



REVIEW ARTICLE

Decoding m⁶A mRNA methylation by reader proteins in liver diseases

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Abstract N⁶-methyladenosine (m⁶A) is a dynamic and reversible epigenetic regulation. As the most prevalent internal post-transcriptional modification in eukaryotic RNA, it participates in the regulation of gene expression through various mechanisms, such as mRNA splicing, nuclear export, localization, translation efficiency, mRNA stability, and structural transformation. The involvement of m⁶A in the regulation of gene expression depends on the specific recognition of m⁶A-modified RNA by reader proteins. In the pathogenesis and treatment of liver disease, studies have found that the expression levels of key genes that promote or inhibit the development of liver disease are regulated by m⁶A modification, in which abnormal expression of reader proteins determines the fate of these gene transcripts. In this review, we introduce m⁶A readers, summarize the recognition and regulatory mechanisms of m⁶A readers on mRNA, and focus on the biological functions and mechanisms of m⁶A readers in liver cancer, viral hepatitis, non-alcoholic fatty liver disease (NAFLD), hepatic fibrosis (HF), acute liver

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injury (ALI), and other liver diseases. This information is expected to be of high value to researchers deciphering the links between m⁶A readers and human liver diseases.

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Introduction

N⁶-methyladenosine (m⁶A) is methylation at the N6 position of adenosine in RNA. It was first discovered in rat messenger RNA (mRNA),¹ and has been successively found in post-transcriptional mRNA modifications in yeast, plants, flies, humans, and other mammals.^{2–5} By the end of 2021, 335 different chemical modifications in RNA had been identified in all living organisms according to MODOMICS,⁶ and the m⁶A modification is considered one of the most important internal post-transcriptional modifications because of its high abundance. As an epigenetic modification occurring widely in both coding^{1,7,8} and non-coding RNA,^{9,10} the m⁶A modification is involved in regulating cellular physiological and pathological processes, which are dynamically reversible processes jointly regulated by three important types of proteins: writers, erasers, and readers. Writer proteins, including core methyltransferase components (METTL3, METTL14, and METTL16¹¹) and their cofactors (WTAP, RBM15/15 B, CBLL1/HAKAI, VIRMA/KIAA1429, and ZC3H13), promote the deposition of m⁶A on mRNAs.¹² The reversibility of m⁶A relies on erasers, including FTO¹³ and ALKBH5,¹⁴ leading to the removal of m⁶A modification.^{13,14} Writers and erasers are important for maintaining proper m⁶A levels and gene expression in human tissues and cells. However, for m⁶A modification to affect gene expression, alterations to the RNA structure or specific recognition by reader proteins are required. Thus, it is essential to explore the reader proteins involved in this process.

The m⁶A readers include five YTH-containing proteins, heterogeneous nuclear ribonucleoproteins (hnRNPs), insulin-like growth factor 2 mRNA-binding proteins (IGF2BPs), fragile X mental retardation protein (FMRP), eukaryotic initiation factor 3 (eIF3), HuR, CNBP, SND1, and PRRC2A. They identify and interpret m⁶A sites on diverse transcripts to regulate the fate of target mRNAs. Highly modified residues are generated by the complex pathway that forms RNA modifications.¹⁵ These surface transcriptome modifications must be recognized by specialized readers to exert the biological effects of RNA modifications. Similarly, the biological function of m⁶A modification is also inseparable from the role of m⁶A readers. m⁶A reader proteins determine the fate of mRNA by regulating mRNA splicing,^{16–18} nuclear output, mRNA translation,^{19–23} stability,^{24–26} and other mRNA metabolic processes, and thus participate in the process of regulating gene expression. Many functions regulated by m⁶A reader proteins highlight the involvement of m⁶A modification in a range of life processes, such as cell fate determination, cell cycle regulation, cell differentiation, neurogenesis, stress response, and circadian maintenance. Many recent studies have found that the dysregulation of key genes

that promote or inhibit the occurrence of liver disease is always accompanied by abnormal expression of m⁶A readers, and multiple mechanisms of gene expression regulation by m⁶A readers involved in the progression of liver disease have been confirmed. This shows that the gene expression reprogramming of abnormally regulated m⁶A reader proteins provides a potential target for the treatment of liver diseases, and targeting m⁶A readers provides a tool for manipulating m⁶A metabolism in liver diseases.

In this review, we describe known m⁶A readers, summarize recent progress in our understanding of their effects on mRNA metabolism, and focus on the biological roles of m⁶A readers in liver cancer, viral hepatitis, non-alcoholic fatty liver disease (NAFLD), hepatic fibrosis (HF), acute liver injury (ALI), and other liver diseases. In summary, the gene expression reprogramming by dysregulated m⁶A reader proteins offers potential targets for the treatment of liver diseases, while targeted m⁶A readers provide tools to manipulate m⁶A metabolism in liver diseases. This review provides the latest and most comprehensive information for investigators focusing on m⁶A regulation in liver diseases.

m⁶A reader members and recognition mechanisms

Writer proteins install m⁶A at a specific domain on target RNAs, and then reader proteins recognize and preferentially bind the RNA to confer its fate and regulate downstream functions. Therefore, m⁶A readers are particularly important for revealing the biological functions of m⁶A-modified RNAs *in vivo* and their roles in the development of various diseases. Eighteen reader proteins have been discovered since the first one was found in 1974. Reader proteins are divided into direct and indirect readers, based on their ability to combine with m⁶A directly and specifically. The recognition mechanism is shown in [Figure 1](#).

Direct readers recognize m⁶A modifications in two main ways. The first way is that reader proteins containing YTH or Tudor regions directly recognize m⁶A modification sites. The second way is that reader proteins can bind to consistent GGm⁶ ACU motifs through specific recognition.²⁷

The YTH domain reader protein is the most representative direct reader protein. YTH domain-containing proteins are categorized into three classes: YTHDC1, YTHDC2, and the YTHDFs (YTHDF1, YTHDF2, and YTHDF3). All five YTH domain proteins share the same YTH domain, and all of them rely on the YTH domain for their recognition roles.^{28,29} YTHDF1–3 and YTHDC2 are mainly located in the cytoplasm and directly bind to the m⁶A site of target RNA to regulate mRNA splicing, translation, and attenuation. As a nuclear m⁶A reader, YTHDC1 exerts nuclear export of m⁶A-modified cellular RNAs, accelerates the decay of certain

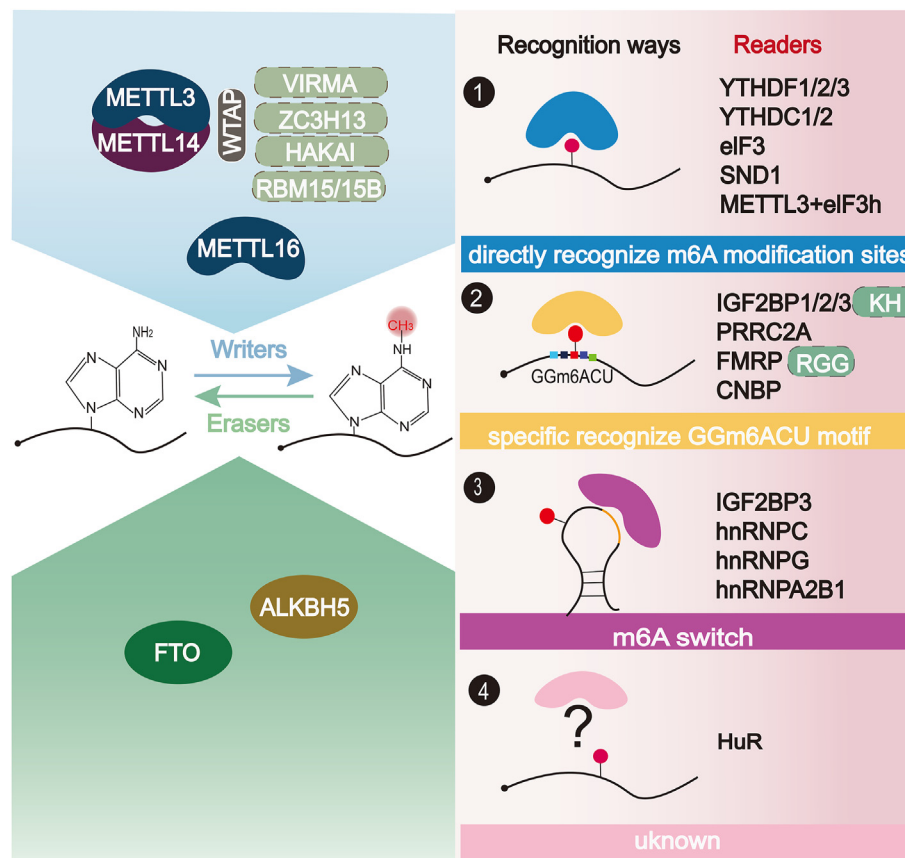


Figure 1 Dynamic and reversible process of m⁶A modification and recognition methods of readers. The methylation of the adenosine N6 site in RNA is accomplished by writers METTL3, METTL14, METTL16, WTAP, RBM15/15 B, VIRMA, HAKAI, and ZC3H1, and the reversal of m⁶A methylation is mediated by erasers FTO and ALKBH5. The m⁶A-modified RNA is recognized by readers in three main ways; they directly combine with m⁶A sites (YTHDF1/2/3, YTHDC1/2, eIF3, SND1, METTL3 + eIF3h), they specifically bind to a consensus GGm⁶ACU motif (IGF2BP1/2/3, PRRC2A, FMRP, CNBP), and they fully expose the internal m⁶A site by opening the “m⁶A switch” (IGF2BP3, hnRNPC, hnRNPG, hnRNPA2B1).

transcripts, and regulates mRNA splicing by recruiting certain splicing factors.^{30–32} Moreover, eIF3 is an m⁶A reader that directly binds to the m⁶A site in the 5' untranslated region (UTR) to drive translation. The identification of eIF3 as an m⁶A reader was originally suggested by the finding that the 48 S complex can be assembled on m⁶A-containing RNA using only eIF1, eIF1A, eIF2, eIF3, and the 40 S subunit. Studies have shown by *in vitro* 48 S reconstitution and m⁶A crosslinking assays that eIF3 preferentially binds to m⁶A residues in their natural sequence context to promote cap-independent translation.³³ Interestingly, acting as a methyltransferase to deposit m⁶A, METTL3 also functions as a direct reader together with eIF3 to identify m⁶A-containing transcripts. METTL3 interacts with eukaryotic translation initiation factor 3 subunit h (eIF3h) to enhance translation when tethered to reporter mRNA at m⁶A sites close to the stop codon.³⁴

Researchers have discovered a novel m⁶A reader protein in nerve cells, PRRC2A, and a new way for the m⁶A reader protein to recognize m⁶A sites. A novel PRRC2A domain, named the GRE domain, specifically binds to the consensus GGm⁶ACU motif to recognize m⁶A-containing transcripts and regulate mRNA stability.³⁵ Similar to PRRC2A, FMRP binds to GGm⁶ACU to regulate gene expression.^{36,37} FMRP,

encoded by the FMR1 gene, is a selective RNA-binding protein associated with translation polysomes.^{38–40} In addition, CNBP, originally thought to be an RNA-binding protein and transcription factor, may also be a novel m⁶A reader. CNBP contains seven highly conserved zinc-finger domains and is involved in RNA transcription, stabilization, and translation.^{41,42} CNBP preferentially binds the GGm⁶ACU site in HeLa cells and mouse 3T3 cells,⁴³ but the mechanism by which CNBP recognizes and binds m⁶A sites requires further study, as CNBP has only been discovered as an m⁶A reader in recent years.

Conversely, indirect readers cannot specifically identify m⁶A-modified sites, and depend on an m⁶A switch, in which m⁶A induces RNA unfolding and enhances the accessibility of its base-paired residues or nearby regions to modulate protein binding.⁴⁴ hnRNPs are a family of RNA-binding proteins (RBPs) prevalent in the human body.⁴⁵ Some of the members of this family act as m⁶A reader proteins, and the process of m⁶A recognition by these reader proteins confirms the above mechanism. hnRNPA2/B1 depends on the m⁶A switch to enhance its affinity to certain adjacent binding sites⁴⁶ and heterogeneous nuclear ribonucleoprotein C (hnRNPC) as an m⁶A reader by binding to a purine-rich motif that becomes unpaired and accessible upon nearby

m⁶A modification.⁴⁷ Similarly, m⁶A increases the accessibility of the surrounding RNA sequence to bind heterogeneous nuclear ribonucleoprotein G (hnRNP G).²⁸ These data indicate that m⁶A affects the binding efficiency of the surrounding sequences and RBPs by changing the structure of RNA.

Among the many m⁶A readers, some reader proteins recognize m⁶A sites by both direct and indirect binding. IGF2BP3 can not only directly recognize m⁶A via a GGm⁶ACU motif but can also act in a manner dependent on the m⁶A structural switch.^{47,48} IGF2BPs are a group of conserved m⁶A readers whose RNA-binding sites contain two RNA recognition motif (RRM) domains and four K-homology (KH) domains. Moreover, the study showed that IGF2BPs depended on KH domains to bind to m⁶A-modified RNA and enhance its stability.^{48,49} In addition, as a newly identified m⁶A reader in recent years, the recognition mechanism of HuR remains unclear. Therefore, further studies of specific identification methods are needed. In conclusion, a variety of recognition methods maximize the potential biological roles of m⁶A, whether directly or indirectly.

m⁶A readers regulate mRNA metabolism

The m⁶A modification is the most abundant and well-studied RNA modification in the epitranscriptome and is involved in various aspects of mRNA metabolism, including mRNA export, translation, stability, and splicing (Table 1).

m⁶A readers regulate mRNA splicing

DNA is transcribed to pre-mRNA, which is converted into mature mRNA with biological function by alternative splicing through the action of various splicing factors.⁶²

m⁶A-modified mRNAs are closely related to m⁶A regulatory factors in the process. In particular, m⁶A readers can indirectly regulate mRNA splicing by interacting with splicing factors (Fig. 2), including exon inclusion and exon skipping. The m⁶A reader YTHDC1 plays an important role in transcriptionally regulated bridging by recruiting different splicing factors; it recruits SRSF3 to the m⁶A site to promote exon inclusion,^{63,30} but antagonizes SRSF10 mRNA binding to facilitate exon skipping.³⁰ Further studies revealed that m⁶A readers can participate in the process of disease development by regulating the alternative splicing of related mRNAs. YTHDC1 can regulate the splicing of tumor suppressor RBM4 to promote or inhibit tumorigenesis.⁶³ hnRNP C itself acts as an effective splicing factor and also acts as an m⁶A reader to recognize m⁶A sites and regulate alternative splicing events to promote PDAC transfer.⁶⁴ In conclusion, the role of m⁶A in the splicing process deserves further attention. There is increasing evidence that splicing disorders are involved in a variety of human diseases, such as indirect disorders that can promote tumorigenesis and cancer development by producing abnormal protein and gene subtypes.

m⁶A readers regulate mRNA nuclear export

Unlike prokaryotes, most of the RNA synthesis in eukaryotic cells takes place in the nucleus, where the newly synthesized RNA is processed. It is then transported to the cytoplasm, where the majority of RNAs participate in different aspects of protein synthesis. However, for all classes of RNA molecules, nuclear export is dependent on the assembly of the RNA into the appropriate ribonucleoprotein complex. Studies have revealed that m⁶A readers can promote the export of mRNA from the nucleus to the cytoplasm (Fig. 2). For instance, the m⁶A reader YTHDC1,

Table 1 The role of m⁶A readers in mRNA metabolism.

m ⁶ A reader	Cellular localization	Mechanism	Reference
IGF2BP1	Nucleus and cytoplasm	Enhances mRNA stability and promotes mRNA translation	48,50
IGF2BP2	Cytoplasm	Enhances mRNA stability and promotes mRNA translation	48,50
IGF2BP3	Nucleus and cytoplasm	Enhances mRNA stability and promotes mRNA translation	48,50
YTHDF1	Cytoplasm	Promotes mRNA translation	51,52
YTHDF2	Cytoplasm	Reduces mRNA stability	51,53
YTHDF3	Cytoplasm	Mediates the translation or degradation	51,53
YTHDC1	Nucleus	Promotes RNA splicing and translocation	30,51,54,55
YTHDC2	Cytoplasm	Enhances the translation of target RNA	51,56
hnRNP C	Nucleus	Participates in the pre-mRNA processing and functions as an "m ⁶ A-switch"	52,57
hnRNP G	Nucleus	Modulates pre-mRNA alternative splicing and acts as an "m ⁶ A-switch"	57
hnRNP A2B1	Nucleus and cytoplasm	Accelerates the processing of primary miRNA, regulates alternative splicing, and acts as an "m ⁶ A-switch"	46,57
eIF3	Cytoplasm	Promotes RNA translation by interacting with YTHDF1	33
METTL3	Nucleus and cytoplasm	Promotes mRNA translation in the cytoplasm	34
Prrc2a	Nucleus and cytoplasm	Unknown	35
FMRP	Nucleus and cytoplasm	Promotes RNA nuclear export	38–40,58
HuR	Cytoplasm	Mediates RNA stability	59
CNBP	Cytoplasm	Unknown	60
SND1	Nucleus and cytoplasm	Mediates RNA stability	61

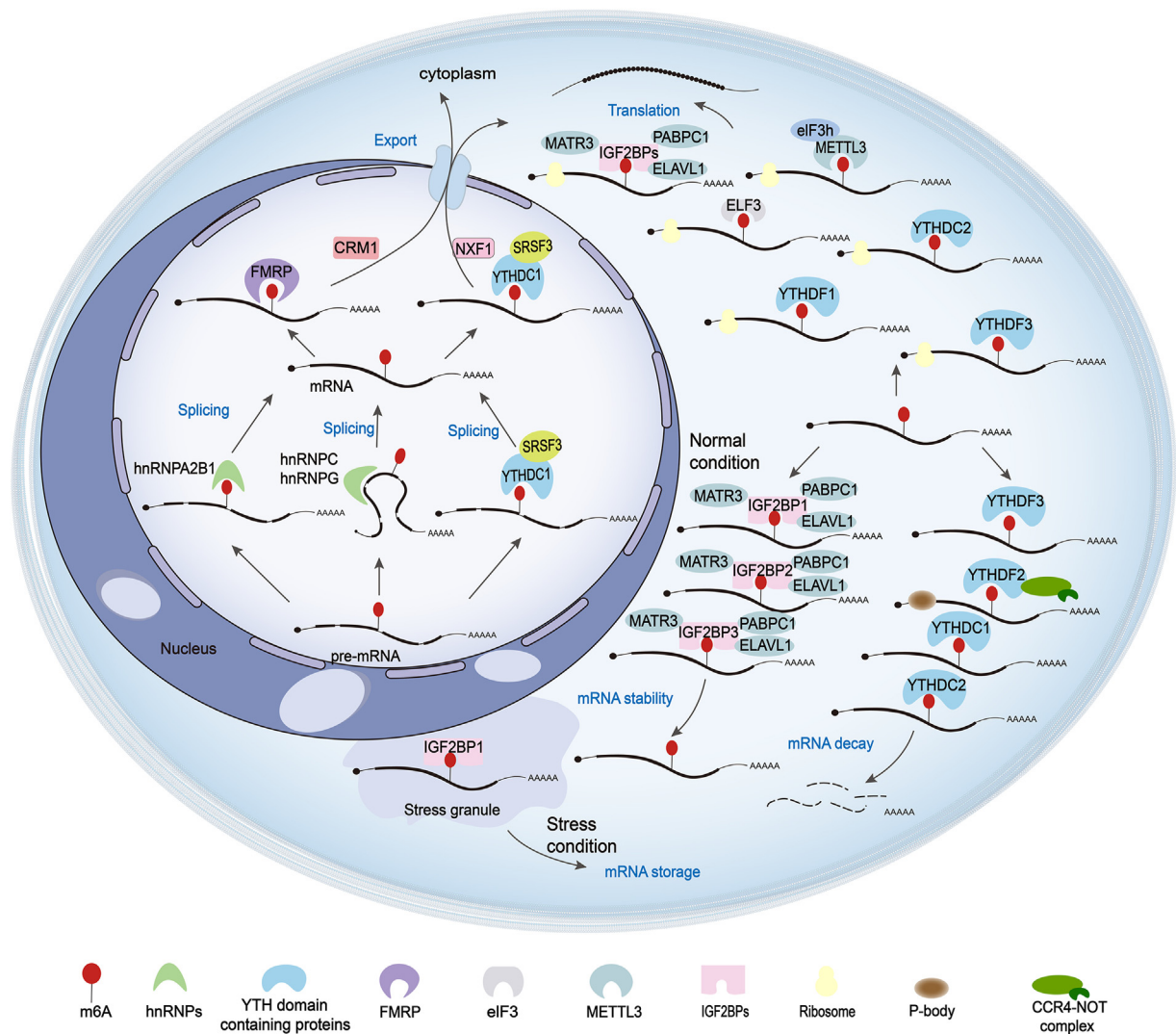


Figure 2 m⁶A readers regulate mRNA metabolism. m⁶A readers are involved in various aspects of RNA metabolism including mRNA splicing, mRNA nuclear export, translation, RNA decay, and RNA stability.

recognizing methylated mRNA, has been found to incorporate target mRNAs into the nuclear export pathway via interaction with SRSF3, allowing mRNA to selectively bind to the nuclear export adaptor protein NXF1 and be exported to the cytoplasm.³² Another reader protein, FMRP, shuttles between the cytoplasm and the nucleus to facilitate the nuclear export of RNAs. FMRP preferentially binds to m⁶A-modified mRNAs and cooperates with the adaptor protein CRM1 to modulate nuclear export of FMRP targets⁶⁵ or interacts with mRNA nuclear export factor NXF2 to export m⁶A-modified transcripts from the nucleus. Taken together, these data indicate that m⁶A readers are required for the transport of some mature mRNA from the nucleus to the cytoplasm.

m⁶A readers regulate mRNA translation

mRNA is processed by the nucleus and transported to the cytoplasm, where it can be loaded into ribosomes for active translation.⁶⁶ The translation efficiency of m⁶A-modified mRNA mainly depends on reader and protein factor

junctions required in the translation process, and m⁶A modifications in different RNA regions play different roles through different modes of action.⁶² m⁶A readers have also been found to enhance mRNA translation in several ways (Fig. 2). On the one hand, reader proteins can recognize the m⁶A modification site that binds the 3' UTR and promote mRNA translation by binding to the translation initiation complex. In the liver, YTHDF1 regulates the translation of EGFR mRNA by binding to the m⁶A site in the 3' UTR of the EGFR transcript, which promotes the development of intrahepatic cholangiocarcinoma.¹⁹ As a protein homolog of YTHDF1, YTHDF2 directly binds the m⁶A modification site of the 6-phosphogluconate dehydrogenase (6PGD) 3' UTR to promote 6PGD mRNA translation in lung cancer cells.⁶⁷ On the other hand, m⁶A localized at the 5' UTR can promote cap-independent translation by relying on reader proteins to recruit eIF3a to nearby translation initiation points. In breast cancer brain metastases, YTHDF3 significantly promotes the binding of eIF3a to m⁶A residues in the 5' UTR of YTHDF3 mRNA, thereby enhancing cap-independent translation.⁶⁸ YTHDC2 promotes gastric cancer progression by

recognizing m⁶A-modified YAP mRNA at the 5' UTR and enhancing YAP mRNA translation.⁶⁹ In addition to YTH proteins, eIF3 serves as an m⁶A reader and promotes cap-independent translation upon induction of cellular stress.³³ Interestingly, in addition to m⁶A writer, METTL3 and METTL16 have a second important role. As a translation-initiation facilitator, METTL16 promotes translation in an m⁶A-independent manner.¹¹ Distinct from METTL16, METTL3 can act as a reader protein promoting the translation of m⁶A-modified RNA in the cytoplasm through an eIF4F-independent mechanism.^{21,34,70} Other readers, such as IGF2BPs, have also been shown to facilitate the translation of m⁶A-modified mRNAs exported to the cytoplasm.⁴⁸

m⁶A readers are involved in the development of many diseases by regulating the translation of related mRNAs. In gastric cancer, YTHDF1 promotes the translation of a key Wnt receptor, frizzled7 (FZD7), in an m⁶A-dependent manner. The increased expression of FZD7 leads to the overactivation of the Wnt/ β -catenin pathway, promoting the occurrence of gastric cancer,⁷¹ and promotes USP14 protein translation, which promotes the occurrence and metastasis of gastric cancer in an m⁶A-dependent manner.⁷² This may provide a potential target for gastric cancer treatment. YTHDF1 also induces intestinal epithelial inflammatory injury by promoting NLRP3 translation⁷³ and mediates the intestinal immune response by regulating TRAF6 translation.⁷⁴ In addition to participating in the occurrence and development of digestive system diseases, YTHDF1 not only promotes the translation of FOXM1 in an m⁶A-dependent manner to promote the progression of breast cancer,⁷⁵ but also regulates the translation of related mRNAs and participates in the occurrence and development of ovarian cancer,⁷⁶ bone marrow mesenchymal stem cell osteogenesis,⁷⁷ lung cancer,⁶⁷ and other diseases. Another m⁶A reader protein, YTHDC2, contributes to colon tumor metastasis by promoting the translation of HIF-1 α .⁷⁸

m⁶A readers regulate mRNA stability

The RNA exported from the nucleus is translated to produce proteins, which can also be sorted into messenger ribonucleoprotein centers, such as processing bodies (P-bodies) and stress granules, for degradation or storage.⁶⁶ m⁶A-modified mRNA in the cytoplasm may be degraded by the action of some readers (Fig. 2). YTHDF2 can specifically recognize the m⁶A site on mRNA and promote its degradation. YTHDF2 is composed of an N-terminal domain and a C-terminal domain which is an RNA-binding domain.⁵¹ After YTHDF2 recognizes m⁶A-modified mRNA through the N-terminal domain, this domain is also responsible for localizing the YTHDF2–mRNA complex to P-bodies and recruiting the CCR4–NOT complex to trigger the adenylation and degradation of transcripts.^{51,79} YTHDF2 is involved in a variety of diseases by regulating RNA degradation. For example, YTHDF2 promotes intrahepatic cholangiocarcinoma progression and desensitizes cisplatin treatment by increasing CDKN1B mRNA degradation,⁸⁰ and YTHDF2-mediated mRNA degradation is also involved in the regulation of lipid metabolism⁸¹ and the occurrence and development of prostate cancer⁸² and other diseases. Interestingly, YTHDF3, alone or in interaction with YTHDF1, stabilizes m⁶A-containing mRNAs and promotes their translation, while YTHDF3

interacts with YTHDF2 to accelerate the decay of m⁶A-containing mRNAs,⁸³ and FMRP regulates the stability of its target RNAs through interaction with YTHDF2.³⁷ YTHDF3 facilitates triple-negative breast cancer progression and metastasis by stabilizing ZEB1 mRNA in an m⁶A-dependent manner.²⁶ In addition to regulating alternative splicing and nuclear export, YTHDC1 also promotes RNA degradation to regulate disease.⁸⁴ In contrast to YTHDF2 and YTHDC1, IGF2BPs contain two RNA recognition motifs (RRMs) and KH domains. KH domain-containing proteins can regulate mRNA stability.⁸⁵ IGF2BPs can bind m⁶A-modified mRNAs through the KH domain, protect m⁶A-modified mRNAs in P-bodies and stress granules from degradation by interacting with ELAV-like RNA binding protein 1 (ELAVL1, also known as HuR), MATR3 (matrin 3), and poly(A) binding protein cytoplasmic 1 (PABPC1),^{50,86} and facilitate mRNA translation⁸⁷ and enhance its stability.^{48,66}

Aberrant expression of m⁶A readers in liver disease

Expression changes in m⁶A regulators have been confirmed to cause obvious pathological and physiological aberrations in liver function. Emerging data have suggested that the aberrant expression of m⁶A readers observed in many types of liver diseases could be strongly associated with the progression and treatment outcomes of liver diseases (Fig. 3).

m⁶A readers in liver cancer

Liver cancer is one of the most common and fatal cancer types in the world.⁸⁸ Among various subtypes of liver cancer, HCC has attracted worldwide attention due to its characteristics of high recurrence rate, high drug resistance, and poor prognosis.^{89–91} Existing studies have indicated that m⁶A is essential for HCC proliferation and progression.⁹²

It was found that IGF2BPs were significantly up-regulated in HCC and indicated a poor prognosis (Table 2).⁴⁸ lnc-CTHCC is regulated by METTL3-mediated m⁶A modification and recognized by IGF2BP1/IGF2BP3; IGF2BP1/IGF2BP3 increases the stability of lnc-CTHCC and maintains its high expression in HCC, thus accelerating the development of the lnc-CTHCC–HNRNP-K–YAP oncogenic axis and promoting the occurrence and progression of hepatocellular carcinoma.⁹³ The role of IGF2BP1 in maintaining the self-renewal of liver cancer stem cells and chemotherapy resistance has been confirmed.^{87,94} IGF2BP1 directly binds to MGAT5 and promotes the stability of MGAT5 mRNA by up-regulating the m⁶A modification of MGAT5 mRNA.⁸⁷ MGAT5 is associated with it with the metastases of cancer stem cells.^{95,96} Deficiency of ALKBH5 leads to an elevated m⁶A level of LYPD1, IGF2BP1 can recognize and stabilize LYPD1 that promotes the proliferation and invasion capabilities of HCC cells, and further drives the tumorigenesis of HCC.⁹⁷ Rbm15-mediated modification of m⁶A promotes post-transcriptional activation of YES proto-oncogene 1 (YES1) in an IGF2BP1-dependent manner and thus plays an important role in promoting proliferation and invasion of HCC.⁹⁸ Overexpression of IGF2BP2 promotes the proliferation of HCC *in vitro* and *in vivo*, and IGF2BP2 recognizes and stabilizes FEN1 mRNA to play a carcinogenic role.⁹⁹ IGF2BP3

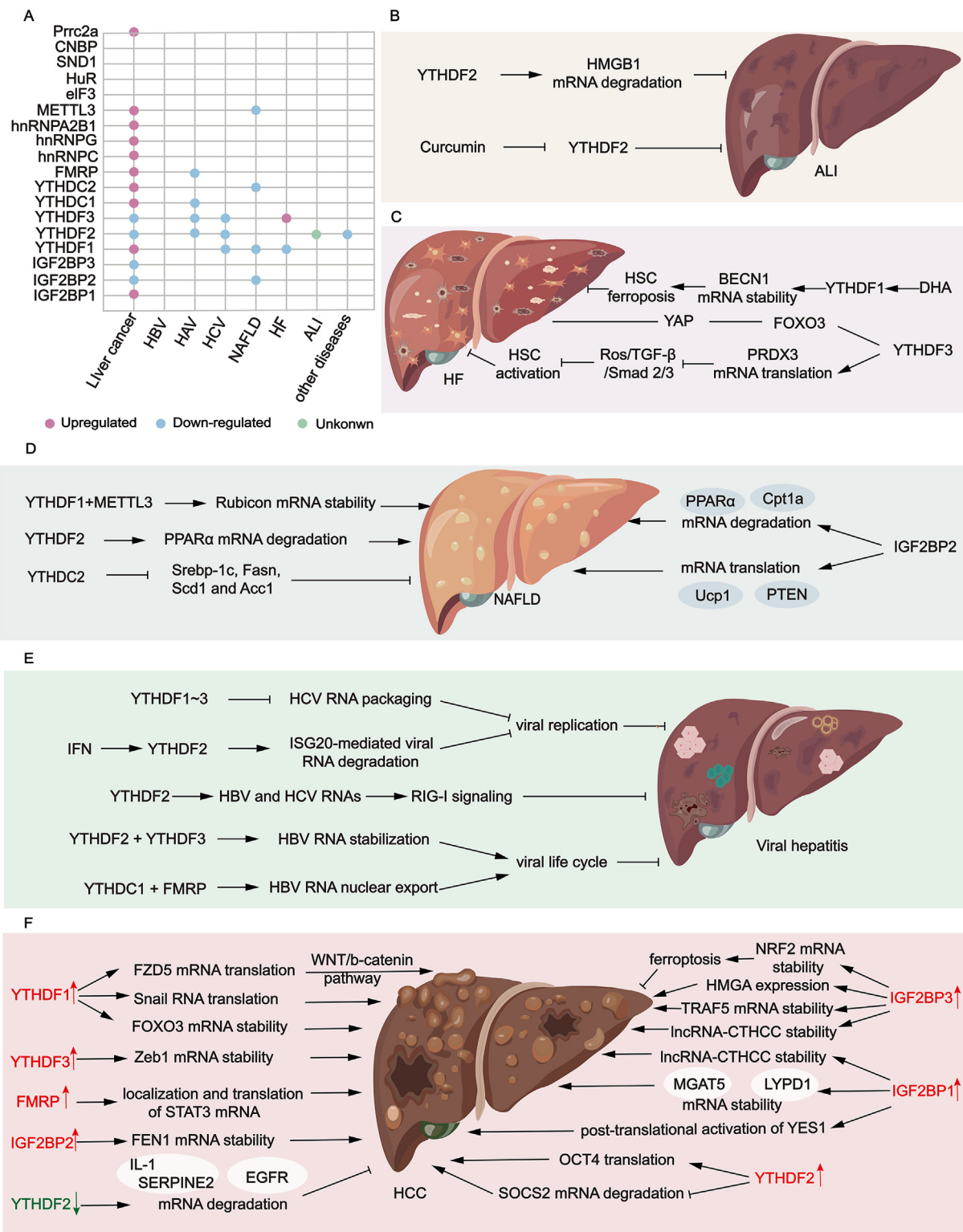


Figure 3 Expression and regulatory mechanism of m⁶A readers in different liver diseases. **(A)** Deregulation of m⁶A readers in various liver diseases. **(B–F)** The specific mechanism of m⁶A readers regulating mRNA metabolism to inhibit or promote the occurrence of various liver diseases. Modifiers in red indicate an oncogenic role and modifiers in green indicate a tumor-suppressive role.

binds to LINC00467 to enhance the stability of TRAF5 mRNA in HCC, thereby mediating HCC progression.¹⁰⁰ Recent studies have demonstrated that the IGF2BP3-NRF2 axis is a key iron decline regulator of Sorafenib on HCC cells.¹⁰¹

IGF2BP3 inhibits ferroptosis by promoting NRF2 mRNA stability in an m⁶A-dependent manner.¹⁰¹

Many studies have suggested that aberrant expression of YTH proteins in HCC could be strongly associated with

Table 2 Functional characterization of m⁶A readers in HCC.

m ⁶ A readers	Expression change	Role	Function	Target RNA	Target RNA change	Mechanism	Reference
IGF2BP1	Up-regulated	Oncogene	Promotes HCC growth and metastasis	lncRNA-CTHCC	Up-regulated	Stabilizes lncRNA-CTHCC and increases its expression	93
			Promotes the liver cancer stem cell phenotype	MGAT5	Up-regulated	Increases MGAT5 mRNA stability and its expression	87
			Promotes the growth and migration/invasion capability of HCC cells	YES1	Up-regulated	IGF2BP1 promotes post-transcriptional activation of YES1	98
			Promotes the proliferation and invasion capabilities of HCC cells	LYPD1	Up-regulated	IGF2BP1 stabilizes LYPD1 and increases its expression	97
			Promotes tumor cell proliferation colony formation ability and cell migration/invasion	MYC, FSCN1, and TK1	Up-regulated	Enhances the stability of MYC, FSCN1, and TK1	48
			Promotes tumor cell proliferation and invasion	SRF	Up-regulated	Enhances SRF mRNA stability	123
			Promotes HCC proliferation	FEN1	Up-regulated	Stabilizes FEN1 mRNA	99
IGF2BP2	Up-regulated	Oncogene	Promotes HCC proliferation	lncRNA-CTHCC	Up-regulated	Stabilizes lncRNA-CTHCC	93
IGF2BP3	Up-regulated	Oncogene	Promotes HCC growth and metastasis				
			Promotes the migration and invasion of tumor stem cells	HMGA2	Up-regulated	Enhances the expression of HMGA2	87,124,125
			Promotes HCC proliferation and metastasis	TRAF5	Up-regulated	Binds with LINC00467 to enhance the mRNA stability of TRAF5	100
			Promotes ferroptosis in HCC cells	NRF2	Up-regulated	Enhances NRF2 mRNA stability	101
YTHDF1	Up-regulated	Oncogene	Regulates EMT progression	Snail	Up-regulated	Promotes translation of Snail	126
			Enhances resistance to sorafenib	FOXO3	Up-regulated	Increases the stability of FOXO3 mRNA	107
			Promotes HCC proliferation and metastasis	FZD5	Up-regulated	Promotes FZD5 mRNA translation and activates the WNT/ β -catenin signaling pathway	52,102–106
YTHDF2	Down-regulated	Tumor suppressor	YTHDF2 silencing provokes inflammation, vascular reconstruction, and metastatic progression of HCC	IL-11, SERPINE2	Not mentioned	Increases the degradation of IL-11 and SERPINE2	105,106
			Suppresses HCC cell proliferation and growth and induces apoptosis	EGFR	Not mentioned	Increases the degradation of EGFR	108

	Up-regulated	Oncogene	Promotes HCC proliferation and migration	SOC52	Up-regulated	Interacts with METTL3 to decrease SOC52 mRNA degradation	109,110
YTHDF3	Up-regulated	Oncogene	Promotes the liver cancer stem cell phenotype and cancer metastasis	OCT4	Upregulated	Promotes OCT4 expression and protein translation	110,111
	Up-regulated	Oncogene	Facilitates HCC migration and invasion, and the EMT process	ZEB1	Up-regulated	Enhances ZEB1 mRNA stability	115
FMRP	Up-regulated	Oncogene	Promotes HCC migration	STAT3	Up-regulated	Facilitates the localization and translation of STAT3 mRNA	127

cancer progression and treatment outcomes (Table 2). YTHDF1, which acts as an oncogene, is significantly over-expressed in HCC and plays an important role in the development of HCC by regulating m⁶A-dependent mRNA translation and enhancing the stability of m⁶A methylated mRNA. YTHDF1 accelerates the translation and output of FZD5 mRNA through the m⁶A mechanism and promotes the proliferation and metastasis of HCC cells through the WNT/b-catenin pathway as an oncogene.^{52,102–106} YTHDF1 disrupts the stability of FOXO3 in the absence of METTL3, thus enhancing the resistance to sorafenib.¹⁰⁷ Down-regulation of YTHDF2 plays the role of tumor suppressor,^{104–106,108} while up-regulation of YTHDF2 has the opposite effect.^{109–111} As a tumor suppressor, YTHDF2 is specifically down-regulated by hypoxia in HCC cells. It directly binds to the m⁶A modification site of EGFR 3'UTR and negatively regulates the stability of EGFR mRNA, suppressing cell proliferation and growth.¹⁰⁸ In contrast, YTHDF2 expression is up-regulated in liver cancer cells. YTHDF2 recognizes m⁶A modification sites and promotes OCT4 protein translation, significantly accelerating HCC development and cancer metastasis.^{110,111} Recent studies have indicated that m⁶A modification methylation regulators have the potential to regulate tumor microenvironment and m⁶A regulators might affect PD-L1 expression and immune cell infiltration in HCC patients.^{112–114} The mRNA and protein expressions of YTHDF2 in LIHC tissues are significantly increased, and YTHDF2 can regulate the polarization of tumor-related macrophages, induce T cell exhaustion, and activate T regulatory cells.¹⁰⁵ As a synergetic factor of YTHDF1 in protein synthesis, YTHDF3 enhances the stability and lifetime of m⁶A-methylated ZEB1 mRNA.¹¹⁵ Up-regulated ZEB1 mediates Circ-KIAA1429 expression, leading to a robust driving force for HCC migration, invasion, and epithelial–mesenchymal transition.¹¹⁵

A large number of m⁶A readers are related to the development of HCC (Table 2). Another reader protein, FMRP, is responsible for the localization of STAT3 mRNA to cell protrusion by interacting with the 3'UTR of STAT3 mRNA, promotes IL-6-mediated STAT3 translation, and promotes HCC metastasis.¹¹⁰ hnRNPs overexpression is also associated with hepatocellular carcinoma metastasis^{111–115}; hnRNP A2B1 is significantly overexpressed in HCC and induces epithelial–mesenchymal transition and epithelial–mesenchymal transition. Snail is a key transcription factor of EMT,^{111,112} YTHDF1 cooperates with METTL3 to enhance snail translation efficiency and accelerate its protein expression, promoting the metastasis of hepatocellular carcinoma.¹¹⁶ In addition, the expression of YTHDC1 and YTHDC2,^{116,117} hnRNPs,^{117–121} and prrc2a¹²² in HCC tumor tissues were higher than those in normal liver tissues, but their roles and mechanisms in HCC remain to be studied.

In conclusion, dysregulation of m⁶A readers plays a crucial role in promoting or suppressing the development of HCC. Hence, they are considered potential targets for prognostic prediction and molecular therapy in HCC.

m⁶A readers in viral hepatitis

The term viral hepatitis refers to liver inflammation related to a viral infection.¹²⁸ Viral hepatitis, secondary to

infection with hepatitis A,¹²⁹ B,¹³⁰ C, D, and E viruses, is a major public health problem and an important cause of morbidity and mortality.¹²⁸ m⁶A modification occurs in cellular RNAs and viral transcripts and regulates the fate of cellular and viral RNAs. In recent years, the role of m⁶A modification in viral hepatitis has gradually attracted the attention of researchers.

Recently, several studies have shown that m⁶A methylation sites have been identified in hepatitis B virus (HBV) transcripts and the hepatitis C virus (HCV) RNA genome.¹³¹ The m⁶A modification can affect viral life cycles in a complex way. On the one hand, m⁶A can play an antiviral or proviral role by recruiting different m⁶A-binding proteins to affect RNA nuclear export during the viral life cycle. YTHDC1 and FMRP, regulating the nuclear export of m⁶A modification, modify HBV transcripts and affect the viral life cycle.¹³² On the other hand, m⁶A reader proteins can affect RNA stability by recognizing the m⁶A modification site of HBV RNA, thereby regulating the expression of viral lifecycle-related proteins. HBV transcripts are m⁶A methylated at an m⁶A consensus motif located within the 5' or 3' stem-loop region present in all HBV RNAs.^{133,134} YTHDF2 and YTHDF3 recognize the m⁶A modification at the 3' stem-loop of HBV RNA transcripts and reduce HBV RNA stabilization, resulting in decreased viral protein expression.¹³¹ In addition, m⁶A can indirectly affect viral replication by regulating the expression of specific genes involved in the viral life cycle. In the process of IFN treatment of HBV, YTHDF2 recognizes ISG20 induced by IFN treatment, which promotes the reduction of viral replication by promoting ISG20-mediated degradation of viral RNA with m⁶A modifications.^{135,136} YTHDF family reader proteins inhibit HCV replication by competing for binding to the Envelope to prevent virus packaging.¹³⁷ Interestingly, YTHDF1–3 proteins recognize the m⁶A-methylated HCV genome and localize HCV RNAs to the lipid droplet fraction to inhibit HCV RNA packaging into virions without affecting viral replication or protein translation.¹³⁸ YTHDF2 interacts with m⁶A sites within RIG-I ligand regions of the HBV and HCV RNAs and inhibits RIG-I signaling, thus participating in HBV and HCV infection.^{131,139–141} These studies demonstrate that m⁶A modification, especially m⁶A reader proteins, plays an important regulatory role in HBV and HCV. In the study of duck hepatitis, ¹⁴² it was confirmed that post-transcriptional regulation of specific transcripts by m⁶A correlates with the virulence of the hepatitis A virus and induces distinct viral responses by promoting the expression of immune regulatory genes during viral infection. However, the methyltransferases that catalyze m⁶A modifications and the reader proteins that recognize m⁶A modifications in hepatitis A are not known.

To sum up, compared with HBV and HCV, the role of m⁶A modification in hepatitis caused by other viruses has been poorly studied. It will be interesting to further investigate the function of m⁶A modification in various types of viral hepatitis. YTH domain proteins affect viral lifecycles and viral replication by regulating RNA metabolism, such as viral RNA stability and nuclear export, which play important regulatory roles in viral hepatitis. It is not clear whether other m⁶A reader proteins play a regulatory role in the development of viral hepatitis.

m⁶A readers in NAFLD

NAFLD is characterized by hepatic steatosis, with excessive accumulation of lipids caused by *de novo* fatty acid (FA) synthesis in hepatocytes. This may develop into non-alcoholic steatohepatitis (NASH), which may lead to cirrhosis and finally HCC.¹⁴³ The functions of m⁶A readers in NAFLD remain largely unknown, and the possible functions are discussed below.

On the one hand, YTH protein regulates hepatic triacylglyceride (TG) homeostasis and lipid synthesis in the liver by mediating RNA metabolism, which may be a target for the treatment of NAFLD. Imbalanced TG homeostasis plays a critical role in the development of NAFLD. YTHDC2 may recognize and bind to m⁶A-modified mRNA, thus mediating the mRNA stability of lipogenic genes to regulate hepatic TG homeostasis.¹⁴⁴ Abundant lysosomes and high levels of autophagy in the liver play an important role in maintaining the homeostasis of lipid metabolism in the liver.^{145–148} Rubicon, a Beclin1-interacting negative regulator for autophagosome-lysosome fusion, inhibits autophagy and accelerates hepatocyte apoptosis and lipid accumulation in NAFLD.¹⁴⁸ YTHDF1, as a partner of METTL3, promotes Rubicon mRNA stability and contributes to the inhibition of autophagic flux and accumulation of LDs.¹⁴⁹ On the other hand, studies have confirmed that abnormally expressed IGF2BP2 can promote the development of NASH and may promote the development of NAFLD to HCC,^{150,151} and can participate in disease occurrence by regulating the expression of related genes. For instance, liver-specific IGF2BP2 overexpression induces steatosis in mice by attenuating PTEN expression and increasing AKT activation downstream.¹⁵² Meanwhile, RNA metabolism regulated by IGF2BP2 also plays an important role in the occurrence of NAFLD. Global IGF2BP2/IMP2 knockout mice are resistant to diet-induced fatty liver disease because of up-regulated Ucp1 mRNA translation,⁴⁹ and in HFD-induced fatty liver, IGF2BP2 may play a role in the degradation of PPAR α and CPT1A mRNA to regulate triglyceride accumulation.¹⁵³ In addition, the circadian clock also affects hepatic lipid metabolism via YTHDF2-mediated PPAR α mRNA decay,⁸¹ which provides new insight into the role of m⁶A readers in the association between the circadian clock and metabolic diseases. These findings provide insights into the underlying molecular mechanisms and evidence that targeting m⁶A readers may have the potential for the development of new strategies in the prevention and treatment of NAFLD and NASH. However, there are few studies on the regulation of m⁶A readers in the occurrence and development of NAFLD, and the association of m⁶A readers with the pathogenesis of NAFLD requires further exploration.

m⁶A readers in HF

Hepatic fibrosis is the result of scar tissue formation in the liver following long-term liver injury and is a critical stage for the development of liver disease into cirrhosis, liver failure, and hepatocellular carcinoma. Evidence is emerging that m⁶A modification participates in HF by regulating the expression of related genes. In mice, DHA treatment alleviated HF by triggering HSC ferroptosis.

YTHDF1 promoted BECN1 mRNA stability in an m⁶A-dependent manner, which in turn induced DHA-mediated ferroptosis.¹⁵⁴ Peroxiredoxin 3 (PRDX3) acts as a master regulator of mitochondrial oxidative stress and exerts hepatoprotective effects. PRDX3 suppresses HSC activation by regulating the mitochondrial ROS/TGF- β 1/Smad2/3 pathway. The m⁶A reader YTHDF3 specifically regulates PRDX3 translation and expression, and HSC-specific YTHDF3 overexpression markedly attenuates CCL4-induced HF, mainly by up-regulating PRDX3 expression.¹⁵⁵ Thus, regulation of the YTHDF3/PRDX3 axis might be a potential therapeutic approach for HF. However, the functions of YTHDF3 in HF might be a global effect related to other targets, such as the fibrosis-related regulators FOXO3 and YAP,^{155–157} and this requires further exploration. Our review may contribute to a deeper understanding of the role of m⁶A readers and ferroptosis in HF. The identified modulation of m⁶A modification provides new insights into the mechanism of DHA against HF, which may be used for further in-depth research on drugs for the prevention and treatment of HF.

m⁶A readers in ALI

The pathogenesis of liver injury is very complex, and there are many factors leading to liver injuries, such as radiation, oxidative stress, cytokines, drugs, and metabolic diseases. m⁶A readers are closely related to the occurrence and treatment of ALI. In irradiation-induced liver injury, YTHDF2 can recognize m⁶A-methylated HMGB1 mRNA and promote its degradation, thus attenuating radiation-induced liver disease.¹⁵⁸ Drug-induced liver injury (DILI) remains the most common cause of acute liver failure (ALF) in the Western world.¹⁵⁹ During the treatment of lipopolysaccharide-induced liver injury by curcumin, m⁶A modification enzymes were abnormal, and the expression levels of METTL3 and METTL14 were increased, while the expression levels of the demethylase and reader protein YTHDF2 were decreased. The protective effect of curcumin on lipopolysaccharide-induced liver injury and hepatic lipid metabolism disorder may be related to the increased m⁶A.¹⁶⁰ However, the role of YTHDF2 in the treatment process and the specific mechanism need to be studied further. Acute liver injury is characterized by inflammation and sudden abnormal liver function. Studies have confirmed that m⁶A reader proteins play a role in regulating inflammation. In LPS-stimulated RAW 264.7 cells, YTHDF2 expression was decreased, and target gene MAP2K4 and MAP4K4 mRNA stability and expression were increased, which consequently triggered the activation of p38, ERK, and NF- κ B signaling, thus promoting the expression of TNF- α , IL-1 β , IL-6, and IL-12.¹⁶¹ Therefore, we suggest that reader proteins such as YTHDF2 may be potential targets for regulating the inflammatory response pathway in ALI and thus play a protective role in the liver.

m⁶A readers in other liver diseases

The liver is the largest digestive and metabolic organ and can be affected by different factors. Sustained damage will lead to various liver diseases. However, abnormal expression of m⁶A readers has been found in many studies on the

occurrence and treatment of liver diseases. The cause of this abnormal expression of m⁶A readers and the resulting responses are of interest. For example, tristetraproline (TTP) can be recruited to the promoter regions of YTHDF2 gene loci and promote their transcription in hepatocytes. TTP relies on YTHDF2 to recognize the m⁶A modification sites on CCL2 and CCL5 mRNA, thereby regulating CCL2 and CCL5 to prevent acute liver failure.¹⁶² m⁶A readers largely determine their roles in the progression of liver diseases. In addition, m⁶A readers play a regulatory role in the induction of many liver diseases. For example, FMRP can control cell death in liver diseases¹⁶³ and selective exosomal miRNA loading during inflammation.¹⁶⁴ However, the specific regulatory mechanism remains unclear.

Conclusions

This paper is the first to comprehensively summarize the current research on m⁶A reader proteins in liver diseases. In normal tissues, m⁶A readers regulate target RNA metabolism to maintain normal life activities. Abnormal expression of m⁶A readers in liver disease tissues leads to metabolic disorders of disease-related factors dependent on m⁶A modification, which leads to accelerated or delayed disease development. This review summarized the current research and concluded that the role and mechanism of reader proteins in regulating RNA metabolism are related to its expression level, enrichment site, biological environment, and cooperative m⁶A regulatory factors. It is essential to identify the intrinsic and extrinsic signals that regulate the recruitment of readers to m⁶A-modified mRNAs and to identify these transcripts in a specific biological environment. In addition, inhibition or overexpression of reader proteins shows therapeutic potential for treating liver diseases by regulating gene expression of disease-related factors, either alone or in combination with other m⁶A regulators. Therefore, the identification or development of small-molecule agents targeting the m⁶A reader to block its interaction with m⁶A-modified mRNAs or other m⁶A regulatory proteins has important medical value. These small molecules may be a potential way to inhibit the abnormal regulation of mRNA metabolism by m⁶A reading proteins in liver diseases.

Future perspectives

Recently, N⁶-methyladenosine, as an important epigenetic modification, has attracted attention for its role in the pathogenesis and treatment of liver diseases. As direct executors for m⁶A-dependent bioprocesses, m⁶A readers can link RNA modifications at specific sites with specific regulatory functions in cells, and their function in liver diseases has attracted attention. This paper summarizes many scientific studies on the abnormal expression of m⁶A readers in liver diseases and the regulation of disease progression, which confirms the important role of m⁶A reader proteins in promoting or inhibiting the development of liver diseases. Therefore, this article reviews the specific proteins, recognition mechanisms, and roles of m⁶A readers in liver diseases, providing a basis for researchers to study m⁶A further as a therapeutic target for liver diseases. There may be the potential in the future to control the

development of liver diseases by regulating the expression of related m⁶A reader proteins *in vivo*, providing a new effective strategy for the clinical treatment of liver diseases. Although research on related agents that regulate m⁶A reader proteins has just begun and numerous challenges remain, this is a very promising avenue of research and we eagerly anticipate future developments.

Author contributions

Lijiao Sun, Xin Chen, and Sai Zhu: writing original draft; Jianan Wang and Xiaofeng Li: visualization, investigation; Shaoxi Diao and Yujin Liu: conceptualization and data curation; Jinjin Xu and Yingyin Sun: investigation and methodology; Cheng Huang and Xiaoming Meng: conceptualization and supervision; Xiongwen Lv and Jun Li: validation, writing, reviewing, and editing.

Conflict of interests

The authors declare no conflict of interests.

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