HFD-induced ER stress in the liver. Inflammation enhances phosphorylation of mTOR and its downstream translational regulators such as p70S6K, EIF4E and EIF4EBP1, which then stimulate translation of CD36, leading to increased levels of CD36 protein in the liver.

SRSF10 protein in hepatocytes (*79*). However, to date, the molecular mechanism of how hepatic SRSF10 is regulated remains largely unknown, and further research is necessary.

3.2. MAFLD

MAFLD is the most common chronic liver disease. Despite increasing advances in the understanding of the pathophysiology of MAFLD, the exact mechanisms towards liver damage development remain unclear. And we urgently need to find therapeutic targets for MAFLD. Here, we summarize and show the first three RBPs with more specific mechanisms and more definite pathways in MAFLD (Figure 4).

3.2.1. Human antigen R

HuR is a member of the Hu RNA-binding protein family and is implicated in metabolism of RNAs (*80*). HuR is involved in a variety of important cellular processes, including inflammation, stress responses, carcinogenesis and apoptosis (*81*). HuR is widely considered to be a main regulator of liver homeostasis and the reduced expression of HuR will cause spontaneous steatosis and promote liver fibrosis (*82*). The study by Siang *et al*. has shown that HuR is an important inhibitor of adipogenesis found in both white adipose tissue and brown adipose tissue (*83*). Downregulation of HuR in adipose tissue greatly increases adipose mass, and glucose-intolerance and insulin-resistance appear at the same time. Mechanistically, HuR can inhibit adipogenesis by binding and stabilizing the mRNA of insulin-induced gene 1 (Insig1), which is a passive regulator of adipogenesis. S-adenosylmethionine (SAM) can increase the expression of Ang II type 1 receptor (AT1R)-associated protein (ATRAP) protein, and HuR also plays a vital role in it. Mechanistically, low SAM levels induced by MAFLD lead to demethylation of HuR, thereby resulting in downstream nuclear shuttling of ATRAP mRNA (*84*). Besides, in the early stages of adipogenesis, HuR can directly bind to the 3'UTR of CCAAT enhancer-binding protein beta (C/EBPβ) mRNA, which participates in the initiation of adipogenesis (*85*). HuR can also directly interact with Apolipoprotein A-IV (APOA4) and stabilize its mRNA expression, which is a plasma lipoprotein that regulates lipid and glucose metabolism by promoting the secretion of TG (*86*). In addition, Tian *et al*. also found that HuR could regulate lipid and sugar metabolism though improving the stability of phosphatase and tensin homolog (PTEN) mRNA. Deletion of HuR selectively decreased the

expression of PTEN, thereby aggravating liver steatosis in mice but also alleviating insulin resistance (*87*). Li *et al*. used adipose-specific HuR knockout (HuRAKO) mice as a model and found that HuRAKO mice showed obesity along with insulin resistance and exacerbated hepatic steatosis (*88*). Mechanistically, they suggested that adipose triglyceride lipase (ATGL), the major lipolytic enzyme, mediated the regulation of lipolysis by HuR. Zhang *et al*. also found that HuR can regulate lipid transport and ATP synthesis to prevent MAFLD using an animal model (*89*). SAM, a principle biological methyl donor involved in many metabolic pathways, is enriched in liver and downregulated in MAFLD patients (*90*). The latest studies noted that hepatic iron deposition is significantly associated with advanced MAFLD and liver fibrosis (*91*). Heparin affin regulatory peptide (HAMP) is an important mediator of iron absorption, transport and release, and is mainly expressed in the liver. HuR can bind to the 3'UTR of HAMP mRNA to upregulate its level (*92*). As discussed above, we can conclude that HuR, as a key regulator of lipid and glucose metabolism, may be a useful therapeutic target for MAFLD.

3.2.2. Cytoplasmic polyadenylation element binding protein

The cytoplasmic polyadenylation element binding protein (CPEB) family is a class of RBPs that regulate mRNA translation under hepatic metabolic stress (*93*). The amount of CPEB varies in different species and each member of the CPEB family has its own identity and role. Cytoplasmic polyadenylation element binding protein 1(CPEB1) is the most extensively studied CPEB, which is associated with meiosis, cell senescence, inflammation, glucose metabolism and liver homeostasis (*94*). When CPEB1 levels are reduced, IL-6, PTEN and signal transducer and activator of transcription 3 will be correspondingly up-regulated, thereby interfering with glucose metabolism and causing insulin resistance, which may lead to the occurrence of some liver diseases such as MAFLD (*95*). Moreover, elevated levels of CPEB1 will induce pathological angiogenesis in chronic liver disease (*96*). CPEB4 is identified to be associated with hepatic steatosis under ER stress and can regulate unfolded protein response (UPR), which is of great significance in the pathogenesis of MAFLD (*97*). A study by Maillo *et al*. has shown that the level of CPEB4 mRNA in the liver is mediated in a special circadian way (*97*). There is a connection between biological rhythm and metabolic homeostasis. Alterations in levels of CPEB4 affect the transduction of UPR to pro-apoptotic signals in hepatocytes, while translational regulation of CPEB4 contributes to alleviation of high fat diet (HFD)-induced hepatic ER stress. Thus, CPEB4 deficiency promotes MAFLD.

3.2.3. Eukaryotic Initiation Factor 4E

Eukaryotic initiation factor 4E (EIF4E), an mRNA cap-binding protein, affects mRNA-ribosome interactions and capture-dependent translation through interacting with eukaryotic initiation factor 4G (*98*). Hepatic inflammatory stress is critical for lipid accumulation and is an independent risk factor for the development of MAFLD. The mammalian target of rapamycin(mTOR) is a widely expressed and highly conserved serine/threonine kinase involved in the progression of metabolic syndrome under inflammatory stress (*99*). The mTOR downstream effectors mainly include EIF4E binding proteins and ribosomal protein S6K kinase (*100*). Recombinant Cluster of Differentiation 36(CD36), a transmembrane glycoprotein can promote the intake of long-chain fatty acids to induce hepatic steatosis, thereby leading to MAFLD (*101*). Wang *et al*. demonstrated that inflammatory stress enhanced phosphorylation of mTOR and its downstream translational regulators such as ribosome S6 protein kinase (p70S6K), EIF4E and EIF4E Binding Protein 1(EIF4EBP1), and then stimulated the translation of CD36, ultimately resulting in increased levels of CD36 protein in the liver (*102*). Rapamycin is a specific mTOR inhibitor that has an effect against lipid deposition in MAFLD treatment. Rapamycin can reduce CD36 protein expression through inhibiting mTOR pathway and phosphorylation of downstream effectors (*103*). All of these results show a molecular mechanism underlying the development of MAFLD and provide new evidence for MAFLD treatment. In addition, EIF4E also may play a vital role in progression from MAFLD to HCC (*100*).

3.3. NASH

NASH is a late-stage MAFLD manifestation, and is also one of the most common causes of liver failure globally. NASH is characterized by persistent liver damage, chronic inflammation and different degrees of liver fibrosis (*104*). Here, we list and summarize several RBPs associated with the development of NASH.

M6A is the most abundant form of internal RNA modifications in messenger RNA, microRNA, and non-coding RNA. Methylation of m6A RNA plays a significant part in hepatic lipid metabolism disorder. Both insulin-like growth factor 2 mRNA binding proteins (IGF2BPs) and YTHDF1 can recognize m6A methylation, and then act as signal transducers and facilitators in MAFLD-NASH-HCC progression (*105,106*). The accumulation of free cholesterol in the liver is an important trigger for the occurrence of severe NASH. Simon *et al*. also elaborated that insulin-like growth factor 2 mRNA binding protein (IGF2BP2) can promote NASH by increasing hepatic iron deposition and free cholesterol (*107*). FTO belongs to the AlkB family of enzymes and is also an important class of RBPs that demethylates specifically mRNA m6A. Studies have shown that upregulation of FTO will induce

increased reactive oxygen species (ROS) release and mitochondrial dysfunction, thereby leading to more severe NASH (*108*). Furthermore, hepatocellular specific inactivation of hnRNPU (a nuclear matrix protein) will exacerbate HFD-induced NASH through aberrantly inducing a truncated Tyrosine Kinase receptor B (TrkB) isoform that promotes liver inflammation and stress-induced cell death, including liver injury, inflammation and fibrosis (*109*). Lack of Blnc1 in the liver can improve NASH, while some RBPs can interact with Blnc1, such as endothelial differentiation-related factor 1 (EDF1), YBX1 and hnRNPU (*49*). Jin *et al*. proposed that circRNA_002581 positively regulates CPEB1 *via* sponge miR-122, a pathway with therapeutic potential for NASH (*110*). Hepatic farnesoid X receptor (FXR) is an important regulator of lipid homeostasis that prevents NASH, and its expression correlates with the severity of NASH (*111*). FXR activation can increase HuR, which maintains hepatocyte homeostasis under normal conditions (*82*). In addition, there are also some RBPs that play a vital role in the fibrosis process of NASH. AEG-1 (astrocyte elevated gene-1), an ER membrane-anchored RBP, which regulates fatty acid β-oxidation (FAO) by inhibiting PPARα activation, and promoted translation of mRNAs encoding fatty acid–synthesizing enzymes, thereby promoting de novo lipogenesis (DNL). Moreover, AEG-1 also can activate NF-κB signal pathway to drive hepatic inflammation and fibrosis (*112*). Gerhard *et al*. demonstrated that adipocyte enhancer binding protein 1 (AEBP1) expression parallels the worsening of fibrosis severity in NASH, which can regulate many differentially expressed genes about NASH (*113*). AEBP1 interacted with miR-372-3p and miR-373-3p, which were shown to be significantly downregulated in NASH fibrosis. Furthermore, a mutual effect between AEBP1 and PTEN has been explored and results revealed a vital function of AEBP1 in hepatic fibrosis in NASH patients. These RBPs are increasingly recognized as viable targets for treating NASH and MAFLD-NASH-HCC. Hence, it is essential to get a deep understanding of their functions and mechanisms.

3.4. Liver Cirrhosis

MAFLD and NASH are considered to be the predecessors of liver fibrosis and eventually cirrhosis. The main feature of liver fibrosis is excessive activation of HSCs (*114*). The dysregulated expression of RBPs also has a strong impact on the occurrence of liver cirrhosis (Figure 5), such as HNRNPA1, LIN28, HuR, TTP and so on. HnRNPA1, which usually is located in the nucleus, is overexpressed in mouse HSCs. ER stress in hepatic

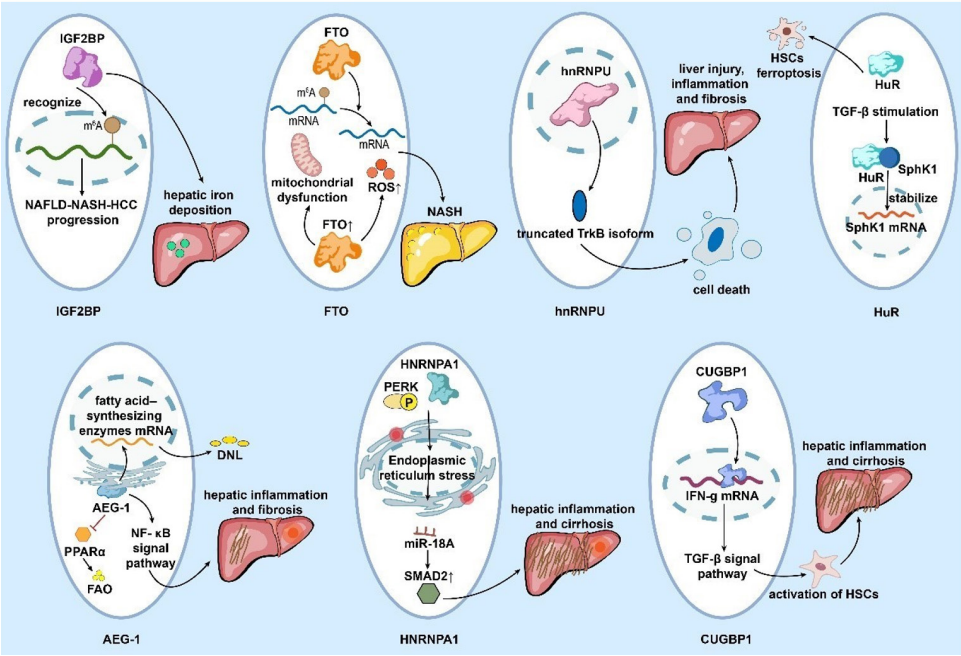

Figure 5. RNA-RBPS interaction in NASH and Liver Cirrhosis. Here, we summarize and show the main mechanisms on RNA-RBPS in NASH and Liver Cirrhosis. IGF2BPs can recognize m6A methylation and facilitate the progression of NAFLD-NASH-HCC. And IGF2BP2 promotes the development of NASH by increasing hepatic iron deposition. FTO specifically demethylates mRNA m6A and upregulation of FTO will initiate increased ROS release and mitochondrial dysfunction, leading to more severe NASH. hnRNPU promotes hepatic inflammation and stress-induced cell death through the induction of truncated TrkB isoforms, leading to liver injury, inflammation and fibrosis. HuR binds to SphK1 and stabilizes its mRNA after TGF-β stimulation. On the other hand, HuR attenuates the effect of HSCs by promoting HSCs ferroptosis. AEG-1 is an ER membrane-anchored RBP that regulates FAO by inhibiting the activation of PPARα and promotes the translation of mRNAs encoding fatty acid synthases, thereby promoting DNL. In addition, AEG-1 activates the NF-κB signaling pathway, driving liver inflammation and fibrosis. Dysregulation of PERK phosphorylation and HNRNPA1 expression mediates endoplasmic reticulum stress in hepatic stellate cells and miR-18A induces SMAD2 overexpression, leading to liver fibrosis and cirrhosis. CUGBP1 binds specifically to IFN-g mRNA and contributes to the TGF-β signaling pathway, thereby promoting the activation of HSCs, ultimately resulting in the development of fibrosis and cirrhosis.

stellate cells is mediated by protein kinase RNA–like endoplasmic reticulum kinase (PERK) phosphorylation and dysregulated expression of HNRNPA1, and then induces recombinant others against decapentaplegic homolog 2(SMAD2) overexpression by miR-18A, thereby causing liver fibrosis and cirrhosis (*115*). LIN28 is a highly conserved RBP involved in many eukaryotic cellular processes and its main function is cell transformation, which is likely to play an important role in liver fibrosis repair (*116*). In the process of MAFLD-NASH-HCC, the expression of LIN28 is abnormally increased. A recent study showed that overexpression of miR-200c bound to LIN28 can promote epithelial-to-mesenchymal transition (EMT) and mesenchymal-to-epithelial transition (MET), and the balance of EMT-MET will decide whether NASH patients recover or develop liver cirrhosis (*117*). Wu *et al*. found that CUG-binding protein 1 (CUGBP1) showed elevated expression in HSCs, which is associated with the severity of liver fibrosis. CUGBP1 specifically binds to interferon (IFN)-γ mRNA and promotes the transforming growth factor (TGF)-β signal pathway, thereby promoting the activation of HSCs, which ultimately leads to the occurrence of fibrosis and liver cirrhosis (*118*). CPEB4, a member of the CPEB family, is highly expressed in the liver and has also been found to prevent HSCs activation and liver fibrosis by silencing (*119*). In the early stage of HSC activation, 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3(PFKFB3) protein is continuously upregulated by CPEB4 and its binding RNA, which has become a potential target for anti-fibrosis and prevention of liver cirrhosis (*120*). An RBP called RBMS3 can specifically bind to the 3'UTR of Peroxiredoxin 1(Prx1) mRNA and increase Prx1 protein expression. Fritz *et al*. found that RBMS3 expression in fibrotic liver was highly expressed, and its expression level increased with increasing HSCs activity(*121*). Moreover, p62 is an RBP class with an RNA-binding motif. Lu *et al*. showed that p62 was sporadically expressed in cirrhotic nodules cells and may be associated with hyperproliferating cells (*122*).

Notably, in recent years, ferroptosis was revealed to be closely associated with liver fibrosis and may become a potential target for liver fibrosis therapy. HuR has long been described as a key player in MAFLD and NASH, and has also been found to contribute to the activation of HSCs and development of liver fibrosis (*113*). In activated HSCs, HuR expression is highly increased (*123*). Upregulation of sphingosine kinase 1 (SphK1) is involved in HSCs activation induced by the TGF-β signaling pathway. On the one hand, HuR can bind to SphK1 and stabilize its mRNA after TGF-β stimulation (*124*). On the other hand, HuR alleviates the effect of HSCs by promoting HSCs ferroptosis (*125*). TTP, which belongs to the same AU-rich element-binding proteins as HuR, has several important roles in different stages of liver fibrosis. TTP has been shown to inhibit HSC ferroptosis by binding to autophagy-related 16-like 1 mRNA (*126*). Finally, gene chip detection identified an lncRNA called linc-SCRG1, which was up-regulated 13.62-fold in human liver cirrhosis (*127*). Linc-SCRG1 can specifically bind to TTP and has the ability to inhibit the phenotypic inactivation of HSCs, thereby delaying the progression of cirrhosis.

4. Non-Coding RNA-RBPs Interaction in MAFLD

MAFLD is closely associated with systemic energy metabolism disorders and can progress from simple steatosis to NASH and eventually to cirrhosis (*2*). Recently, many studies found that the relationship between non-coding RNAs (ncRNAs) and RBP was involved in the occurrence and development of MAFLD. Here, we introduced several key ncRNA-RBP interactions for MAFLD.

HuR mentioned above is an important RBP for MAFLD. Studies have shown that HuR can upregulate multiple lncRNAs, such as lncRNA NEAT1, LINC00336 and lncRNA UFC1, further promoting cell proliferation and invasion and inhibiting cell apoptosis (*85*). Apolipoprotein A-IV (APOA4), a plasma lipoprotein, can regulate glucose and lipid metabolism by promoting the secretion of TG. A study found that both antisense lncRNA APOA4-AS and APOA4 are significantly upregulated in MAFLD, and APOA4-AS can interact with HuR directly. After knockdown of HuR, APOA4-AS levels were significantly reduced, resulting in lower plasma TG and TC levels. CPEB1 has been shown to have an important role in chronic liver disease. MiR-122, the most common miRNA in adult liver, has been identified to be a downstream target of circRNA_002581 and an upstream regulator of CPEB1. Jinet al. confirmed the existence of circRNA_002581-miR-122-CPEB1 axis *in vitro*, which is involved in NASH pathogenesis by inhibiting autophagy related to the PTEN-AMPK-mTOR pathway (*110*). SIRT1 plays an important role in many metabolic diseases, including MAFLD. Among them, SIRT1 in liver can regulate the expression of QKI 5 by the PPARα/FoxO1 signal pathway, which belongs to the STAR family of RNA-binding proteins, and SIRT1 siRNA can induce acetylation of QKI 5 (*11*). A recent study by Chen *et al*. showed that silencing circRNA_0000660 can significantly inhibit miR-693 to upregulate IGFBP1 level, thereby reducing lipid accumulation in liver and alleviating MAFLD (*128*). In addition, several lncRNAs have been demonstrated to be tightly associated with RBPs. Hepatic lncSHGL can participate in MAFLD development by promoting fasting hyperglycemia and lipid deposition in mice. Wang *et al*. also found that lncSHGL can enhance HnRNPA1 to promote CaM mRNA translation (*129*). Moreover, studies found that lncRNA Blnc1 was elevated abnormally in MAFLD mice, which was associated with hepatic steatosis and insulin resistance. Proteomic analysis found that EDF1, YBX1 and hnRNPU all

Table 2. Potential Drugs Targeting RBPs in the development of MAFLD

Drugs	Disease	Target	Function	*Ref.*
SAM	MAFLD	HuR↑	maintain metabolic homeostasis	(*84*)
Betaine	MAFLD	FTO↓	decrease de novo lipogenesis, increase lipolysis	(*137*)
Exenatide	MAFLD	FTO↓	reverse lipid accumulation, promote inflammation regression	(*140*)
eIFsixty-1,4,6	HCC	EIF4E↓	inhibit HCC formation	(*100*)
PHA-781089	HCC	TTP↓	induce apoptosis of HCC	(*145*)

RBPs, RNA binding proteins; MAFLD, metabolic-associated fatty liver disease; NASH, nonalcoholic steatohepatitis; SAM, S-adenosylmethionine; FTO, fat mass and obesity-associated protein; HCC, hepatocellular carcinoma; eIF6, eukaryotic translation initiation factor 6; TTP, Tristetraprolin.

interacted with Blnc1.

Except for RNA-RBP interactions, some RBPs also can directly interact with RBPs or proteins, thereby affecting the development of MAFLD. For instance, Tian *et al*. have showed that HuR can regulate intracellular cholesterol homeostasis by regulating the expression of ATP-binding cassette transporter A1 (ABCA1) (*87*). And Woodhoo *et al*. have found that HuR also reduces profibrotic effects induced by TGF-β through significantly silencing the expression of alpha smooth muscle actin (a-SMA) (*130*). Also, downregulation of CUGBP1 can also suppress a-SMA expression, and can further promote the IFN-γ signaling pathway, which is associated with the progression of liver fibrosis (*118*). Moreover, PINX1 (Pin2/TRF1 interacting protein) has been found to be overexpressed in patients with cirrhosis. A study by Huang *et al*. has demonstrated that inhibition of PinX1 can significantly increase telomere length and telomerase activity, thereby attenuating the progression of MAFLD *in vivo* and *in vitro* (*131*). In addition, DDX1, an RBP that regulates insulin, can increase blood glucose through mediated inhibition of insulin translation. DDX1 can interact with another RBP called eIF4B, which is a well-established factor for translation initiation(*132*). Therefore, it is tempting to speculate about the importance of future studies on the interactions between RBPs and other molecules, and it will help us to elucidate the pathological mechanism of MAFLD.

5. Potential Drugs Targeting RBPs in MAFLD

Nowadays, the development of drugs targeted for RBPs has come to the fore given its significance in the evolution of MAFLD (*7*). This section will discuss the potential therapeutic drugs being studied for MAFLD targeted at RBPs (as shown in Table 2).

Therapeutic targets for improving lipid metabolism of MAFLD can be developed in two ways: 1) to lower the metabolizing substrates in the liver; 2) to accelerate the effective metabolism of lipids in the liver (*133*). For example, SAM is a major biological methyl donor in mammalian cells, which is a defining factor of the subcellular localization of HuR (*134*). ATRAP was shown to potentially prevent abnormal metabolism of tissue, including lipid deposition and hepatic fibrosis (*135*). The low SAM concentration induced by MAFLD will cause HuR demethylation, which directly breaks the dynamic balance of nucleocytoplasmic shuttling of ATRAP mRNA. When SAM supplementation is provided, SAM maintains the nucleocytoplasmic shuttling of ATRAP mRNA by regulating HuR methylation and upregulates the expression of ATRAP, thereby reducing the disorder of lipid metabolism and insulin resistance (*84*). One of the main physiological effects of betaine is to get involved in the methionine cycle in the human liver as a methyl donor (*136*). In addition, experiments in a mouse model have shown that the detected low methylation status of m6A and increased FTO expression could be corrected by betaine supplementation. This suggests that betaine supplementation can significantly reduce the liver function lesions and morphology damage caused by high fat as well as ectopic fat accumulation to prevent MAFLD (*137*). And most remarkably, betaine is a main component of many foods, including wheat, shellfish, spinach, and beets, which enlightens us that perhaps MAFLD can be treated by diet therapy (*138*). Moreover, exenatide is a glucagon like peptide-1 receptor agonist antidiabetic drug that can ameliorate insulin resistance and reduce hepatic steatosis (*139*). Li *et al*. established animal models with MAFLD induced by a HFD and the related cell culture models and studied the protective effect of exenatide on the fatty liver through FTO genes *in vivo* and *in vitro* technologies. Histological analysis indicates that exenatide significantly reverses HF-induced lipid accumulation and inflammatory evolution, accompanied by a dropping expression of FTO mRNA and protein, which may represent an effective treatment strategy for MAFLD (*140*).

The abnormally accumulated fat will advance the development of HCC and the metabolism of fat in the liver is closely related to the regulation of functional proteins (*141*). Translation factors such as EIF4E act continuously on the evolution from MAFLD to HCC (*142*). In this regard, it is identified in a study that three compounds (eIFsixty-1, eIFsixty-4, eIFsixty-6) can inhibit the binding of eIF-6 and 60S and the translation of Lipogenic enzymes, which can delay the formation and growth of HCC nodules without obvious negative side effects (*100*). The RNA-binding protein TTP can regulate about 2500 genes, the functional modification of which represents a promising therapeutic strategy

for HCC (*143*). It has been shown that the consumption of TTP in HCC cell lines prevents HCC cells from apoptosis (*144*). PHA-781089, a MAPKAP2 (MK2) inhibitor, was used as an inhibitor of TTP functions in a study, which showed that mRNA expression of the TTP target can be restored in the presence of MK2 inhibitors and this is a sign that the MK2/TTP pathway plays a role in the proliferation and maintenance of HCC (*145*).

However, we should be careful about the side effects of targeting RBPs in MAFLD therapy. For example, HuR is involved in the production of cellular inflammation. One inflammatory phenotype is largely the driving force behind HuR's implications in heart-related diseases including vascular inflammation and atherosclerosis (*146*). Besides, some pathogenic microorganisms like Hepatitis C virus use HuR biology to promote disease progression (*147*) .

In conclusion, a network of gene expression regulation through multiple approaches involved in RBPs will contribute to the new drug design and biomarker discoveries of MAFLD and other related liver diseases that evolved from MAFLD. The advanced therapies for MAFLD derived from RBP are being tested and will be gradually applied.

6. Conclusion

In recent years, the vital roles of RBPs have been demonstrated in the development of MAFLD. With the development of biological and molecular science, more and more RBPs and target RNAs in MAFLD have been discovered, allowing us to better understand the occurrence of MAFLD. In this review, we conclude that these RBPs are associated with insulin resistance and liver fat deposition, MAFLD, NASH and liver cirrhosis. Therefore, we discuss the ncRBP-RNA interactions in MAFLD and several drugs related to RBPs in MAFLD therapy.

Currently, the research on the specific mechanism of RBP is not well studied, and drug strategies targeting RBPs are still in the start-up stage, just like MAFLD. Notably, the different alternative splicing regulation of RBPs will lead to the complexity of their protein functions. Protein functional structure of targeted RBPs with high selection may be effective in reducing the occurrence of side effects. From what has been discussed above, posttranscriptional modulation by RBPs is becoming an important constructive mechanism in the occurrence and development of MAFLD, which still needs further studies to elucidate the complex regulatory network in MAFLD and other metabolic diseases.

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[§]These authors contributed equally to this work.

**Address correspondence to:*
Peng Yu, The Second Clinical Medical College / Department of Endocrinology and Metabolism, The Second Affiliated Hospital of Nanchang University, Nanchang, Jiangxi, China.
E-mail: yu8220182@163.com

Jing Zhang, The Second Clinical Medical College / Department of Anesthesiology, The Second Affiliated Hospital of Nanchang University, Nanchang, China.
E-mail: zhangjing666doc@163.com

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