




REVIEW

N⁶-methyladenosine Steers RNA Metabolism and Regulation in Cancer

Shenghua Dong¹  | Yutong Wu¹ | Yadi Liu¹ | Hengyou Weng²  | Huilin Huang¹ 

¹ Sun Yat-sen University Cancer Center, State Key Laboratory of Oncology in South China, Collaborative Innovation Center for Cancer Medicine, Guangzhou, Guangdong 510060, P. R. China

² Bioland Laboratory, Guangzhou Regenerative Medicine and Health Guangdong Laboratory, Guangzhou 510005, P. R. China

Correspondence

Huilin Huang, Sun Yat-sen University Cancer Center, State Key Laboratory of Oncology in South China, Collaborative Innovation Center for Cancer Medicine, Guangzhou 510060, Guangdong, P. R. China

Email: huanghl1@sysucc.org.cn

Hengyou Weng, Bioland Laboratory, Guangzhou Regenerative Medicine and Health Guangdong Laboratory, Guangzhou, 510005, P. R. China

Email: weng_hengyou@grmh-gdl.cn

Funding information

Guangdong Basic and Applied Basic Research Foundation, Grant/Award Number: 2021A1515010425; National Key R&D Program of China, Grant/Award Number: 2020YFA0112403; Ministry of Science and Technology of the People's Republic of China

Abstract

As one of the most studied ribonucleic acid (RNA) modifications in eukaryotes, N⁶-methyladenosine (m⁶A) has been shown to play a predominant role in controlling gene expression and influence physiological and pathological processes such as oncogenesis and tumor progression. Writer and eraser proteins, acting opposite to deposit and remove m⁶A epigenetic marks, respectively, shape the cellular m⁶A landscape, while reader proteins preferentially recognize m⁶A modifications and mediate fate decision of the methylated RNAs, including RNA synthesis, splicing, exportation, translation, and stability. Therefore, RNA metabolism in cells is greatly influenced by these three classes of m⁶A regulators. Aberrant expression of m⁶A regulators has been widely reported in various types of cancer, leading to cancer initiation, progression, and drug resistance. The close links between m⁶A and cancer shed light on the potential use of m⁶A methylation and its regulators as prognostic biomarkers and drug targets for cancer therapy. Given the notable effects of m⁶A in reversing chemoresistance and enhancing immune therapy, it is a promising target for combined therapy. Herein, we summarize the recent discoveries on m⁶A and its regulators, emphasizing their influences on RNA metabolism, their dysregulation and impacts in diverse malignancies, and discuss the clinical implications of m⁶A modification in cancer.

KEYWORDS

cancer therapy, chemoresistance, immunotherapy, m⁶A methylation, oncogenesis, prognostic biomarkers, RNA epigenetics, RNA metabolism

1 | BACKGROUND

Analogous to deoxyribonucleic acid (DNA) and protein, RNA has more than 100 chemical modifications, which tremendously propels our understanding on gene expression control [1]. The most remarkable RNA modification is

N⁶-methyladenosine (m⁶A), methylated adenosine at the N⁶ position, which was first discovered in the 1970s [2, 3]. Although m⁶A is one of the most abundant messenger RNA (mRNA) modifications in mammals, its significance was not fully acknowledged until the identification of *fat mass and obesity-associated protein (FTO)* as a

This is an open access article under the terms of the [Creative Commons Attribution-NonCommercial-NoDerivs](https://creativecommons.org/licenses/by-nc-nd/4.0/) License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2021 The Authors. *Cancer Communications* published by John Wiley & Sons Australia, Ltd. on behalf of Sun Yat-sen University Cancer Center

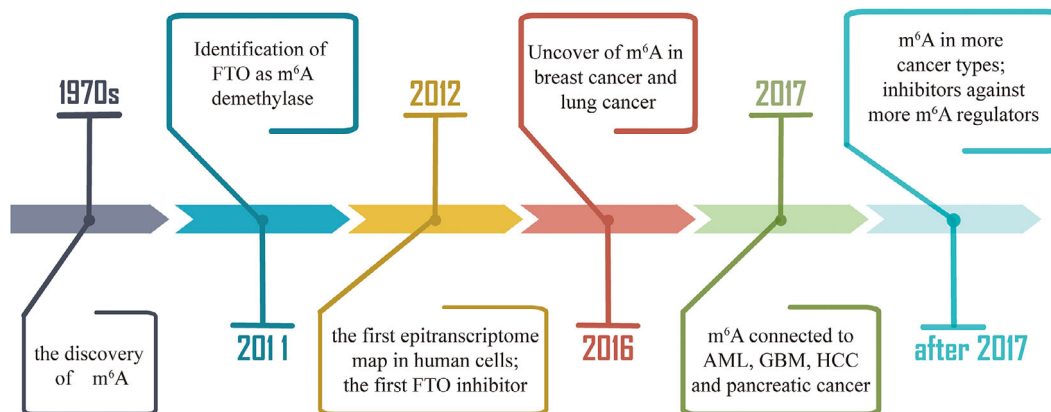


FIGURE 1 The timeline of RNA epigenetics. m⁶A was first discovered in the 1970s. In 2011, FTO was identified as an m⁶A demethylase. In 2012, the antibody-based transcriptome-wide sequencing method was developed to obtain m⁶A profiling in the human transcriptome. The first *FTO* inhibitor was found in the same year. Association of m⁶A with cancer began to be reported in breast cancer and lung cancer in 2016, and the cancer types expanded to AML, GBM, HCC, and pancreatic cancer in 2017. Up to now, m⁶A has been found to play critical roles in most cancer types, and inhibitors against more m⁶A regulators are in development. Abbreviations: RNA, ribonucleic acid; m⁶A, N⁶-methyladenosine; FTO, fat mass and obesity-associated protein; AML, acute myeloid leukemia; GBM, glioblastoma; HCC, hepatocellular carcinoma

demethylase and the advent of transcriptome-wide m⁶A mapping techniques that depicts the full scope of m⁶A profile (Figure 1) [4, 5]. Next-generation sequencing (NGS) revealed that the distribution of m⁶A on mRNA is widespread and not random. The consensus sequence RRACH (R indicates guanosine (G) or adenosine (A), while H indicates A, cytidine (C) or uridine (U)) and the enrichment in certain regions (3' untranslated region and coding sequence) are common characteristics of the m⁶A epitranscriptome [4, 5]. Owing to the high abundance and reversible feature of m⁶A, more attention has been gained to the wide-ranging regulation of m⁶A in physiological and pathological processes, especially in oncogenesis and tumor progression. Given the important roles of m⁶A in cancer, we discuss the functions of m⁶A and its regulators in RNA metabolism control, their oncogenic or tumor-suppressive roles in diverse malignancies, as well as the potential application of m⁶A methylation in cancer diagnosis and therapeutics.

2 | m⁶A AND ITS REGULATORS IN RNA METABOLISM

The m⁶A modification is critical for RNA fate decision as it can influence almost all aspects of RNA metabolism, including synthesis (i.e. transcription), splicing, nuclear exportation, translation, and degradation. In this section, we summarize m⁶A regulators and their functions in RNA metabolism (Figure 2 and Table 1).

2.1 | m⁶A regulators

The m⁶A modification on mRNA is installed by the m⁶A methyltransferase complex (MTC, also known as m⁶A “writers”). A heterodimer consisting of *methyltransferase-like 3* (*METTL3*) and *methyltransferase-like 14* (*METTL14*) constitutes the core of MTC, in which *METTL3* is the catalytic subunit while *METTL14* mediates substrate RNA recognition and binding [6–9]. Other essential components of the MTC complex, including *Willms tumor 1 associated protein* (*WTAP*), *RNA Binding Motif Protein 15* (*RBM15*), *RNA Binding Motif Protein 15B* (*RBM15B*), *Zinc Finger CCCH-Type Containing 13* (*ZC3H13*), and *Vir like m⁶A methyltransferase associated* (*VIRMA*), anchor MTC to target RNAs [10–15].

The m⁶A modification is reversible and can be removed by m⁶A demethylases (also known as m⁶A “erasers”). As the first characterized RNA m⁶A demethylase, *FTO* also has oxidative demethylation activity towards multiple other types of DNA and RNA methylations, including m³T, m³U, m⁶Am, and m¹A [16, 17]. Nonetheless, m⁶A is the major physiological substrate of *FTO* [16]. The *alkB homolog 5* (*ALKBH5*) is the second m⁶A eraser which specifically demethylates RNA m⁶A [18].

The effect of m⁶A on gene expression is mediated by the m⁶A binding proteins, also known as m⁶A “readers”, which selectively interact with methylated RNAs and affect RNA metabolism. There are three well-known families of m⁶A readers, *YT521-B homology* (*YTH*) domain family, *insulin-like growth factor 2 mRNA-binding*

TABLE 1 The function of m⁶A in RNA metabolism

RNA metabolism	m ⁶ A readers	Function	Mechanism	References
<i>RNA synthesis</i>	<i>YTHDC1</i>	Enhance transcription	Regulate carRNAs and H3K9me2 to affect chromatin structure	[45, 46]
<i>Splicing</i>	<i>YTHDC1</i>	Mediate alternative splicing	Recruit splicing factor SRSF3 and prevent SRSF10	[23]
	<i>hnRNPA2B1</i>	Mediate alternative splicing	Function as splicing factor by itself	[33]
	<i>hnRNPG</i>	Mediate alternative splicing	m ⁶ A-switch mechanism	[31]
	<i>hnRNPC</i>	Mediate alternative splicing	m ⁶ A-switch mechanism	[30]
<i>Nuclear exportation</i>	<i>YTHDC1</i>	Promote exportation	Facilitate <i>NXF1-mediated export</i>	[24]
	<i>FMRP</i>	Promote exportation	Facilitate <i>XPO-mediated export</i>	[34, 35]
<i>RNA stability</i>	<i>YTHDF2</i>	RNA decay	Recruit CCR4-NOT deadenylase complex	[25, 36]
	<i>YTHDF3</i>	RNA decay		[27]
	<i>YTHDF1</i>	RNA decay		
	<i>YTHDC2</i>	RNA decay	RNA decay	[37]
	<i>IGF2BPs</i>	Stabilize RNA	Recruit huR, PABPC1, MATR3	[29]
	<i>FMRP</i>	Stabilize RNA		[38, 40]
<i>Translation</i>	<i>PRRC2A</i>	Stabilize RNA		[39]
	<i>YTHDF1</i>	Enhance translation	Facilitate cap-dependent ribosome recruitment	[26]
	<i>YTHDF3</i>	Enhance translation	Interact with YTHDF1	[27, 28]
	<i>YTHDC2</i>	Enhance translation	couple active translation with prevention of mRNA decay	[37]
	<i>METTL3</i>	Enhance translation	Interact with eIF3h and form a loop machinery	[41, 42]
	<i>IGF2BPs</i>	Enhance translation	Couple active translation with prevention of mRNA decay	[29]

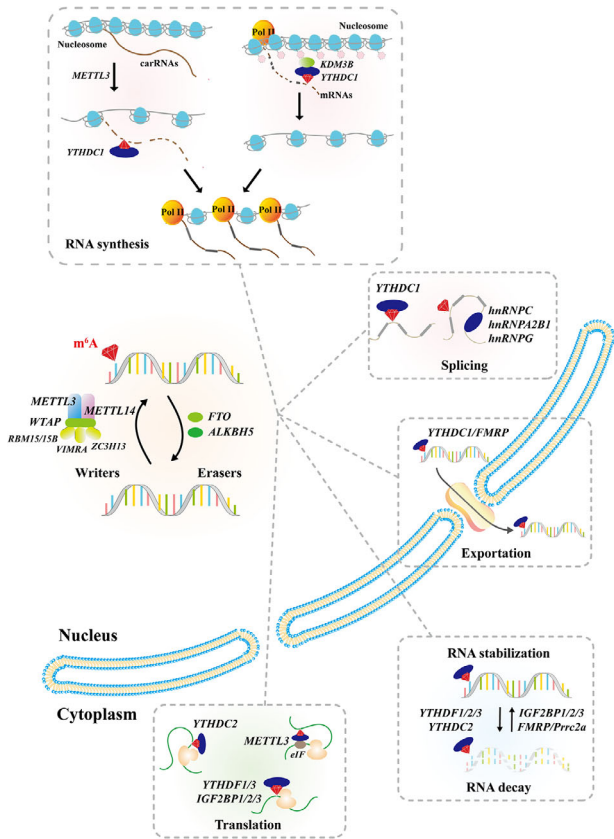


FIGURE 2 The functions of m^6A and its machinery in RNA metabolism. The m^6A modification is installed by m^6A methyltransferases (Writers), consisting of *METTL3/14*, *WTAP*, *VIRMA*, *RBM15/15B*, and *ZC3H13*, and is removed by RNA demethylases (Erasers), including *FTO* and *ALKBH5*. The m^6A reader proteins, including *YTHDF1/2*, *YTHDF1/2/3*, *IGF2BP1/2/3*, *hnRNPA2B1/C/G*, *FMRP*, *Prnc2a* and *METTL3*, work with m^6A to participate in RNA synthesis, splicing, exportation, translation, and degradation. Abbreviations: m^6A , N^6 -methyladenosine; RNA, ribonucleic acid; *METTL3/14*, methyltransferase-like 3/14; *WTAP*, Willms tumor 1 associated protein; *VIRMA*, Vir like m^6A methyltransferase associated; *RBM15/15B*, RNA Binding Motif Protein 15/15B; *ZC3H13*, Zinc Finger CCCH-Type Containing 13; *FTO*, fat mass and obesity-associated protein; *ALKBH5*, alkB homolog 5; *YTHDF1/2/3*, YTH domain-containing protein 1/2/3; *IGF2BP1/2/3*, insulin-like growth factor 2 mRNA-binding protein 1/2/3; *hnRNPA2B1/C/G*, heterogeneous nuclear ribonucleoproteins B1/C/G; *FMRP*, Fragile X mental retardation protein; *Prnc2a*, Proline-Rich Coiled-Coil 2A; *carRNAs*, chromosome-associated regulatory RNAs; *Pol II*, polymerase II; *mRNAs*, messenger RNAs; *eIF*, eukaryotic translation initiation factor

proteins (*IGF2BPs*), and *heterogeneous nuclear ribonucleoproteins (HNRNPs)* [19–22]. Members of the YTH domain family, including *YTH domain-containing protein 1 (YTHDC1)*, *YTH domain-containing protein 2 (YTHDC2)*,

YTH domain-containing family protein 1 (YTHDF1), *YTH domain-containing family protein 2 (YTHDF2)*, and *YTH domain-containing family protein 3 (YTHDF3)*, have been identified as direct m^6A readers harboring m^6A binding pockets [23–28]. *YTHDC1* is localized in the nucleus and regulates RNA splicing and nuclear exportation [23, 24] while cytoplasmic *YTHDF1*, *YTHDF2*, *YTHDF3*, and *YTHDC2* modulate RNA decay and translation cooperatively [25–28]. *IGF2BPs*, on the other hand, preferentially recognize and bind to m^6A methylated mRNAs to promote their stability and translation [29]. Unlike these two families of m^6A readers, the HNRNP family members, including *heterogeneous nuclear ribonucleoprotein C (hnRNPC)* and *heterogeneous nuclear ribonucleoprotein G (hnRNPG)*, recognize their targets through an “ m^6A switch” mechanism in which methylated A on the opposite side of a U-tract alters the structure and accessibility of hairpin RNAs [30, 31].

In short, writers and erasers work together to modulate m^6A dynamics and maintain its homeostasis in cells, while the activity of readers allows m^6A to exert its influence in each step of the RNA life cycle.

2.2 | m^6A -mediated precursor mRNA (pre-mRNA) splicing

Splicing is a fundamental step of gene expression regulation by removing introns and joining exons co-transcriptionally. The alternative selection of exons results in the production of multiple mRNA variants and ultimately diverse protein products from a single gene, contributing to proteome diversity. The influence of m^6A on alternative splicing was described by Dominissini et al. [5] and was further supported by studies showing that *METTL3*, *WTAP*, *FTO* and *ALKBH5* all modulated alternative splicing [10, 18, 32]. The m^6A methylated pre-mRNAs indeed undergo alternative splicing through the activity of *YTHDC1*, *heterogeneous nuclear ribonucleoproteins A2/B1 (hnRNPA2B1)*, or an “ m^6A switch” mechanism. As a founding member of the YTH domain family, *YTHDC1* binds methylated pre-mRNAs and promotes exon inclusion by recruiting splicing factor *serine/arginine-rich splicing factor 3 (SRSF3)* and repelling *serine/arginine-rich splicing factor (SRSF10)* [23]. Similarly, *hnRNPA2B1* binds to m^6A -bearing RNA and modulates a subset of *METTL3*- and m^6A -mediated alternative splicing events [33]. In the “ m^6A switch” mechanism, m^6A affects RNA structure and enhances the accessibility of *hnRNPC* and *hnRNPG* to the flanking U-tract, while loss of m^6A or *hnRNPC/hnRNPG* can alter the splicing pattern of neighbor exons [30, 31].

2.3 | m⁶A-mediated RNA nuclear exportation

Fully spliced mRNAs are allowed to translocate from the nucleus to the cytoplasm, which is under tight control. The interaction of *YTHDC1* and nuclear export adaptor protein *SRSF3* facilitates RNA binding to *nuclear RNA export factor 1 (NXF1)* which assists in nuclear translocation [24]. *Fragile X mental retardation protein (FMRP)*, also known as *FMRI*, is also required for the *Exportin 1 (XPO1)*-mediated nuclear export of methylated mRNAs [34, 35]. The functional studies of these reader proteins support the earlier observation that accumulation of polyadenylated (polyA) RNA in the cytoplasm is associated with an increase in m⁶A methylation by *ALKBH5* silencing [18], and the notion that m⁶A is a determinant for the subcellular location of mRNAs.

2.4 | m⁶A-modulated RNA stability

The steady level of mRNA is established by a balance between its production and degradation, thus, the stability of mRNA is of great importance on modulating mRNA metabolism and gene expression. Members of the YTH domain family play a crucial role in controlling mRNA turnover. As the first well-defined m⁶A reader, *YTHDF2* mediates the instability of the transcriptome in an m⁶A dependent manner [25]. The C-terminal domain of *YTHDF2* selectively binds m⁶A-marked RNA while the N-terminal domain mediates the anchoring of *YTHDF2*-bound mRNA to RNA degradation site and the recruitment of *carbon catabolite repression-negative on TATA-less (CCR4-NOT)* deadenylase complex, leading to the shortening of mRNA half-life [25, 36]. Interestingly, a coordinated functional interaction among *YTHDF* proteins was reported, in which *YTHDF3* could affect decay and translation of m⁶A-modified RNA with combined efforts of *YTHDF2* and *YTHDF1*, respectively [27]. *YTHDC2* also plays an essential role in the translation and decay of methylated mRNA [37]. Considering the above-mentioned m⁶A readers that mediate RNA degradation, mRNAs with declined m⁶A modification are supposed to be more stable. However, the opposite phenomenon was observed in a portion of mRNAs, especially for the transcripts of some oncogenes, suggesting an alternative mechanism of m⁶A-dependent regulation on RNA half-life. Before long, *IGF2BP* family proteins were identified as a new class of m⁶A readers that enhance mRNA stability by interacting with mRNA stabilizers, such as *ELAV like RNA binding protein 1 (ELAVL1)*, also known as *huR*, *poly(a) binding protein cytoplasmic 1 (PABPC1)* and *Matrin 3 (MATR3)*, and thereby, influencing gene expression [29]. In addition to

IGF2BPs, *FMRP* and *Proline-Rich Coiled-Coil 2A (PRRC2A)* have been reported to bind to m⁶A marked mRNAs and play a role in maintaining mRNA stability, further demonstrating that m⁶A could function as a double-edged sword in controlling mRNA half-life [29, 38-40].

2.5 | m⁶A-mediated RNA translation

Protein translation, a process in which the genetic codes are translated into amino acid sequences, is also tightly controlled. m⁶A has been widely reported to be involved in translation regulation. In the canonical cap-dependent translation, *YTHDF1* facilitates cap-dependent ribosome recruitment to mRNA by forming a loop structure mediated by *eukaryotic translation initiation factor 4G (eIF4G)* and the interaction of *YTHDF1* with *eukaryotic translation initiation factor 3 (eIF3)* [26]. *YTHDF3* was later proven to have a coordinated translation-promoting function with *YTHDF1* [27, 28]. In addition, both *IGF2BP* proteins and *YTHDC2* couple active translation with the prevention of mRNA decay [29, 37]. Notably, *METTL3* plays a methyltransferase-independent function to promote translation by interacting with *eukaryotic translation initiation factor 3h (eIF3h)* and forming mRNA loop machinery [41, 42]. It was also reported that m⁶A in 5' untranslated region (5'UTR) of mRNAs or the body of circular RNAs (circRNAs) could promote translation in a cap-independent manner [43, 44].

2.6 | m⁶A-associated RNA synthesis

Although it was thought that m⁶A mainly affects gene expression post-transcriptionally, emerging evidence has shown that m⁶A carries a lot of weight in transcriptional control. Liu et al. [45] reported that *METTL3* methylated chromosome-associated regulatory RNAs (carRNAs), while *YTHDC1* mediated the nuclear degradation of the methylated carRNAs. Loss of m⁶A methylation via *Mettl3* knockout in mouse embryonic stem cells increased carRNAs levels and therefore facilitated chromatin accessibility and transcription activity [45]. Moreover, m⁶A on mRNAs could facilitate the open state of corresponding chromatin regions through *YTHDC1*-mediated recruitment of histone H3 lysine 9 dimethylation (H3K9me2) demethylase *lysine demethylase 3B (KDM3B)*, leading to the removal of the repressive H3K9me2 histone mark and the promotion of transcription [46].

In summary, m⁶A methylation has been widely associated with every aspect of RNA metabolism and gene expression regulation, attributing to the extensive research

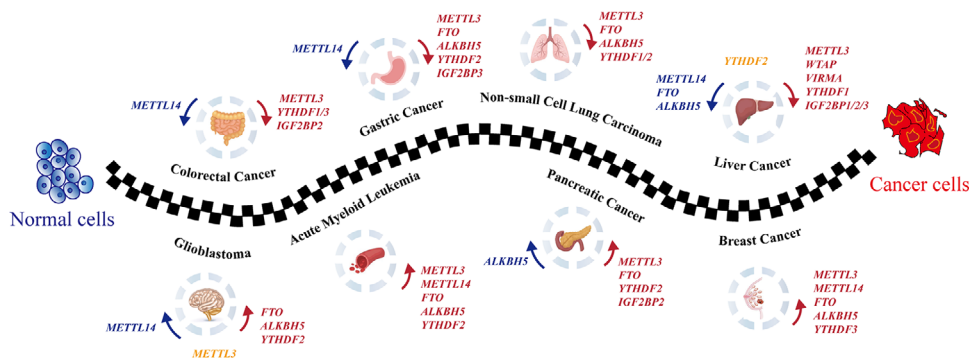


FIGURE 3 The roles of m^6A regulators on tumorigenesis. The proteins promoting tumorigenesis are in red, the ones with tumor-suppressive roles are in blue, while the ones with controversial function are in orange. Abbreviations: m^6A , N^6 -methyladenosine. METTL3/14, methyltransferase-like 3/14; YTHDF1/2/3 YTH domain-containing protein 1/2/3; IGF2BP1/2/3, insulin-like growth factor 2 mRNA-binding protein 1/2/3; FTO, fat mass and obesity-associated protein; ALKBH5, alkB homolog 5; WTAP, Willms tumor 1 associated protein; VIRMA, Vir like m^6A methyltransferase associated

on the identification of m^6A regulators and the exploration of their functions. We have been updated by rapidly expanding research in this field. For instance, the three homologs of *YTHDF*, *YTHDF1*, *YTHDF2* and *YTHDF3*, were deemed to play distinct roles in controlling RNA decay and translation [25–28]. However, a similar function of these three proteins on RNA decay and the compensation effect among them have been revealed very recently in certain cell contexts and bioprocesses, such as the ovarian development of zebrafish and the early development of mice [47–49]. Fully studying the interaction network of m^6A regulators, including *YTHDF* proteins and others, under different contexts will give us a comprehensive insight into the effects of m^6A modification on RNA metabolism.

3 | ABERRANT m^6A METHYLATION IN HUMAN CANCERS

Given the importance of m^6A in controlling RNA metabolism, aberrant methylation usually causes dysregulation of gene expression, including activation of oncogenes and repression of tumor suppressors, which plays fundamental roles in the initiation, development, and progression of various cancer types (Figure 3 and Table 2).

3.1 | Acute myeloid leukemia

Acute myeloid leukemia (AML) is known for its devastating outcome and low 5-year overall survival rate (<40%) in patients aged under 60 years old. It is originated from a disordered clone of hematopoietic stem and progenitor cells

(HSPCs), leading to the blockage of myeloid differentiation and the production of leukemic stem cells (LSCs) with self-renewal capacity that dominates the initiation of AML and the development of drug resistance [50]. The impact of *FTO* on AML when first discovered linked m^6A RNA modification to AML [51]. Specifically, the overexpression of *FTO* promoted oncofusion proteins-induced leukemogenesis through the demethylation of *ankyrin repeat and sox box containing 2 (ASB2)* and *retinoic acid receptor alpha (RARA)* mRNA transcripts [51]. The oncogenic function of *FTO* could be selectively inhibited by R-2-hydroxyglutarate (R-2HG) in *isocitrate dehydrogenase (IDH)* wild-type AML cells or by small molecular inhibitors FB23-2 and CS1/2 in a broad panel of AML cells [52, 53], resulting in the suppression of cell growth, LSC maintenance and immune evasion [52, 54]. Another m^6A demethylase *ALKBH5* was also recently found to play oncogenic roles in AML through the *KDM4C (lysine demethylase 4C)-ALKBH5-AXL (tyrosine-protein kinase receptor UFO)* and *ALKBH5- m^6A -TACC3 (transforming acidic coiled-coil containing protein 3)* axes [55, 56].

On the other hand, the m^6A methyltransferase machinery has also been linked to AML. Barbieri et al. [57] reported that *METTL3* was recruited to transcriptional start sites by *CCAAT enhancer-binding protein zeta (CEBPZ)*, thus, promoted m^6A deposition in the coding region and enhanced the translation of associated mRNA transcripts which helped to maintain leukemic state. Depletion of *METTL3* in AML cells restrained translation of *c-MYC*, *B-cell lymphoma 2 (BCL2)*, and *phosphatase and tensin homolog (PTEN)* through m^6A -mediated effects, leading to accelerated cell differentiation and apoptosis coupled with lower proliferative ability [58]. As another core component of the MTC, *METTL14* also plays a critical role in leukemogenesis. The m^6A

TABLE 2 The roles of m⁶A regulators in human cancer

Cancer types	Regulators	Target genes	Roles	References
Liver cancer	<i>METTL3</i>	<i>SOCS2, SNAIL, LINC00958</i>	Oncogene	[64, 214, 215]
	<i>METTL4</i>	<i>pri-miR-126</i>	Tumor suppressor	[62]
	<i>WTAP</i>	<i>ETSI</i>	Oncogene	[65]
	<i>VIRMA</i>	<i>ID2, GATA3</i>	Oncogene	[66, 67]
	<i>ALKBH5</i>	<i>LYPD1</i>	Tumor suppressor	[68]
	<i>FTO</i>	<i>GNAO1</i>	Tumor suppressor	[69]
	<i>YTHDF1</i>		Oncogene	[63, 73]
	<i>YTHDF2</i>	<i>EGFR, ILL1, SRPINE2</i>	Controversial	[70–72]
	<i>IGF2BP1/2/3</i>	<i>MYC, FSCNI, TKI, MARCRSL1</i>	Oncogene	[29]
	<i>IGF2BP1</i>	<i>SRF</i>	Oncogene	[74]
Non-small Cell Lung Carcinoma	<i>METTL3</i>	<i>YAP, MALATI, EGFR, TAZ, BRD4</i>	Oncogene	[41, 42, 120]
	<i>FTO</i>	<i>MZF1, USP7</i>	Oncogene	[121, 122]
Gastric Cancer	<i>ALKBH5</i>	<i>UBE2C, FOXM1, YAP</i>	Oncogene	[123–125]
	<i>YTHDF1</i>	<i>KEAPI</i>	Oncogene	[126]
	<i>YTHDF2</i>	<i>6PGD</i>	Oncogene	[127]
	<i>METTL3</i>	<i>ZMYM1, HDGF, SEC62, ARHGAP5-AS1</i>	Oncogene	[76–81]
	<i>METTL4</i>		Tumor suppressor	[82]
	<i>FTO</i>		Oncogene	[82]
Colorectal Cancer	<i>ALKBH5</i>	<i>NEATI</i>	Oncogene	[83]
	<i>YTHDF2</i>		Oncogene	[216]
	<i>IGF2BP3</i>	<i>HDGF</i>	Oncogene	[77]
	<i>METTL3</i>	<i>SOX2, pri-miR-1246</i>	Oncogene	[97, 98]
	<i>METTL4</i>	<i>pri-miR-375, lncRNA XIIST, SOX4</i>	Tumor suppressor	[94–96]
Glioblastoma	<i>YTHDF1</i>		Oncogene	[99, 100]
	<i>YTHDF3</i>	<i>GAS5 lncRNA</i>	Oncogene	[101]
	<i>IGF2BP2</i>	<i>SOX2, MYC</i>	Oncogene	[98, 102]
	<i>METTL3</i>	<i>ADAM19, SRSFs, SOX2</i>	Controversial	[105–107]
	<i>METTL4</i>	<i>ADAM19</i>	Tumor suppressor	[107]
Acute Myeloid Leukemia	<i>FTO</i>		Oncogene	[107]
	<i>ALKBH5</i>	<i>FOXMI</i>	Oncogene	[108]
	<i>YTHDF2</i>	<i>MYC, VEGFA</i>	Oncogene	[109]
	<i>METTL3</i>	<i>MYC, BCL2, PTEN, SP1, SP2</i>	Oncogene	[57, 58]

(Continues)

TABLE 2 (Continued)

Cancer types	Regulators	Target genes	Roles	References
	<i>METTL4</i>	MYB, MYC	Oncogene	[59]
	<i>FTO</i>	<i>ASB2</i> , <i>RARA</i> , <i>MYC</i> , <i>CEBPA</i>	Oncogene	[51, 52]
	<i>ALKBH5</i>	<i>AXL</i> , <i>TACC3</i>	Oncogene	[55, 56]
	<i>YTHDF2</i>	<i>TNFRSF2</i>	Oncogene	[60]
Pancreatic Cancer	<i>METTL3</i>	<i>pri-miR-25</i>	Oncogene	[90, 91, 152]
	<i>FTO</i>	<i>MYC</i>	Oncogene	[89]
	<i>ALKBH5</i>	<i>KCNK15-AS1</i> , <i>WIFI1</i> , <i>PER1</i>	Tumor suppressor	[86–88]
	<i>YTHDF2</i>	<i>YAP</i>	Oncogene	[92]
	<i>IGFBP2</i>	<i>DANCR</i>	Oncogene	[93]
Breast Cancer	<i>METTL3</i>	<i>HBXIP</i> , <i>BCL2</i> , <i>AK4</i>	Oncogene	[111, 217, 218]
	<i>METTL4</i>	<i>transforming growth factor β signaling pathway genes</i>	Oncogene	[113]
	<i>FTO</i>	<i>BNIP3</i> , <i>miR-181b-3p</i>	Oncogene	[117, 118]
	<i>ALKBH5</i>	<i>NANOG</i>	Oncogene	[114–116, 219]
	<i>YTHDF3</i>	<i>ST6GALNAC5</i> , <i>GJAI</i> , <i>EGFR</i>	Oncogene	[119]

modification and stability of *transcriptional activator Myb* (*MYB*) and *MYC* transcripts are under tight control by the *SPI1* (*transcription factor PU.1*)-*METTL4* axis during normal hematopoiesis, while elevated expression of *METTL4* leads to myeloid malignancy and enhanced self-renewal capacity of leukemia stem cells by m⁶A-mediated stabilization of *MYB* and *MYC* oncogenic transcripts [59].

Apart from m⁶A erasers and writers, the relationship between an m⁶A reader, *YTHDF2*, and leukemogenesis has also been uncovered. Paris et al. [60] reported that a decrease in *Ythdf2* resulted in higher stability of the *tumor necrosis factor receptor superfamily member 2* (*Tnfrsf2*) and more apoptosis of LSCs. They further found that depletion of *YTHDF2* could promote hematopoietic stem cell (HSC) expansion, making *YTHDF2* an additional promising anti-leukemia target. Although m⁶A writers, erasers and readers are clearly associated with AML, how these regulators cooperate in the network is to be elucidated.

3.2 | Liver cancer

As the sixth commonly diagnosed cancer, liver carcinoma is the fourth cause of tumor-associated death globally [61]. The current existing challenge of liver cancer lies in the detection of late-stage disease, recurrence, and distant metastasis. Therefore, growing efforts are being made to further understand the underlying mechanisms of liver cancer development and progression from various aspects, including RNA epigenetics.

By examination of m⁶A level in paired tumor and adjacent tissues, Ma et al. [62] found that m⁶A levels of polyA RNAs were decreased in hepatocellular carcinoma (HCC), the most common type of primary liver cancer. Further, they found that downregulation of *METTL4* was associated with metastasis and could serve as a prognostic factor in HCC. Mechanistically, *METTL4* could interact with the primary microRNA (miRNA) processing protein *microprocessor complex subunit DGCR8* (*DGCR8*) and regulate primary *miRNA-126* processing in an m⁶A-dependent manner [62]. In contrast, *METTL3* is highly expressed in HCC and promotes HCC tumorigenicity and progression by regulating the suppressor of *cytokine signaling 2* (*SOCS2*) and *Snail family transcriptional repressor 1* (*Snail1*) mRNAs homeostasis [63, 64]. In addition to mRNA, the dysregulation of RNA methylation on the long non-coding RNAs (lncRNAs) also contributes to the oncogenic function of *METTL3*. An HCC specific lncRNA, *LINC00958*, was stabilized by *METTL3*-mediated m⁶A modification and was found to facilitate HCC lipogenesis and progression through the sponging of *miRNA-3619-5p*, and thus upregulated *hepatoma-derived growth factor* (*HDGF*) expression [64]. A nanoplatfrom delivering *LINC00958* small inter-

fering RNA (siRNA) was then developed for anti-HCC purposes [64]. As regulatory components of MTC, WTAP guides HuR-mediated *ETS Proto-Oncogene 1 (ETS1)* instability in an m⁶A-dependent pattern [65] while VIRMA (also named KIAA1429) mediates the installation of m⁶A on the mRNA of *DNA-binding protein inhibitor ID-2 (ID2)* and the antisense lncRNA of *GATA binding protein 3 (GATA3)* [66, 67], thereby contributing to liver cancer development.

Besides MTC, the functions of other m⁶A modulators have been revealed in liver cancer as well. Declined *ALKBH5* caused more m⁶A on *LY6/PLAUR domain containing 1 (LYPD1)*, and the latter was recognized and stabilized by *IGF2BP1*, resulting in a more malignant HCC phenotype [68]. *Sirtuin 1 (SIRT1)*-induced *FTO* SUMOylation (small ubiquitin-related modifier, SUMO) leads to the degradation of *FTO* protein, alleviating *FTO*-mediated *G protein subunit alpha O1 (GNAO1)* demethylation and increasing its expression, which has been shown to promote hepatocarcinogenesis [69].

Controversial roles of *YTHDF2* have been reported in HCC. Yang et al. [70] reported that *YTHDF2* was essential for HCC cell survival. By contrast, Hou et al. [71] reported that low expression of *YTHDF2* could provoke inflammation, vascular reconstruction, and metastatic progression in HCC. This function could be blocked by *hypoxia-inducible factor (HIF)-2 α* , revealing a molecular 'rheostat' role of *YTHDF2* in the epitranscriptome and HCC progression [71]. Coincidentally, Zhong et al. [72] found *YTHDF2* could suppress HCC cell proliferation by destabilizing the *epidermal growth factor receptor (EGFR)* mRNA and was inhibited by the hypoxia environment of HCC. For *YTHDF1*, its function in promoting the translation of *Snail* mRNA and driving epithelial-to-mesenchymal transition (EMT) seems to be consistent with the poor prognosis associated with its high expression level in HCC patients [63, 73]. The role of another reader protein, *IGF2BP1*, was also described in HCC, where *IGF2BP1* protected *serum response factor (SRF)* mRNA from miRNA-mediated decay in an m⁶A dependent manner, supporting *IGF2BPs* as oncogenic drivers in cancer [29, 74]. Overall, the above-mentioned research lay the foundation for treating liver cancer from the RNA epigenetics view.

3.3 | Gastrointestinal carcinoma

Gastric cancer is the fifth most-diagnosed neoplasm globally, with approximately 1 million patients being newly diagnosed each year [75]. Considering its rapid progression and tendency to metastasis, scientists have been trying to find out intrinsic mechanisms of gastric cancer, and progress has been made in revealing the relationship between m⁶A regulators and the metastatic property of

gastric cancer. For instance, *METTL3* installs m⁶A on *zinc finger MYM-type containing 1 (ZMYM1)* to increase its stability, and *ZMYM1* recruits the *C-terminal-binding protein (CtBP)/ lysine-specific histone demethylase 1 (LSD1)/ REST corepressor 1 (CoREST)* complex to repress *E-cadherin* (also named *cadherin-1, CDH1*) transcription, thus strengthening the EMT program and metastasis [76]. The activation of *METTL3* transcription increases m⁶A modification on *HDGF* mRNA, facilitating the binding of *IGF2BP3*. Both of the secreted and nuclear *HDGF* contribute to gastric tumorigenesis and development [77]. The oncogenic role of *METTL3* in gastric cancer was also demonstrated by other researchers [78–81]. *METTL14*, in contrast, has a tumor-suppressive function, and the knockdown of it activates the Wnt/ *PI3K (phosphoinositide 3 kinase)- Akt (protein kinase B)* signaling to promote tumor progression [82]. Other m⁶A-related proteins, including *IGF2BP3* and *ALKBH5*, are both shown to play oncogenic roles in the development of gastric carcinoma [77, 83].

Pancreatic cancer has the lowest survival rate (9%) among all cancer types [84, 85]. Providing insights into the development of pancreatic cancer from the aspect of RNA epigenetics is also of great significance. Three studies suggested a tumor-suppressive role of *ALKBH5* in pancreatic cancer. He et al. [86] reported that *ALKBH5* inhibited pancreatic cancer motility by regulating the m⁶A level of *anti-sense RNA 1 of KCNK15 (KCNK15-AS1)* lncRNA. Tang et al. [87] found that *ALKBH5* was downregulated in pancreatic ductal adenocarcinoma (PDAC) cells and its overexpression sensitized cells to chemotherapy, with *Wnt inhibitory factor 1 (WIF-1)* being identified as the target of *ALKBH5*. Recently, another research demonstrated that *ALKBH5* led to demethylation of *period circadian regulator 1 (PER1)* mRNA and lifted *PER1* level in a *YTHDF2*-dependent manner, thereby reactivating the *ATM (A-T mutated)-CHK2 (serine/threonine-protein kinase)-P53 (tumor protein 53)/CDC25C (cell division cycle 25C)* pathway [88]. In contrast to *ALKBH5*, other m⁶A regulators, including *FTO*, *METTL3*, *YTHDF2* and *IGF2BP2*, were all shown to exhibit oncogenic roles in pancreatic cancer by promoting cell proliferation, EMT, invasion, or chemo- and radio-resistance [89–93].

As one of the most common types of carcinoma, colorectal cancer (CRC) is known for its increasing incidence globally [61]. m⁶A modification has been found to be involved in the pathogenesis of CRC in recent years. Reduction of *METTL14* was found to be correlated with unfavorable prognosis of CRC patients. Mechanistically, less m⁶A modification on the oncogenic lncRNA (*X inactive specific transcript*) *XIST* or *SRY-box transcription factor 4 (SOX4)* mRNA due to low level of *METTL14* inhibited *YTHDF2* binding, preventing the decay of *XIST* or *SOX4*, and resulting in the malignant phenotype [94, 95]. In addition, the

processing of primary *miRNA-375* was inhibited in CRC with decreased expression of *METTL14*, which contributed to CRC progression [96]. Interestingly, the demethylation of histone H3 lysine 4 trimethylation (H3K4me3) on the *METTL14* promoter is responsible for the repression of *METTL14* transcription in CRC [95]. In contrast, *METTL3* generally contributes to tumor development in CRC [97, 98]. For example, *METTL3* was reported to have not only stemness-inducing function but also tumorigenesis and metastasis-promoting activity in CRC [98]. *SRY-box transcription factor 2 (SOX2)*, the downstream target of *METTL3*, was recognized and stabilized by *IGF2BP2* in an m⁶A dependent manner [98]. In terms of m⁶A readers, *YTHDF1* was regulated by *MYC* and promoted CRC development [99, 100], while *YTHDF3*, a well-known target of *Yes-associated protein (YAP)*, formed a feedback loop by mediating the degradation of lncRNA *growth arrest specific 5 (GAS5)*. The latter could facilitate *YAP* nuclear translocation, phosphorylation, and ubiquitin-dependent decay in CRC [101]. In addition, *long intergenic noncoding RNA for IGF2BP2 stability (LINRIS)* was found able to inhibit ubiquitination of *IGF2BP2* at lysine 139 and prevent its degradation via the autophagy-lysosome pathway, thus promoting tumorigenesis through the *LINRIS-IGF2BP2-MYC* axis in CRC [102].

3.4 | Glioblastoma

Glioblastoma (GBM) is a type of commonly occurred and aggressive brain tumor, in which m⁶A modification has been demonstrated to play a role as well [103, 104]. *METTL3* is a predictive prognostic marker in GBM and plays a role in glioma stem-like cells (GSCs) maintenance by depositing m⁶A modification in the *SOX2* 3' untranslated region (3'UTR) region and leading to the overexpression of *SOX2* [105]. Consistently, Li et al. [106] found that elevated expression of *METTL3* correlated with the clinical aggressiveness of malignant gliomas. m⁶A modification of the splicing factor *serine/arginine-rich splicing factor (SRSF)* decreased upon *METTL3* knockdown, leading to *YTHDC1*-dependent nonsense-mediated mRNA decay of the *SRSF* transcripts and alternative splicing isoform switches in glioblastoma [106]. However, an opposite role of *METTL3* in GBM has also been reported, in which *METTL3* and *METTL14* were considered as tumor suppressors by targeting *metallopeptidase domain 19 (ADAM19)*, suggesting more in-depth studies are remained to be done [107]. High *forkhead box M1 (FOXM1)* level caused by *ALKBH5*-induced m⁶A reduction on *FOXM1* mRNA contributes to GSC and tumorigenesis, while *antisense RNA of FOXM1 (FOXM1-AS)* enhances the binding of *ALKBH5* and *FOXM1* [108]. More recently, Dixit et al. [109] reported

a dependency of GSCs on *YTHDF2*, which surprisingly stabilized *MYC* and *vascular endothelial growth factor A (VEGFA)* transcripts in an m⁶A-dependent manner, distinct from the well-recognized role of *YTHDF2* in mediating mRNA decay.

3.5 | Breast cancer

As a highly heterogeneous neoplasm, breast cancer is the second cause of tumor-associated death for women globally [110]. A positive feedback loop of HBXIP (Hepatitis B X-interacting protein)/let-7g (lethal-7g)/*METTL3*/HBXIP, in which m⁶A modification was involved in gene expression regulation, was demonstrated to drive the aggressiveness of breast cancer [111]. In another study [112], the depletion of *METTL3* induced *adenylate kinase 4 (AK4)* overexpression, reactive oxygen species (ROS) reduction, and less resistance of MCF-7 cells to tamoxifen. *METTL14* and *ALKBH5* were shown to promote breast cancer growth and invasion by regulating m⁶A levels of key EMT and angiogenesis-associated transcripts. Interestingly, the authors reported that *METTL14* and *ALKBH5* controlled each other's expression and inhibited *YTHDF3*, and the latter could in turn block RNA demethylase activity, forming a writer-eraser-reader collaborative loop [113]. The role of *ALKBH5* in breast cancer has also been reported by other groups [114–116]. Notably, Zhang et al. [114, 116] found that the expression of *ALKBH5* could be stimulated by *HIF-1 α* and *HIF-2 α* upon exposure to hypoxia, which increased breast cancer stem cells by reducing m⁶A modification on *NANOG* mRNA and increased *NANOG* protein level. Similar to *ALKBH5*, *FTO* also promotes breast cancer progression, with *BCL2 interacting protein 3 (BNIP3)* and miR-181b-3p being identified as targets of *FTO* [117, 118]. In addition, Chang et al. [119] revealed the involvement of *YTHDF3*-mediated epitranscriptomic regulation in breast cancer brain metastasis. *YTHDF3* overexpression was able to promote the translation of m⁶A-modified *ST6 N-acetylgalactosaminide alpha-2,6-sialyltransferase 5 (ST6GALNAC5)*, *gap junction protein alpha 1 (GJA1)*, and *EGFR*, which are related to brain metastasis. Further, they found that the overexpression of *YTHDF3* was the combined consequence of increased gene copy number and the autoregulation of *YTHDF3* cap-independent translation by binding to m⁶A residues within its own 5'UTR.

3.6 | Other cancers

Although advanced detection techniques and combined treatment have been used, lung carcinoma, especially non-small cell lung cancer (NSCLC), is still the main

cause of cancer-associated death globally [61]. Most of the m⁶A modulators, including *METTL3* [41, 42, 120], *FTO* [121, 122], *ALKBH5* [123–125], *YTHDF1* [126] and *YTHDF2* [127], were demonstrated to be oncogenic in NSCLC, a main subtype accounted for 80%–85% of lung cancer. It's worth mentioning that the oncogenic function of *METTL3* is attributed to its methyltransferase-dependent and -independent activities, suggested by recent studies [41, 42, 120]. As a writer, *METTL3* installs m⁶A on mRNAs, such as *YAP*, and lncRNAs, such as *metastasis-associated lung adenocarcinoma transcript 1 (MALAT1)*, and promotes the invasion and metastasis of NSCLC via the activation of the *YAP* pathway [120]. On the other hand, *METTL3* functions like a reader to recognize m⁶A-modified mRNAs and promote the production of oncoproteins, such as *EGFR*, *tafazzin (TAZ)*, *mitogen-activated protein kinase 2 (MAPK2)*, *DNA methyltransferase 3A (DNMT3A)*, and *bromodomain-containing protein 4 (BRD4)* [41, 42]. Whether the oncogenic roles of *METTL3* rely on its writer or reader activity in other cancer types is unclear and needs to be elucidated. In contrast to *METTL3*, *ALKBH5* inhibits tumor growth and metastasis by lessening *YAP* level in a *YTHDFs* dependent manner and impairing *YAP* function with the help of the *miRNA-107/LATS2 (large tumor suppressor kinase 2)* axis in lung cancer [125].

Diffuse large B-cell lymphoma (DLBCL) is a subtype of lymphoid malignancy with heterogenous characteristics in clinical manifestation, pathology, and biology [128]. *METTL3* was found to be upregulated in DLBCL tissues and promoted DLBCL progression by depositing m⁶A methylation on *pigment epithelium-derived factor (PEDF)* transcript, though the detailed mechanism needs to be further studied [129]. A PIWI-interacting RNA (piRNA), *piRNA-30473*, was illustrated to have oncogenic activity in DLBCL through an m⁶A-dependent manner [130]. Further, it was found that *piRNA-30473* could increase *WTAP* level, which facilitated m⁶A modifications on downstream targets, such as *hexokinase 2 (HK2)*.

Ovarian cancer and endometrial cancer are highly aggressive gynecologic cancers [131, 132]. m⁶A regulators, including *ALKBH5* [133], *METTL3* [134], *IGF2BP1* [74] and *YTHDF1* [135], were suggested to be critical factors in promoting ovarian cancer. For instance, multi-omics analysis has been used to explore the crucial component of m⁶A-related modulators in ovarian cancer and identified a subunit of eIF3, eIF3C, as a direct *YTHDF1* target [135]. Interestingly, the protein but not the RNA level of eIF3C was increased and positively correlated with the protein level of *YTHDF1* in ovarian cancer patients, suggesting that modification of *eIF3C* mRNA could be more relevant to its role in cancer. Up to 70% of endometrial cancers exhibited m⁶A hypomethylation, possibly attributed to either a hotspot R298P (R is arginine, P is proline) mutation in *METTL14* or

a decline of *METTL3* level [136]. The changes of these two key m⁶A modulators advanced endometrial tumor development via the *AKT* pathway.

Overall, dysregulation of m⁶A modifiers in cancer is frequently observed and plays crucial roles in cancer initiation, development, and drug resistance, through modulating/recognizing m⁶A on critical target transcripts.

4 | FACTORS AFFECTING m⁶A IN CANCER

There is no doubt that m⁶A regulators dominate the layer of epitranscriptomic regulation; nonetheless, internal or external factors are able to regulate m⁶A incorporation in different contexts, especially in cancer. Here, we sum up factors that have an impact on m⁶A in cancer.

4.1 | Genetic and epigenetic factors

Noncoding RNAs (ncRNAs) are a kind of RNA traditionally regarded as molecules that are not translated but have regulatory activities in gene expression. Accumulating data have shown that m⁶A methylation affects the production and/or functions of ncRNAs, including lncRNAs, circular RNAs, and miRNAs [12, 30, 44, 64, 94, 137–140]. On the other hand, ncRNAs also play a role in m⁶A-mediated gene expression regulation. For instance, *ALKBH5* acted as an oncogene in GSCs by demethylating *FOXMI* mRNA. Interestingly, this process was strengthened by *FOXMI-AS*, a lncRNA antisense to *FOXMI* [108]. The discovery of *FOXMI-AS* as a pivotal modulator in *ALKBH5*-dependent GSC proliferation emphasizes the role of ncRNA in GSC. *RNA-binding regulatory peptide (RBRP)* is a peptide encoded by lncRNA *LINC00266-1*, and its interaction with the m⁶A reader *IGF2BP1* intensified the function of *IGF2BP1*, thus, reinforced the expression of *MYC* and the process of tumorigenesis. More importantly, higher *RBRP* level in patients was associated with shorter overall survival, confirming its oncogenic effect and the potential applications as a therapeutic target in treating cancers [141]. In colorectal cancer, the inhibition of *miRNA-455-3p* rescued β -*catenin* depletion-induced reduction of *heat shock transcription factor 1 (HSF1)* m⁶A modification and *METTL3* interaction [142]. Taken together, ncRNAs exist as critical modulators of m⁶A-dependent gene expression control, and more of their regulatory roles and mechanisms remain to be explored.

The RNA methylation also has crosstalk with histone modifications. To be specific, m⁶A peaks are enriched in the region of histone H3 lysine 36 trimethylation (H3K36me3) and are declined with the reduction of

H3K36me3. Mechanistically, *METTL14* recognizes and binds to H3K36me3, after which MTC interacts with RNA Polymerase II and further installs m⁶A to actively transcribed pre-mRNA [143]. *Histone acetyltransferase P300 (EP300)*-mediated histone H3 lysine 27 acetylation (H3K27ac) activates *METTL3* transcription which stimulates m⁶A modification on *HDGF* mRNA and enhances its stability, and finally leads to tumor growth and liver metastasis in human gastric cancer [77]. Similarly, *lysine demethylase 5c (KDM5C)*-induced H3K4me3 demethylation in the promoter of *METTL14* attenuates *METTL14* transcription, resulting in reduced m⁶A deposition on *SOX4* and the upregulation of the tumor suppressor *SOX4* in colorectal cancer [95].

The posttranslational modifications on m⁶A regulators have also been identified and were found to play crucial roles in controlling the activity of the m⁶A machinery and therefore, the epitranscriptome. In HCC, *SIRT1* activated the SUMO E3 ligase *RAN binding protein 2 (RANBP2)* which mediated SUMOylation and degradation of *FTO*, and resulted in more m⁶A on *GNAOI*, an anti-tumor molecule in HCC [69]. *METTL3* could be activated by *ATM*-mediated phosphorylation at serine 43 and localized to double-strand break sites, and *YTHDC1* was subsequently recruited due to *METTL3*-induced m⁶A deposition. Interference with this *METTL3*-m⁶A-*YTHDC1* axis enhanced the sensitivity of cancer cells to DNA damage-based therapy [144].

To sum up, the complicated network connecting m⁶A modification and other genetic and epigenetic factors integrates comprehensive information from various sources and strengthens gene expression control more accurately.

4.2 | Environmental exposure affects m⁶A methylation

In addition to internal factors, external exposure also has an influence on m⁶A methylation. Human carcinogens in different content elicit detrimental effects to human bodies in genotoxic or non-genotoxic ways [145]. Evidence has shown that cigarette smoke causes oncogenic mutations and epigenetic changes [146, 147]. Tobacco smoking can alter miRNA encoding genes [148–150]. Genes with aberrant levels further participate in a myriad of pathological processes, including tumorigenesis and tumor progression [151]. Cigarette smoke condensate induced hypo-methylation in *METTL3* promoter caused *METTL3* overexpression and subsequently more m⁶A modification which promoted maturation of *miRNA-25*. The latter activated the *AKT-p70S6 kinase* pathway and played an oncogenic role in pancreatic cancer [152].

Reduced global m⁶A level was observed in A549 lung epithelial cells in response to sodium arsenite and partic-

ulate matter, and change in m⁶A level was associated with the concentration of environmental toxicants [153]. In contrast, chronic exposure of human bronchial epithelial cells to sodium arsenite-induced malignant phenotype with increased m⁶A modification which was synergistically regulated by m⁶A modulators [154]. Dynamic m⁶A incorporation was found in chemical carcinogen-induced cellular transformation, in which the *METTL3*-m⁶A-*CDCPI (CUB domain-containing protein 1)* axis contributed a lot to cell proliferation and progression, consistent with the effect of chemical carcinogenesis [155].

As a well-known oncogenic virus, Epstein–Barr virus (EBV) is the culprit of about 2% of all malignancies via regulating numerous host cell activities. Lang et al. [156] have observed the interplay between EBV and m⁶A decoration. *EBV nuclear antigen 3C (EBNA3C)*, the viral-encoded latent oncoprotein, was upregulated by *METTL14*-mediated m⁶A modification, and could in turn activate *METTL14* transcription and directly interact with *METTL14* to promote its protein stability. Therefore, *METTL14* appears to be an important factor in EBV-induced oncogenesis. In addition, m⁶A modification plays a role in the lifecycle and infection of the hepatitis virus which predominantly contributes to chronic liver diseases and the tumorigenesis of HCC [157, 158]. It was found that Hepatitis B virus (HBV) pregenomic RNA (pgRNA) was m⁶A modified in the RRACH motif within the epsilon stem-loop and bound by *YTHDF2/3* proteins [157]. Blocking m⁶A methylation by either silencing *METTL3* and *METTL14* or mutating this adenosine base to cytosine affected the stability of pgRNA and suppressed reverse transcription. The infection of Hepatitis C virus (HCV), a single-stranded RNA virus, was proven to be regulated by m⁶A modification as well [158]. The m⁶A machinery in host cells is present not only in the nucleus but also in the cytoplasm where they can modify the HCV RNA. Silencing of *METTL3* and *METTL14* in Huh7 hepatoma cells increased the production of infectious HCV particles and the percentage of HCV-positive cells, while depletion of *FTO* inhibited HCV particle production and infection. Taken together, these studies indicate the important roles of m⁶A modification during the pathogenesis, development, and progression of virus-related cancers, implicating that modulation of m⁶A modification could serve as prevention or therapeutic strategies in virus-related cancers.

5 | CLINICAL IMPLICATIONS OF m⁶A IN CANCERS

A growing body of research on m⁶A methylation reveals a new layer of epigenetic regulation in oncogenesis and provides implications for the use of m⁶A in innovative and effective diagnostic and therapeutic approaches.

5.1 | Implications of m⁶A in cancer diagnosis and prognosis

Effective biomarkers, along with sensitive and specific detection methods, will greatly contribute to the early diagnosis of cancers, thus, improve the survival of patients. Recently, m⁶A methylation and its regulators have become emerging biomarkers for cancer diagnosis and prognosis [21, 159]. Owing to metabolic reversibility, high abundance and stability, methylated nucleosides could be accessible in biological fluids (e.g., serum and urine) or circulating cells [160, 161]. Huang et al. [161] developed a liquid chromatography-electrospray ionization tandem mass spectrometric (LC-ESI-MS/MS) method to determine m⁶A level in single cells and found increased RNA m⁶A methylation in circulating tumor cells from the blood of lung cancer patients. Pei et al. [162] also detected elevated m⁶A level in the peripheral blood leukocyte from non-small cell lung cancer patients by flow cytometry, indicating the potential use of m⁶A as a non-invasive biomarker. Furthermore, m⁶A regulators, including *METTL3* [77, 163–169], *WTAP* [170–173], *FTO* [174–177], *IGF2BPs* [178–184], and *YTHDFs* [73, 185–192], have been proven to associate with favorable or unfavorable prognosis in different types of cancers (detailed in Section 3). It should be noted that prognosis is not simply associated with the expression of a certain gene but a comprehensive signature of multiple m⁶A regulators in cancers, including lung cancer [163, 164], pancreatic cancer [166, 193], and HCC [194]. Despite that m⁶A and its regulators exhibit powerful potential as biomarkers, it is still challenging for clinical application due to the heterogeneity of m⁶A in patients and the lack of assays to detect site-specific m⁶A from low-input clinical samples. Future single-cell sequencing techniques might provide powerful assistance in solving such problems.

5.2 | m⁶A modification and chemosensitivity

Drug resistance is the major cause of therapeutic failure and recurrence in chemotherapy. Recent studies have indicated that m⁶A modification was associated with drug response and chemoresistance [21, 159]. Mutations of receptor tyrosine kinases, such as *BCR-ABL* (*breakpoint cluster region, BCR; tyrosine-protein kinase, ABL*), *c-kit proto-oncogene (KIT)* and *fms like tyrosine kinase 3 (FLT3)* frequently occur in leukemia and are effective therapeutic targets in the clinic [195–197]. The tolerance of tyrosine kinase inhibitors (TKIs), a big challenge in leukemia treatment, is mediated by m⁶A demethylation resulting from elevated *FTO* in leukemia cells [198]. Decreased *ALKBH5* was found in gemcitabine-treated patient-derived

xenograft (PDX) model and predicted poor clinical outcome in PDAC, while overexpression of *ALKBH5* could sensitize PDAC to chemotherapy [87]. Moreover, *METTL3*-induced m⁶A installation was found to contribute to oxaliplatin resistance in colon cancer [199], cisplatin resistance in NSCLC [120] and tamoxifen resistance in breast cancer [112]. Therefore, silencing of *METTL3* could reverse drug resistance in the above scenarios [112, 120, 199], and could enhance the sensitivity of DNA damage-based therapy *in vivo* and *in vitro* [144]. Collectively, robust evidence unveils the participation of m⁶A modulators in drug resistance, shedding light on the application of these regulators as predictive markers in chemotherapy or drug targets in combination with chemotherapy.

5.3 | The m⁶A modification and cancer immunotherapy

Although immunotherapy has been considered as a promising treatment in defeating cancer, lacking durable effects in some groups of patients limits its efficacy [200–202]. Intriguingly, the absence of *YTHDF1* in mice enhances antigen-specific *cluster of differentiation 8 (CD8)*-positive T-cell anti-tumor reaction due to promoted tumor antigen cross-presentation in classical dendritic cells (cDCs) [203]. As a result, the therapeutic efficacy of *programmed death-ligand 1 (PD-L1)* checkpoint blockade is enhanced in *YTHDF1*-deficient mice [203]. What's more, the decline of *FTO* also improves the low response of melanoma cells to interferon-gamma and enhances the reaction to anti-PD-1 (*programmed cell death protein 1, PDI*) blockade in mice [204]. These studies suggest that *YTHDF1* and *FTO* might be potential drug targets in combination with immunotherapy.

5.4 | Targeting m⁶A and its regulators in cancer therapy

Given the benefits of targeting m⁶A methylation in cancer therapy, as discussed above, researchers never cease exploring effective inhibitors of m⁶A enzymes. The most well representative one is the development of small-molecule agents targeting *FTO*. Initially, a natural compound named Rhein was found to bind to *FTO* catalytic domain and competitively inhibited the recognition of m⁶A substrate [205]. An ascorbic acid analog was then designed in 2014 to inhibit the 2-oxoglutarate-dependent hydroxylase activity of *FTO* and elevate m⁶A level [206]. Later, meclofenamic acid (MA) and an acylhydrazine compound, *FTO* inhibitor 12, have been identified to have inhibitory activity on *FTO* over *ALKBH5* [207, 208].

By targeting *FTO*, R-2HG and FB23-2 exhibited promising inhibitory effects in the treatment of AML [52, 53]. More recently, two potent agents, CS1 and CS2, have been developed by high-throughput screening from over 260,000 compounds and showed anti-tumor effects in multiple cancers by suppressing the self-renewal of cancer stem cells and immune evasion [54]. The inhibitors for m⁶A writers and readers are also of great interest to the researchers. By screening a library of 4000 analogs and derivatives of S-adenosyl-methionine (SAM), UZH1a has been found to be an effective *METTL3* inhibitor and could modulate transcriptomic m⁶A signal in the MOLM13 leukemia cells; however, its *in vivo* effect still needs to be elucidated [209]. As a small molecule inhibitor, BTYNB was reported to disrupt the association between *IGF2BP1* and target RNA, which resulted in the decrease of *E2 factor* (*E2F*)-driven cell cycle transition and inhibition of tumor progression [210]. With more and more druggable m⁶A targets being proven with proof-of-concept evidence to combat cancers, the identification of specific inhibitors and the application of these inhibitors in the clinic, especially in combination with other therapies, are of great importance and in urgent need. Instead of altering the transcriptome-wide m⁶A level, “m⁶A editing” is a CRISPR (clusters of regularly interspaced short palindromic repeats)-CAS9 (CRISPR-associated protein-9)-based method to mediate programmable RNA methylation or demethylation at a specific locus. To achieve site-specific removal of m⁶A, Liu et al. [211] engineered m⁶A ‘erasers’ by fusing catalytic dead Cas9 (*dCas9*) with *ALKBH5* or *FTO*, while Li et al. [212] chose the RNA-targeting CRISPR-Cas system, *dCas13*, to engineer *ALKBH5*. Recently, *dcas13* fusions with truncated *METTLE3* or modified *METTL3-METTL14* complex have been established to direct site-specific m⁶A incorporation [213]. To date, these new techniques allow precise manipulation of a single methylation site, in the hope of evaluating the exact function of a single methylation site and targeting of single m⁶A for cancer treatment.

6 | PERSPECTIVES AND CONCLUSION

Fast-growing research in the RNA epigenetics field delineates a comprehensive picture about how m⁶A methylation is tightly controlled by its enzymes (writers and erasers) and works with reader proteins to participate in almost every step of RNA metabolism. Hypo- or hyper-methylation might lead to aberrant gene expression, abnormal cellular function, and diseases, such as cancer. The direct links between m⁶A and various cancers not only provide an insight into the mechanism of tumorigenesis but also are valuable for guiding clinical applica-

tions in fighting against cancers. Considering the broad effects of m⁶A in strengthening the anti-cancer effect of chemotherapy and immunotherapy, the combination of m⁶A-targeting agents with traditional chemotherapeutic drugs or PD-1/PD-L1 inhibitors holds great therapeutic promise. However, there is still a debate whether targeting the total abundance/level of m⁶A methylation (i.e., targeting enzymes) or targeting gene-/site-specific m⁶A methylation is a better choice, which warrants more proof-of-concept studies. Overall, m⁶A modification is a rising star in the epigenetic field and holds therapeutic promise for a broad range of cancer.

Abbreviations

RNA, ribonucleic acid; m⁶A, N⁶-methyladenosine; DNA, deoxyribonucleic acid; mRNA, messenger RNA; *FTO*, fat mass and obesity-associated protein; NGS, Next-generation sequencing; G, guanosine; A, adenosine; C, cytidine; U, uridine; MTC, methyltransferase complex; *METTL3*, methyltransferase-like 3; *METTL14*, methyltransferase-like 14; *WTAP*, Willms tumor 1 associated protein; *RBM15*, RNA Binding Motif Protein 15; *RBM15B*, RNA Binding Motif Protein 15B; *ZC3H13*, Zinc Finger CCCH-Type Containing 13; *VIRMA*, Vir like m6A methyltransferase associated; *ALKBH5*, alkB homolog 5; *YTH*, YTH domain-containing protein; *YTHDC1*, YTH domain-containing protein 1; *YTHDC2*, YTH domain-containing protein 2; *YTHDF1*, YTH domain-containing family protein 1; *YTHDF2*, YTH domain-containing family protein 2; *YTHDF3*, YTH domain-containing family protein 3; *hnRNPC*, heterogeneous nuclear ribonucleoprotein C; *hnRNPG*, heterogeneous nuclear ribonucleoprotein G; *hnRNPA2B1*, heterogeneous nuclear ribonucleoproteins A2/B1; *SRSF3*, serine/arginine-rich splicing factor 3; *SRSF10*, serine/arginine-rich splicing factor; *NXF1*, nuclear RNA export factor 1; *FMRP*, Fragile X mental retardation protein; *XPO1*, Exportin 1; polyA, polyadenylated; *ELAVL1*, ELAV like RNA binding protein 1; *PABPC1*, poly(a) binding protein cytoplasmic 1; *MATR3*, Matrin 3; *PRRC2A*, Proline Rich Coiled-Coil 2A; *eIF4G*, eukaryotic translation initiation factor 4G; *eIF3*, eukaryotic translation initiation factor 3; *eIF3h*, eukaryotic translation initiation factor 3h; 5'UTR, 5' untranslated region; circRNAs, circular RNAs; carRNAs, chromosome-associated regulatory RNAs; H3K9me2, histone H3 lysine 9 dimethylation; *KDM3B*, lysine demethylase 3B; AML, acute myeloid leukemia; HSPCs, hematopoietic stem and progenitor cells; LSCs, leukemic stem cells; *ASB2*, ankyrin repeat and socs box containing 2; *RARA*, retinoic acid receptor alpha; R-2HG, R-2-hydroxyglutarate; *IDH*, isocitrate dehydrogenase; *KDM4C*, lysine demethylase 4C; *AXL*, tyrosine-protein kinase receptor UFO; *TACC3*,

transforming acidic coiled-coil containing protein 3; CEBPZ, CCAAT enhancer binding protein zeta; BCL2, B-cell lymphoma 2; PTEN, phosphatase and tensin homolog; SPI1, transcription factor PU.1; Tnfrsf2, tumor necrosis factor receptor superfamily member 2; HSC, hematopoietic stem cell; HCC, hepatocellular carcinoma; miRNA, microRNA; DGCR8, microprocessor complex subunit DGCR8; SOCS2, cytokine signaling 2; Snail1, snail family transcriptional repressor 1; lncRNAs, long non-coding RNAs; HDGF, hepatoma-derived growth factor; siRNA, small interfering RNA; ETS1, HuR-mediated ETS Proto-Oncogene 1; ID2, DNA-binding protein inhibitor ID-2; GATA3, GATA binding protein 3; LYPD1, LY6/PLAUR domain containing 1; SIRT1, Sirtuin 1; SUMO, small ubiquitin-related modifier; GNAO1, G protein subunit alpha O1; HIF, hypoxia-inducible factor; EGFR, epidermal growth factor receptor; EMT, epithelial-to-mesenchymal transition; SRF, serum response factor; ZMYM1, zinc finger MYM-type containing 1; CtBP, C-terminal-binding protein; LSD1, lysine-specific histone demethylase 1; CoREST, REST corepressor 1; PI3K, phosphoinositide 3 kinase; Akt, protein kinase B; KCNK15-AS1, antisense RNA 1 of KCNK15; PDAC, pancreatic ductal adenocarcinoma; WIF-1, Wnt inhibitory factor 1; PER1, period circadian regulator 1; ATM, A-T mutated; CHK2, serine/threonine-protein kinase; P53, tumor protein 53; CDC25C, cell division cycle 25C; CRC, colorectal cancer; SOX4, SRY-box transcription factor 4; H3K4me3, histone H3 lysine 4 trimethylation; SOX2, SRY-box transcription factor 2; YAP, Yes-associated protein; GAS5, growth arrest specific 5; LINRIS, long intergenic noncoding RNA for IGF2BP2 stability; GBM, Glioblastoma; GSCs, glioma stem-like cells; 3'UTR, 3' untranslated region; SRSF, serine/arginine-rich splicing factor; ADAM19, ADAM metalloproteinase domain 19; FOXM1, forkhead box M1; FOXM1-AS, antisense RNA of FOXM1; VEGFA, vascular endothelial growth factor A; HBXIP, Hepatitis B X-interacting protein; let-7g, lethal-7g; AK4, adenylate kinase 4; ROS, reactive oxygen species; BNIP3, BCL2 interacting protein 3; ST6GALNAC5, ST6 N-acetylgalactosaminide alpha-2,6-sialyltransferase 5; GJA1, gap junction protein alpha 1; NSCLC, non-small cell lung cancer; MALAT1, metastasis associated lung adenocarcinoma transcript 1; TAZ, tafazzin; MAPK2, mitogen-activated protein kinase 2; DNMT3A, DNA methyltransferase 3A; BRD4, bromodomain-containing protein 4; ATS2, large tumor suppressor kinase 2; DLBCL, Diffuse large B-cell lymphoma; PEDF, pigment epithelium-derived factor; piRNA, PIWI-interacting RNA; HK2, hexokinase 2; ncRNAs, noncoding RNAs; RBRP, RNA-binding regulatory peptide; HSF1, heat shock transcription factor 1; H3K36me3, histone H3 lysine 36 trimethylation; EP300, histone acetyltransferase P300; H3K27ac, histone H3 lysine 27 acetylation; KDM5C,

lysine demethylase 5c; RANBP2, RAN binding protein 2; CDCP1, CUB domain containing protein 1; EBV, Epstein-Barr virus; EBNA3C, EBV nuclear antigen 3C; HBV, Hepatitis B virus; pgRNA, pregenomic RNA; HCV, Hepatitis C virus; LC-ESI-MS/MS, liquid chromatography electrospray ionization tandem mass spectrometric; BCR, breakpoint cluster region; ABL, tyrosine-protein kinase; KIT, c-kit proto-oncogene; FLT3, fms like tyrosine kinase 3; TKIs, tyrosine kinase inhibitors; PDX, patient-derived xenograft; CD8, cluster of differentiation 8; cDCs, classical dendritic cells; PD-L1, programmed death-ligand 1; PD1, programmed cell death protein 1; MA, meclofenamic acid; SAM, S-adenosyl-methionine; E2F, E2 factor; CRISPR, clusters of regularly interspaced short palindromic repeats; CAS9, CRISPR-associated protein-9; dCas9, catalytic dead Cas9;

DECLARATIONS

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

COMPETING INTERESTS

The authors declare that they have no competing interests.

ACKNOWLEDGMENTS

This work was supported in part by the grant 2021A1515010425 (H.H.) from Guangdong Basic and Applied Basic Research Foundation, and the National Key R&D Program of China 2020YFA0112403 (H.W.) from the Ministry of Science and Technology of the People's Republic of China.

AUTHORS' CONTRIBUTIONS

SHD, HYW and HLH wrote the manuscript. SHD made the figures. SHD, YTW, YDL, HYW and HLH revised and approved the final manuscript.

ORCID

Shenghua Dong  <https://orcid.org/0000-0001-8490-8144>

Hengyou Weng  <https://orcid.org/0000-0001-6474-2861>

Huilin Huang  <https://orcid.org/0000-0003-2608-572X>

REFERENCES

1. Roundtree IA, Evans ME, Pan T and He C. Dynamic RNA Modifications in Gene Expression Regulation. *Cell*. 2017;169(7):1187-1200.

2. Desrosiers R, Friderici K, Rottman F. Identification of methylated nucleosides in messenger RNA from Novikoff hepatoma cells. *Proc Natl Acad Sci U S A*. 1974;71(10):3971-5.
3. Perry RP, Kelley DE. Existence of methylated messenger RNA in mouse L cells. *Cell*. 1974;1(1):37-42.
4. Meyer KD, Saletore Y, Zumbo P, Elemento O, Mason CE, Jaffrey SR. Comprehensive analysis of mRNA methylation reveals enrichment in 3' UTRs and near stop codons. *Cell*. 2012;149(7):1635-46.
5. Dominissini D, Moshitch-Moshkovitz S, Schwartz S, Salmon-Divon M, Ungar L, Osenberg S, et al. Topology of the human and mouse m6A RNA methylomes revealed by m6A-seq. *Nature*. 2012;485(7397):201-6.
6. Liu J, Yue Y, Han D, Wang X, Fu Y, Zhang L, et al. A METTL3-METTL14 complex mediates mammalian nuclear RNA N6-adenosine methylation. *Nat Chem Biol*. 2014;10(2):93-5.
7. Wang P, Duxtader KA, Nam Y. Structural Basis for Cooperative Function of Mettl3 and Mettl14 Methyltransferases. *Mol Cell*. 2016;63(2):306-317.
8. Wang X, Feng J, Xue Y, Guan Z, Zhang D, Liu Z, et al. Structural basis of N(6)-adenosine methylation by the METTL3-METTL14 complex. *Nature*. 2016;534(7608):575-8.
9. Śledź P, Jinek M. Structural insights into the molecular mechanism of the m(6)A writer complex. *Elife*. 2016;5.
10. Ping XL, Sun BF, Wang L, Xiao W, Yang X, Wang WJ, et al. Mammalian WTAP is a regulatory subunit of the RNA N6-methyladenosine methyltransferase. *Cell Res*. 2014;24(2):177-89.
11. Schwartz S, Mumbach MR, Jovanovic M, Wang T, Maciag K, Bushkin GG, et al. Perturbation of m6A writers reveals two distinct classes of mRNA methylation at internal and 5' sites. *Cell Rep*. 2014;8(1):284-96.
12. Patil DP, Chen CK, Pickering BF, Chow A, Jackson C, Guttman M, et al. m(6)A RNA methylation promotes XIST-mediated transcriptional repression. *Nature*. 2016;537(7620):369-373.
13. Yue Y, Liu J, Cui X, Cao J, Luo G, Zhang Z, et al. VIRMA mediates preferential m(6)A mRNA methylation in 3'UTR and near stop codon and associates with alternative polyadenylation. *Cell Discov*. 2018;4:10.
14. Wen J, Lv R, Ma H, Shen H, He C, Wang J, et al. Zc3h13 Regulates Nuclear RNA m(6)A Methylation and Mouse Embryonic Stem Cell Self-Renewal. *Mol Cell*. 2018;69(6):1028-1038.e6.
15. Knuckles P, Lence T, Haussmann IU, Jacob D, Kreim N, Carl SH, et al. Zc3h13/Flacc is required for adenosine methylation by bridging the mRNA-binding factor Rbm15/Spenito to the m(6)A machinery component Wtap/FI(2)d. *Genes Dev*. 2018;32(5-6):415-429.
16. Jia G, Fu Y, Zhao X, Dai Q, Zheng G, Yang Y, et al. N6-methyladenosine in nuclear RNA is a major substrate of the obesity-associated FTO. *Nat Chem Biol*. 2011;7(12):885-7.
17. Jia G, Yang CG, Yang S, Jian X, Yi C, Zhou Z, et al. Oxidative demethylation of 3-methylthymine and 3-methyluracil in single-stranded DNA and RNA by mouse and human FTO. *FEBS Lett*. 2008;582(23-24):3313-9.
18. Zheng G, Dahl JA, Niu Y, Fedorcsak P, Huang CM, Li CJ, et al. ALKBH5 is a mammalian RNA demethylase that impacts RNA metabolism and mouse fertility. *Mol Cell*. 2013;49(1):18-29.
19. Yang Y, Hsu PJ, Chen YS, Yang YG. Dynamic transcriptomic m(6)A decoration: writers, erasers, readers and functions in RNA metabolism. *Cell Res*. 2018;28(6):616-624.
20. Zaccara S, Ries RJ, Jaffrey SR. Reading, writing and erasing mRNA methylation. *Nat Rev Mol Cell Biol*. 2019;20(10):608-624.
21. Huang H, Weng H, Chen J. m(6)A Modification in Coding and Non-coding RNAs: Roles and Therapeutic Implications in Cancer. *Cancer Cell*. 2020;37(3):270-288.
22. Zhou KI, Pan T. An additional class of m(6)A readers. *Nat Cell Biol*. 2018;20(3):230-232.
23. Xiao W, Adhikari S, Dahal U, Chen YS, Hao YJ, Sun BF, et al. Nuclear m(6)A Reader YTHDC1 Regulates mRNA Splicing. *Mol Cell*. 2016;61(4):507-519.
24. Roundtree IA, Luo GZ, Zhang Z, Wang X, Zhou T, Cui Y, et al. YTHDC1 mediates nuclear export of N(6)-methyladenosine methylated mRNAs. *Elife*. 2017. 6.
25. Wang X, Lu Z, Gomez A, Hon GC, Yue Y, Han D, et al. N6-methyladenosine-dependent regulation of messenger RNA stability. *Nature*. 2014;505(7481):17-20.
26. Wang X, Zhao BS, Roundtree IA, Lu Z, Han D, Ma H, et al. N(6)-methyladenosine Modulates Messenger RNA Translation Efficiency. *Cell*. 2015;161(6):1388-99.
27. Shi H, Wang X, Lu Z, Zhao BS, Ma H, Hsu PJ, et al. YTHDF3 facilitates translation and decay of N(6)-methyladenosine-modified RNA. *Cell Res*. 2017;27(3):315-328.
28. Li A, Chen YS, Ping XL, Yang X, Xiao W, Yang Y, et al. Cytoplasmic m(6)A reader YTHDF3 promotes mRNA translation. *Cell Res*. 2017;27(3):444-447.
29. Huang H, Weng H, Sun W, Qin X, Shi H, Wu H, et al. Recognition of RNA N(6)-methyladenosine by IGF2BP proteins enhances mRNA stability and translation. *Nat Cell Biol*. 2018;20(3):285-295.
30. Liu N, Dai Q, Zheng G, He C, Parisien M, Pan T. N(6)-methyladenosine-dependent RNA structural switches regulate RNA-protein interactions. *Nature*. 2015;518(7540):560-4.
31. Zhou KI, Shi H, Lyu R, Wylder AC, Matuszek Ż, Pan JN, et al. Regulation of Co-transcriptional Pre-mRNA Splicing by m(6)A through the Low-Complexity Protein hnRNPG. *Mol Cell*. 2019;76(1):70-81.e9.
32. Zhao X, Yang Y, Sun BF, Shi Y, Yang X, Xiao W, et al. FTO-dependent demethylation of N6-methyladenosine regulates mRNA splicing and is required for adipogenesis. *Cell Res*. 2014;24(12):1403-19.
33. Alarcon CR, Goodarzi H, Lee H, Liu X, Tavazoie S, Tavazoie SF. HNRNPA2B1 Is a Mediator of m(6)A-Dependent Nuclear RNA Processing Events. *Cell*. 2015;162(6):1299-308.
34. Hsu PJ, Shi H, Zhu AC, Lu Z, Miller N, Edens BM, et al. The RNA-binding protein FMRP facilitates the nuclear export of N (6)-methyladenosine-containing mRNAs. *J Biol Chem*. 2019;294(52):19889-19895.
35. Edens BM, Vissers C, Su J, Arumugam S, Xu Z, Shi H, et al. FMRP Modulates Neural Differentiation through m(6)A-Dependent mRNA Nuclear Export. *Cell Rep*. 2019;28(4):845-854.e5.
36. Du H, Zhao Y, He J, Zhang Y, Xi H, Liu M, et al. YTHDF2 destabilizes m(6)A-containing RNA through direct recruitment of the CCR4-NOT deadenylase complex. *Nat Commun*. 2016;7:12626.

37. Hsu PJ, Zhu Y, Ma H, Guo Y, Shi X, Liu Y, et al. Ythdc2 is an N(6)-methyladenosine binding protein that regulates mammalian spermatogenesis. *Cell Res.* 2017;27(9):1115-1127.
38. Zhang F, Kang Y, Wang M, Li Y, Xu T, Yang W, et al. Fragile X mental retardation protein modulates the stability of its m6A-marked messenger RNA targets. *Hum Mol Genet.* 2018;27(22):3936-3950.
39. Wu R, Li A, Sun B, Sun JG, Zhang J, Zhang T, et al. A novel m(6)A reader Prrc2a controls oligodendroglial specification and myelination. *Cell Res.* 2019;29(1):23-41.
40. Edupuganti RR, Geiger S, Lindeboom RGH, Shi H, Hsu PJ, Lu Z, et al. N(6)-methyladenosine (m(6)A) recruits and repels proteins to regulate mRNA homeostasis. *Nat Struct Mol Biol.* 2017;24(10):870-878.
41. Lin S, Choe J, Du P, Triboulet R, Gregory RI. The m(6)A Methyltransferase METTL3 Promotes Translation in Human Cancer Cells. *Mol Cell.* 2016;62(3):335-345.
42. Choe J, Lin S, Zhang W, Liu Q, Wang L, Ramirez-Moya J, et al. mRNA circularization by METTL3-eIF3h enhances translation and promotes oncogenesis. *Nature.* 2018;561(7724):556-560.
43. Coots RA, Liu XM, Mao Y, Dong L, Zhou J, Wan J, et al. m(6)A Facilitates eIF4F-Independent mRNA Translation. *Mol Cell.* 2017;68(3):504-514.e7.
44. Yang Y, Fan X, Mao M, Song X, Wu P, Zhang Y, et al. Extensive translation of circular RNAs driven by N(6)-methyladenosine. *Cell Res.* 2017;27(5):626-641.
45. Liu J, Dou X, Chen C, Chen C, Liu C, Xu MM, et al. N(6)-methyladenosine of chromosome-associated regulatory RNA regulates chromatin state and transcription. *Science.* 2020;367(6477):580-586.
46. Li Y, Xia L, Tan K, Ye X, Zuo Z, Li M, et al. N(6)-Methyladenosine co-transcriptionally directs the demethylation of histone H3K9me2. *Nat Genet.* 2020;52(9):870-877.
47. Kontur C, Jeong M, Cifuentes D, Giraldez AJ. Ythdf m(6)A Readers Function Redundantly during Zebrafish Development. *Cell Rep.* 2020;33(13):108598.
48. Zaccara S, Jaffrey SR. A Unified Model for the Function of YTHDF Proteins in Regulating m(6)A-Modified mRNA. *Cell.* 2020;181(7):1582-1595.e18.
49. Lasman L, Krupalnik V, Viukov S, Mor N, Aguilera-Castrejon A, Schneir D, et al. Context-dependent functional compensation between Ythdf m(6)A reader proteins. *Genes Dev.* 2020;34(19-20):1373-1391.
50. Döhner H, Weisdorf DJ, Bloomfield CD. Acute Myeloid Leukemia. *N Engl J Med.* 2015;373(12):1136-52.
51. Li Z, Weng H, Su R, Weng X, Zuo Z, Li C, et al. FTO Plays an Oncogenic Role in Acute Myeloid Leukemia as a N(6)-Methyladenosine RNA Demethylase. *Cancer Cell.* 2017;31(1):127-141.
52. Su R, Dong L, Li C, Nachtergaele S, Wunderlich M, Qing Y, et al. R-2HG Exhibits Anti-tumor Activity by Targeting FTO/m(6)A/MYC/CEBPA Signaling. *Cell.* 2018;172(1-2):90-105.e23.
53. Huang Y, Su R, Sheng Y, Dong L, Dong Z, Xu H, et al. Small-Molecule Targeting of Oncogenic FTO Demethylase in Acute Myeloid Leukemia. *Cancer Cell.* 2019;35(4):677-691.e10.
54. Su R, Dong L, Li Y, Gao M, Han L, Wunderlich M, et al. Targeting FTO Suppresses Cancer Stem Cell Maintenance and Immune Evasion. *Cancer Cell.* 2020;38(1):79-96.e11.
55. Wang J, Li Y, Wang P, Han G, Zhang T, Chang J, et al. Leukemogenic Chromatin Alterations Promote AML Leukemia Stem Cells via a KDM4C-ALKBH5-AXL Signaling Axis. *Cell Stem Cell.* 2020;27(1):81-97.e8.
56. Shen C, Sheng Y, Zhu AC, Robinson S, Jiang X, Dong L, et al. RNA Demethylase ALKBH5 Selectively Promotes Tumorigenesis and Cancer Stem Cell Self-Renewal in Acute Myeloid Leukemia. *Cell Stem Cell.* 2020;27(1):64-80.e9.
57. Barbieri I, Tzelepis K, Pandolfini L, Shi J, Millan-Zambrano G, Robson SC, et al. Promoter-bound METTL3 maintains myeloid leukaemia by m(6)A-dependent translation control. *Nature.* 2017;552(7683):126-131.
58. Vu LP, Pickering BF, Cheng Y, Zaccara S, Nguyen D, Minuesa G, et al. The N(6)-methyladenosine (m(6)A)-forming enzyme METTL3 controls myeloid differentiation of normal hematopoietic and leukemia cells. *Nat Med.* 2017;23(11):1369-1376.
59. Weng H, Huang H, Wu H, Qin X, Zhao BS, Dong L, et al. METTL14 Inhibits Hematopoietic Stem/Progenitor Differentiation and Promotes Leukemogenesis via mRNA m(6)A Modification. *Cell Stem Cell.* 2018;22(2):191-205.e9.
60. Paris J, Morgan M, Campos J, Spencer GJ, Shmakova A, Ivanova I, et al. Targeting the RNA m(6)A Reader YTHDF2 Selectively Compromises Cancer Stem Cells in Acute Myeloid Leukemia. *Cell Stem Cell.* 2019;25(1):137-148.e6.
61. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin.* 2018. 68(6):394-424.
62. Ma JZ, Yang F, Zhou CC, Liu F, Yuan JH, Wang F, et al. METTL14 suppresses the metastatic potential of hepatocellular carcinoma by modulating N(6)-methyladenosine-dependent primary MicroRNA processing. *Hepatology.* 2017;65(2):529-543.
63. Lin X, Chai G, Wu Y, Li J, Chen F, Liu J, et al. RNA m(6)A methylation regulates the epithelial mesenchymal transition of cancer cells and translation of Snail. *Nat Commun.* 2019;10(1):2065.
64. Zuo X, Chen Z, Gao W, Zhang Y, Wang J, Wang J, et al. M6A-mediated upregulation of LINC00958 increases lipogenesis and acts as a nanotherapeutic target in hepatocellular carcinoma. *J Hematol Oncol.* 2020;13(1):5.
65. Chen Y, Peng C, Chen J, Chen D, Yang B, He B, et al. WTAP facilitates progression of hepatocellular carcinoma via m6A-HuR-dependent epigenetic silencing of ETS1. *Mol Cancer.* 2019;18(1):127.
66. Cheng X, Li M, Rao X, Zhang W, Li X, Wang L, et al. KIAA1429 regulates the migration and invasion of hepatocellular carcinoma by altering m6A modification of ID2 mRNA. *Onco Targets Ther.* 2019;12:3421-3428.
67. Lan T, Li H, Zhang D, Xu L, Liu H, Hao X, et al. KIAA1429 contributes to liver cancer progression through N6-methyladenosine-dependent post-transcriptional modification of GATA3. *Mol Cancer.* 2019;18(1):186.
68. Chen Y, Zhao Y, Chen J, Peng C, Zhang Y, Tong R, et al. ALKBH5 suppresses malignancy of hepatocellular carcinoma via m(6)A-guided epigenetic inhibition of LYPD1. *Mol Cancer.* 2020;19(1):123.
69. Liu X, Liu J, Xiao W, Zeng Q, Bo H, Zhu Y, et al. SIRT1 Regulates N(6)-Methyladenosine RNA Modification in

Hepatocarcinogenesis by Inducing RANBP2-Dependent FTO SUMOylation. *Hepatology*. 2020.

70. Yang Z, Li J, Feng G, Gao S, Wang Y, Zhang S, et al. MicroRNA-145 Modulates N(6)-Methyladenosine Levels by Targeting the 3'-Untranslated mRNA Region of the N(6)-Methyladenosine Binding YTH Domain Family 2 Protein. *J Biol Chem*. 2017;292(9):3614-3623.
71. Hou J, Zhang H, Liu J, Zhao Z, Wang J, Lu Z, et al. YTHDF2 reduction fuels inflammation and vascular abnormalization in hepatocellular carcinoma. *Mol Cancer*. 2019;18(1):163.
72. Zhong L, Liao D, Zhang M, Zeng C, Li X, Zhang R, et al. YTHDF2 suppresses cell proliferation and growth via destabilizing the EGFR mRNA in hepatocellular carcinoma. *Cancer Lett*. 2019;442:252-261.
73. Zhao X, Chen Y, Mao Q, Jiang X, Jiang W, Chen J, et al. Overexpression of YTHDF1 is associated with poor prognosis in patients with hepatocellular carcinoma. *Cancer Biomark*. 2018;21(4):859-868.
74. Müller S, Glaß M, Singh AK, Haase J, Bley N, Fuchs T, et al. IGF2BP1 promotes SRF-dependent transcription in cancer in a m6A- and miRNA-dependent manner. *Nucleic Acids Res*. 2019;47(1):375-390.
75. Smyth EC, Nilsson M, Grabsch HI, van Grieken NC, Lordick F. Gastric cancer. *Lancet*. 2020;396(10251):635-648.
76. Yue B, Song C, Yang L, Cui R, Cheng X, Zhang Z, et al. METTL3-mediated N6-methyladenosine modification is critical for epithelial-mesenchymal transition and metastasis of gastric cancer. *Mol Cancer*. 2019;18(1):142.
77. Wang Q, Chen C, Ding Q, Zhao Y, Wang Z, Chen J, et al. METTL3-mediated m(6)A modification of HDGF mRNA promotes gastric cancer progression and has prognostic significance. *Gut*. 2020;69(7):1193-1205.
78. Zhu L, Zhu Y, Han S, Chen M, Song P, Dai D, et al. Impaired autophagic degradation of lncRNA ARHGAP5-AS1 promotes chemoresistance in gastric cancer. *Cell Death Dis*. 2019;10(6):383.
79. Lin S, Liu J, Jiang W, Wang P, Sun C, Wang X, et al. METTL3 Promotes the Proliferation and Mobility of Gastric Cancer Cells. *Open Med (Wars)*. 2019;14:25-31.
80. He H, Wu W, Sun Z, Chai L. MiR-4429 prevented gastric cancer progression through targeting METTL3 to inhibit m(6)A-caused stabilization of SEC62. *Biochem Biophys Res Commun*. 2019;517(4):581-587.
81. Liu T, Yang S, Sui J, Xu SY, Cheng YP, Shen B, et al. Dysregulated N6-methyladenosine methylation writer METTL3 contributes to the proliferation and migration of gastric cancer. *J Cell Physiol*. 2020;235(1):548-562.
82. Zhang C, Zhang M, Ge S, Huang W, Lin X, Gao J, et al. Reduced m6A modification predicts malignant phenotypes and augmented Wnt/PI3K-Akt signaling in gastric cancer. *Cancer Med*. 2019;8(10):4766-4781.
83. Zhang J, Guo S, Piao HY, Wang Y, Wu Y, Meng XY, et al. ALKBH5 promotes invasion and metastasis of gastric cancer by decreasing methylation of the lncRNA NEAT1. *J Physiol Biochem*. 2019;75(3):379-389.
84. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. *CA Cancer J Clin*. 2020;70(1):7-30.
85. Wang ZQ, Zhang F, Deng T, Zhang L, Feng F, Wang FH, et al. The efficacy and safety of modified FOLFIRINOX as first-line chemotherapy for Chinese patients with metastatic pancreatic cancer. *Cancer Commun. (Lond)*, 2019;39(1):26.
86. He Y, Hu H, Wang Y, Yuan H, Lu Z, Wu P, et al. ALKBH5 Inhibits Pancreatic Cancer Motility by Decreasing Long Non-Coding RNA KCN15-AS1 Methylation. *Cell Physiol Biochem*. 2018;48(2):838-846.
87. Tang B, Yang Y, Kang M, Wang Y, Wang Y, Bi Y, et al. m(6)A demethylase ALKBH5 inhibits pancreatic cancer tumorigenesis by decreasing WIF-1 RNA methylation and mediating Wnt signaling. *Mol Cancer*. 2020;19(1):3.
88. Guo X, Li K, Jiang W, Hu Y, Xiao W, Huang Y, et al. RNA demethylase ALKBH5 prevents pancreatic cancer progression by posttranscriptional activation of PER1 in an m6A-YTHDF2-dependent manner. *Mol Cancer*. 2020;19(1):91.
89. Tang X, Liu S, Chen D, Zhao Z, Zhou J. The role of the fat mass and obesity-associated protein in the proliferation of pancreatic cancer cells. *Oncol Lett*. 2019;17(2):2473-2478.
90. Taketo K, Konno M, Asai A, Koseki J, Toratani M, Satoh T, et al. The epitranscriptome m6A writer METTL3 promotes chemo- and radioresistance in pancreatic cancer cells. *Int J Oncol*. 2018;52(2):621-629.
91. Xia T, Wu X, Cao M, Zhang P, Shi G, Zhang J, et al. The RNA m6A methyltransferase METTL3 promotes pancreatic cancer cell proliferation and invasion. *Pathol Res Pract*. 2019;215(11):152666.
92. Chen J, Sun Y, Xu X, Wang D, He J, Zhou H, et al. YTH domain family 2 orchestrates epithelial-mesenchymal transition/proliferation dichotomy in pancreatic cancer cells. *Cell Cycle*. 2017;16(23):2259-2271.
93. Hu X, Peng WX, Zhou H, Jiang J, Zhou X, Huang D, et al. IGF2BP2 regulates DANCR by serving as an N6-methyladenosine reader. *Cell Death Differ*. 2020;27(6):1782-1794.
94. Yang X, Zhang S, He C, Xue P, Zhang L, He Z, et al. METTL14 suppresses proliferation and metastasis of colorectal cancer by down-regulating oncogenic long non-coding RNA XIST. *Mol Cancer*. 2020;19(1):46.
95. Chen X, Xu M, Xu X, Zeng K, Liu X, Pan B, et al. METTL14-mediated N6-methyladenosine modification of SOX4 mRNA inhibits tumor metastasis in colorectal cancer. *Mol Cancer*. 2020;19(1):106.
96. Chen X, Xu M, Xu X, Zeng K, Liu X, Sun L, et al. METTL14 Suppresses CRC Progression via Regulating N6-Methyladenosine-Dependent Primary miR-375 Processing. *Mol Ther*. 2020;28(2):599-612.
97. Peng W, Li J, Chen R, Gu Q, Yang P, Qian W, et al. Upregulated METTL3 promotes metastasis of colorectal cancer via miR-1246/SPRED2/MAPK signaling pathway. *J Exp Clin Cancer Res*. 2019;38(1):393.
98. Li T, Hu PS, Zuo Z, Lin JF, Li X, Wu QN, et al. METTL3 facilitates tumor progression via an m(6)A-IGF2BP2-dependent mechanism in colorectal carcinoma. *Mol Cancer*. 2019;18(1):112.
99. Nishizawa Y, Konno M, Asai A, Koseki J, Kawamoto K, Miyoshi N, et al. Oncogene c-Myc promotes epitranscriptome m(6)A reader YTHDF1 expression in colorectal cancer. *Oncotarget*. 2018;9(7):7476-7486.
100. Bai Y, Yang C, Wu R, Huang L, Song S, Li W, et al. YTHDF1 Regulates Tumorigenicity and Cancer Stem Cell-Like Activity in Human Colorectal Carcinoma. *Front Oncol*. 2019;9:332.

101. Ni W, Yao S, Zhou Y, Liu Y, Huang P, Zhou A, et al. Long noncoding RNA GAS5 inhibits progression of colorectal cancer by interacting with and triggering YAP phosphorylation and degradation and is negatively regulated by the m(6)A reader YTHDF3. *Mol Cancer*. 2019;18(1):143.
102. Wang Y, Lu JH, Wu QN, Jin Y, Wang DS, Chen YX, et al. LncRNA LINRIS stabilizes IGF2BP2 and promotes the aerobic glycolysis in colorectal cancer. *Mol Cancer*. 2019;18(1):174.
103. Ceccarelli M, Barthel FP, Malta TM, Sabedot TS, Salama SR, Murray BA, et al. Molecular Profiling Reveals Biologically Discrete Subsets and Pathways of Progression in Diffuse Glioma. *Cell*. 2016;164(3):550-63.
104. Chen J, McKay RM, Parada LF. Malignant glioma: lessons from genomics, mouse models, and stem cells. *Cell*. 2012;149(1):36-47.
105. Visvanathan A, Patil V, Arora A, Hegde AS, Arivazhagan A, Santosh V, et al. Essential role of METTL3-mediated m(6)A modification in glioma stem-like cells maintenance and radioresistance. *Oncogene*. 2018;37(4):522-533.
106. Li F, Yi Y, Miao Y, Long W, Long T, Chen S, et al. N(6)-Methyladenosine Modulates Nonsense-Mediated mRNA Decay in Human Glioblastoma. *Cancer Res*. 2019;79(22):5785-5798.
107. Cui Q, Shi H, Ye P, Li L, Qu Q, Sun G, et al. m(6)A RNA Methylation Regulates the Self-Renewal and Tumorigenesis of Glioblastoma Stem Cells. *Cell Rep*. 2017;18(11):2622-2634.
108. Zhang S, Zhao BS, Zhou A, Lin K, Zheng S, Lu Z, et al. m(6)A Demethylase ALKBH5 Maintains Tumorigenicity of Glioblastoma Stem-like Cells by Sustaining FOXM1 Expression and Cell Proliferation Program. *Cancer Cell*. 2017;31(4):591-606.e6.
109. Dixit D, Prager BC, Gimple RC, Poh HX, Wang Y, Wu Q, et al. The RNA m6A Reader YTHDF2 Maintains Oncogene Expression and Is a Targetable Dependency in Glioblastoma Stem Cells. *Cancer Discov*. 2021;11(2):480-499.
110. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. *CA Cancer J Clin*. 2019. 69(1):7-34.
111. Cai X, Wang X, Cao C, Gao Y, Zhang S, Yang Z, et al. HBXIP-elevated methyltransferase METTL3 promotes the progression of breast cancer via inhibiting tumor suppressor let-7g. *Cancer Lett*. 2018;415:11-19.
112. Liu X, Gonzalez G, Dai X, Miao W, Yuan J, Huang M, et al. Adenylate Kinase 4 Modulates the Resistance of Breast Cancer Cells to Tamoxifen through an m(6)A-Based Epitranscriptomic Mechanism. *Mol Ther*. 2020.
113. Panneerdoss S, Eedunuri VK, Yadav P, Timilsina S, Rajamanickam S, Viswanadhapalli S, et al. Cross-talk among writers, readers, and erasers of m(6)A regulates cancer growth and progression. *Sci Adv*. 2018;4(10):eaar8263.
114. Zhang C, Samanta D, Lu H, Bullen JW, Zhang H, Chen I, et al. Hypoxia induces the breast cancer stem cell phenotype by HIF-dependent and ALKBH5-mediated m⁶A-demethylation of NANOG mRNA. *Proc Natl Acad Sci U S A*. 2016;113(14):E2047-56.
115. Fry NJ, Law BA, Ilkayeva OR, Carraway KR, Holley CL, Mansfield KD. N(6)-methyladenosine contributes to cellular phenotype in a genetically-defined model of breast cancer progression. *Oncotarget*. 2018;9(58):31231-31243.
116. Zhang C, Zhi WI, Lu H, Samanta D, Chen I, Gabrielson E, et al. Hypoxia-inducible factors regulate pluripotency factor expression by ZNF217- and ALKBH5-mediated modulation of RNA methylation in breast cancer cells. *Oncotarget*. 2016;7(40):64527-64542.
117. Niu Y, Lin Z, Wan A, Chen H, Liang H, Sun L, et al. RNA N6-methyladenosine demethylase FTO promotes breast tumor progression through inhibiting BNIP3. *Mol Cancer*. 2019;18(1):46.
118. Xu Y, Ye S, Zhang N, Zheng S, Liu H, Zhou K, et al. The FTO/miR-181b-3p/ARL5B signaling pathway regulates cell migration and invasion in breast cancer. *Cancer Commun (Lond)*. 2020;40(10):484-500.
119. Chang G, Shi L, Ye Y, Shi H, Zeng L, Tiwary S, et al. YTHDF3 Induces the Translation of m(6)A-Enriched Gene Transcripts to Promote Breast Cancer Brain Metastasis. *Cancer Cell*. 2020.
120. Jin D, Guo J, Wu Y, Du J, Yang L, Wang X, et al. m(6)A mRNA methylation initiated by METTL3 directly promotes YAP translation and increases YAP activity by regulating the MALAT1-miR-1914-3p-YAP axis to induce NSCLC drug resistance and metastasis. *J Hematol Oncol*. 2019;12(1):135.
121. Li J, Han Y, Zhang H, Qian Z, Jia W, Gao Y, et al. The m6A demethylase FTO promotes the growth of lung cancer cells by regulating the m6A level of USP7 mRNA. *Biochem Biophys Res Commun*. 2019;512(3):479-485.
122. Liu J, Ren D, Du Z, Wang H, Zhang H, Jin Y. m(6)A demethylase FTO facilitates tumor progression in lung squamous cell carcinoma by regulating MZF1 expression. *Biochem Biophys Res Commun*. 2018;502(4):456-464.
123. Chao Y, Shang J, Ji W. ALKBH5-m(6)A-FOXM1 signaling axis promotes proliferation and invasion of lung adenocarcinoma cells under intermittent hypoxia. *Biochem Biophys Res Commun*. 2020;521(2):499-506.
124. Guo J, Wu Y, Du J, Yang L, Chen W, Gong K, et al. Deregulation of UBE2C-mediated autophagy repression aggravates NSCLC progression. *Oncogenesis*. 2018;7(6):49.
125. Jin D, Guo J, Wu Y, Yang L, Wang X, Du J, et al. m(6)A demethylase ALKBH5 inhibits tumor growth and metastasis by reducing YTHDFs-mediated YAP expression and inhibiting miR-107/LATS2-mediated YAP activity in NSCLC. *Mol Cancer*. 2020;19(1):40.
126. Shi Y, Fan S, Wu M, Zuo Z, Li X, Jiang L, et al. YTHDF1 links hypoxia adaptation and non-small cell lung cancer progression. *Nat Commun*. 2019;10(1):4892.
127. Sheng H, Li Z, Su S, Sun W, Zhang X, Li L, et al. YTH domain family 2 promotes lung cancer cell growth by facilitating 6-phosphogluconate dehydrogenase mRNA translation. *Carcinogenesis*. 2020;41(5):541-550.
128. Miao Y, Medeiros LJ, Li Y, Li J, Young KH. Genetic alterations and their clinical implications in DLBCL. *Nature Reviews Clinical Oncology*. 2019;16(10):634-652.
129. Cheng Y, Fu Y, Wang Y, Wang J. The m6A Methyltransferase METTL3 Is Functionally Implicated in DLBCL Development by Regulating m6A Modification in PEDF. *Front Genet*. 2020;11:955.
130. Han H, Fan G, Song S, Jiang Y, Qian C, Zhang W, et al. piRNA-30473 contributes to tumorigenesis and poor prognosis by regulating m6A RNA methylation in DLBCL. *Blood*. 2020.
131. Bowtell DD, Böhm S, Ahmed AA, Aspúria PJ, Bast RC, Jr., Beral V, et al. Rethinking ovarian cancer II: reducing

- mortality from high-grade serous ovarian cancer. *Nat Rev Cancer*. 2015;15(11):668-79.
132. Brooks RA, Fleming GF, Lastra RR, Lee NK, Moroney JW, et al. Current recommendations and recent progress in endometrial cancer. *CA Cancer J Clin*. 2019;69(4):258-279.
 133. Zhu H, Gan X, Jiang X, Diao S, Wu H, Hu J. ALKBH5 inhibited autophagy of epithelial ovarian cancer through miR-7 and BCL-2. *J Exp Clin Cancer Res*. 2019;38(1):163.
 134. Hua W, Zhao Y, Jin X, Yu D, He J, Xie D, et al. METTL3 promotes ovarian carcinoma growth and invasion through the regulation of AXL translation and epithelial to mesenchymal transition. *Gynecol Oncol*. 2018;151(2):356-365.
 135. Liu T, Wei Q, Jin J, Luo Q, Liu Y, Yang Y, et al. The m6A reader YTHDF1 promotes ovarian cancer progression via augmenting EIF3C translation. *Nucleic Acids Res*. 2020;48(7):3816-3831.
 136. Liu J, Eckert MA, Harada BT, Liu SM, Lu Z, Yu K, et al. m(6)A mRNA methylation regulates AKT activity to promote the proliferation and tumorigenicity of endometrial cancer. *Nat Cell Biol*. 2018;20(9):1074-1083.
 137. Wu Y, Yang X, Chen Z, Tian L, Jiang G, Chen F, et al. m(6)A-induced lncRNA RPI1 triggers the dissemination of colorectal cancer cells via upregulation of Zeb1. *Mol Cancer*. 2019;18(1):87.
 138. Yang D, Qiao J, Wang G, Lan Y, Li G, Guo X, et al. N6-Methyladenosine modification of lincRNA 1281 is critically required for mESC differentiation potential. *Nucleic Acids Res*. 2018;46(8):3906-3920.
 139. Mauer J, Luo X, Blanjoie A, Jiao X, Grozhik AV, Patil DP, et al. Reversible methylation of m(6)A(m) in the 5' cap controls mRNA stability. *Nature*. 2017;541(7637):371-375.
 140. Chen RX, Chen X, Xia LP, Zhang JX, Pan ZZ, Ma XD, et al. N(6)-methyladenosine modification of circNSUN2 facilitates cytoplasmic export and stabilizes HMGA2 to promote colorectal liver metastasis. *Nat Commun*. 2019;10(1):4695.
 141. Zhu S, Wang JZ, Chen D, He YT, Meng N, Chen M, et al. An oncopeptide regulates m(6)A recognition by the m(6)A reader IGF2BP1 and tumorigenesis. *Nat Commun*. 2020;11(1):1685.
 142. Song P, Feng L, Li J, Dai D, Zhu L, Wang C, et al. β -catenin represses miR455-3p to stimulate m6A modification of HSF1 mRNA and promote its translation in colorectal cancer. *Mol Cancer*. 2020;19(1):129.
 143. Huang H, Weng H, Zhou K, Wu T, Zhao BS, Sun M, et al. Histone H3 trimethylation at lysine 36 guides m(6)A RNA modification co-transcriptionally. *Nature*. 2019;567(7748):414-419.
 144. Zhang C, Chen L, Peng D, Jiang A, He Y, Zeng Y, et al. METTL3 and N6-Methyladenosine Promote Homologous Recombination-Mediated Repair of DSBs by Modulating DNA-RNA Hybrid Accumulation. *Mol Cell*. 2020;79(3):425-442.e7.
 145. Luch A. Nature and nurture - lessons from chemical carcinogenesis. *Nat Rev Cancer*. 2005;5(2):113-25.
 146. Clark SJ, Molloy PL. Smoke-Induced Changes to the Epigenome Provide Fertile Ground for Oncogenic Mutation. *Cancer Cell*. 2017;32(3):278-280.
 147. Vaz M, Hwang SY, Kagiampakis I, Phallen J, Patil A, O'Hagan HM, et al. Chronic Cigarette Smoke-Induced Epigenomic Changes Precede Sensitization of Bronchial Epithelial Cells to Single-Step Transformation by KRAS Mutations. *Cancer Cell*. 2017;32(3):360-376.e6.
 148. Xi S, Inchauste S, Guo H, Shan J, Xiao Z, Xu H, et al. Cigarette smoke mediates epigenetic repression of miR-217 during esophageal adenocarcinogenesis. *Oncogene*. 2015;34(44):5548-59.
 149. Teschendorff AE, Yang Z, Wong A, Pipinikas CP, Jiao Y, Jones A, et al. Correlation of Smoking-Associated DNA Methylation Changes in Buccal Cells With DNA Methylation Changes in Epithelial Cancer. *JAMA Oncol*. 2015;1(4):476-85.
 150. Xi S, Xu H, Shan J, Tao Y, Hong JA, Inchauste S, et al. Cigarette smoke mediates epigenetic repression of miR-487b during pulmonary carcinogenesis. *J Clin Invest*. 2013;123(3):1241-61.
 151. Lu J, Getz G, Miska EA, Alvarez-Saavedra E, Lamb J, Peck D, et al. MicroRNA expression profiles classify human cancers. *Nature*. 2005;435(7043):834-8.
 152. Zhang J, Bai R, Li M, Ye H, Wu C, Wang C, et al. Excessive miR-25-3p maturation via N(6)-methyladenosine stimulated by cigarette smoke promotes pancreatic cancer progression. *Nat Commun*. 2019;10(1):1858.
 153. Cayir A, Barrow TM, Guo L, Byun HM. Exposure to environmental toxicants reduces global N6-methyladenosine RNA methylation and alters expression of RNA methylation modulator genes. *Environ Res*. 2019;175:228-234.
 154. Gu S, Sun D, Dai H, Zhang Z. N(6)-methyladenosine mediates the cellular proliferation and apoptosis via microRNAs in arsenite-transformed cells. *Toxicol Lett*. 2018;292:1-11.
 155. Yang F, Jin H, Que B, Chao Y, Zhang H, Ying X, et al. Dynamic m(6)A mRNA methylation reveals the role of METTL3-m(6)A-CDCP1 signaling axis in chemical carcinogenesis. *Oncogene*. 2019;38(24):4755-4772.
 156. Lang F, Singh RK, Pei Y, Zhang S, Sun K, Robertson ES. EBV epitranscriptome reprogramming by METTL4 is critical for viral-associated tumorigenesis. *PLoS Pathog*. 2019;15(6):e1007796.
 157. Imam H, Khan M, Gokhale NS, McIntyre ABR, Kim GW, Jang JY, et al. N6-methyladenosine modification of hepatitis B virus RNA differentially regulates the viral life cycle. *Proc Natl Acad Sci U S A*. 2018;115(35):8829-8834.
 158. Gokhale NS, McIntyre ABR, McFadden MJ, Roder AE, Kennedy EM, Gandara JA, et al. N6-Methyladenosine in Flaviviridae Viral RNA Genomes Regulates Infection. *Cell Host Microbe*. 2016;20(5):654-665.
 159. Huang H, Weng H, Deng X, Chen J. RNA Modifications in Cancer: Functions, Mechanisms, and Therapeutic Implications. *Annual Review of Cancer Biology*. 2020;4(1):221-240.
 160. Gehrke CW, Kuo KC, Waalkes TP, Borek E. Patterns of urinary excretion of modified nucleosides. *Cancer Res*. 1979;39(4):1150-3.
 161. Huang W, Qi CB, Lv SW, Xie M, Feng YQ, Huang WH, et al. Determination of DNA and RNA Methylation in Circulating Tumor Cells by Mass Spectrometry. *Anal Chem*. 2016;88(2):1378-84.
 162. Pei Y, Lou X, Li K, Xu X, Guo Y, Xu D, et al. Peripheral Blood Leukocyte N6-methyladenosine is a Noninvasive Biomarker for Non-small-cell Lung Carcinoma. *Onco Targets Ther*. 2020;13:11913-11921.
 163. Zhu J, Wang M, Hu D. Deciphering N(6)-Methyladenosine-Related Genes Signature to Predict Survival in Lung Adenocarcinoma. *Biomed Res Int*. 2020. **2020**:2514230.
 164. Liu GM, Zeng HD, Zhang CY, Xu JW. Identification of METTL3 as an Adverse Prognostic Biomarker in Hepatocellular Carcinoma. *Dig Dis Sci*. 2020.

165. Qu N, Qin S, Zhang X, Bo X, Liu Z, Tan C, et al. Multiple m(6)A RNA methylation modulators promote the malignant progression of hepatocellular carcinoma and affect its clinical prognosis. *BMC Cancer*. 2020;20(1):165.
166. Hou J, Wang Z, Li H, Zhang H, Luo L. Gene Signature and Identification of Clinical Trait-Related m(6) A Regulators in Pancreatic Cancer. *Front Genet*. 2020;11:522.
167. Jiang L, Zhang M, Wu J, Wang S, Yang X, Yi M, et al. Exploring diagnostic m6A regulators in endometriosis. *Aging (Albany NY)*. 2020;12(24):25916-25938.
168. Li N, Zhan X. Identification of pathology-specific regulators of m(6)A RNA modification to optimize lung cancer management in the context of predictive, preventive, and personalized medicine. *Epma j*. 2020;11(3):485-504.
169. Hu BB, Wang XY, Gu XY, Zou C, Gao ZJ, Zhang H, et al. N(6)-methyladenosine (m(6)A) RNA modification in gastrointestinal tract cancers: roles, mechanisms, and applications. *Mol Cancer*. 2019;18(1):178.
170. Li BQ, Huang S, Shao QQ, Sun J, Zhou L, You L, et al. WT1-associated protein is a novel prognostic factor in pancreatic ductal adenocarcinoma. *Oncol Lett*. 2017;13(4):2531-2538.
171. Xi Z, Xue Y, Zheng J, Liu X, Ma J, Liu Y. WTAP Expression Predicts Poor Prognosis in Malignant Glioma Patients. *J Mol Neurosci*. 2016;60(2):131-6.
172. Galardi S, Michienzi A, Ciafrè SA. Insights into the Regulatory Role of m(6)A Epitranscriptome in Glioblastoma. *Int J Mol Sci*. 2020;21(8).
173. Zhuang J, Lin C, Ye J. m(6) A RNA methylation regulators contribute to malignant progression in rectal cancer. *J Cell Physiol*. 2020;235(9):6300-6306.
174. Li Y, Zheng D, Wang F, Xu Y, Yu H, Zhang H. Expression of Demethylase Genes, FTO and ALKBH1, Is Associated with Prognosis of Gastric Cancer. *Dig Dis Sci*. 2019;64(6):1503-1513.
175. Guan K, Liu X, Li J, Ding Y, Li J, Cui G, et al. Expression Status And Prognostic Value Of M6A-associated Genes in Gastric Cancer. *J Cancer*. 2020;11(10):3027-3040.
176. Wang JY, Chen LJ, Qiang P. The Potential Role of N6-Methyladenosine (m6A) Demethylase Fat Mass and Obesity-Associated Gene (FTO) in Human Cancers. *Onco Targets Ther*. 2020;13:12845-12856.
177. Xu D, Shao W, Jiang Y, Wang X, Liu Y, Liu X. FTO expression is associated with the occurrence of gastric cancer and prognosis. *Oncol Rep*. 2017;38(4):2285-2292.
178. Yan X, Wan H, Hao X, Lan T, Li W, Xu L, et al. Importance of gene expression signatures in pancreatic cancer prognosis and the establishment of a prediction model. *Cancer Manag Res*. 2019;11:273-283.
179. Guo W, Huai Q, Wan H, Guo L, Song P, Gao S, et al. Prognostic Impact of IGF2BP3 Expression in Patients with Surgically Resected Lung Adenocarcinoma. *DNA Cell Biol*. 2021;40(2):316-331.
180. He X, Li W, Liang X, Zhu X, Zhang L, Huang Y, et al. IGF2BP2 Overexpression Indicates Poor Survival in Patients with Acute Myelocytic Leukemia. *Cell Physiol Biochem*. 2018;51(4):1945-1956.
181. Cao J, Mu Q, Huang H. The Roles of Insulin-Like Growth Factor 2 mRNA-Binding Protein 2 in Cancer and Cancer Stem Cells. *Stem Cells Int*. 2018. **2018**:4217259.
182. Dahlem C, Barghash A, Puchas P, Haybaeck J, Kessler SM. The Insulin-Like Growth Factor 2 mRNA Binding Protein IMP2/IGF2BP2 is Overexpressed and Correlates with Poor Survival in Pancreatic Cancer. *Int J Mol Sci*. 2019. 20(13).
183. Wang X, Guan D, Wang D, Liu H, Wu Y, Gong W, et al. Genetic variants in m(6)A regulators are associated with gastric cancer risk. *Arch Toxicol*. 2021;95(3):1081-1088.
184. Lederer M, Bley N, Schleifer C, Hüttelmaier S. The role of the oncofetal IGF2 mRNA-binding protein 3 (IGF2BP3) in cancer. *Semin Cancer Biol*. 2014;29:3-12.
185. Xu D, Shao J, Song H, Wang J. The YTH Domain Family of N6-Methyladenosine "Readers" in the Diagnosis and Prognosis of Colonic Adenocarcinoma. *Biomed Res Int*. 2020. 2020:9502560.
186. Liu M, Zhou S, Wang J, Zhang Q, Yang S, Feng J, et al. Identification of genes associated with survival of breast cancer patients. *Breast Cancer*. 2019;26(3):317-325.
187. Liu T, Yang S, Cheng YP, Kong XL, Du DD, Wang X, et al. The N6-Methyladenosine (m6A) Methylation Gene YTHDF1 Reveals a Potential Diagnostic Role for Gastric Cancer. *Cancer Manag Res*. 2020;12:11953-11964.
188. Zhang B, Gu Y, Jiang G. Expression and Prognostic Characteristics of m(6) A RNA Methylation Regulators in Breast Cancer. *Front Genet*. 2020;11:604597.
189. Liu L, Liu X, Dong Z, Li J, Yu Y, Chen X, et al. N6-methyladenosine-related Genomic Targets are Altered in Breast Cancer Tissue and Associated with Poor Survival. *J Cancer*. 2019;10(22):5447-5459.
190. Shao XY, Dong J, Zhang H, Wu YS, Zheng L. Systematic Analyses of the Role of the Reader Protein of N (6)-Methyladenosine RNA Methylation, YTH Domain Family 2, in Liver Hepatocellular Carcinoma. *Front Mol Biosci*. 2020;7:577460.
191. Wang JY, Lu AQ. The biological function of m6A reader YTHDF2 and its role in human disease. *Cancer Cell Int*. 2021;21(1):109.
192. Liu S, Li G, Li Q, Zhang Q, Zhuo L, Chen X, et al. The roles and mechanisms of YTH domain-containing proteins in cancer development and progression. *Am J Cancer Res*. 2020;10(4):1068-1084.
193. Meng Z, Yuan Q, Zhao J, Wang B, Li S, Offringa R, et al. The m(6)A-Related mRNA Signature Predicts the Prognosis of Pancreatic Cancer Patients. *Mol Ther Oncolytics*. 2020;17:460-470.
194. Hu C, Yu M, Li C, Wang Y, Li X, Ulrich B, et al. miR-550-1 functions as a tumor suppressor in acute myeloid leukemia via the hippo signaling pathway. *Int J Biol Sci*. 2020;16(15):2853-2867.
195. Du Z, Lovly CM. Mechanisms of receptor tyrosine kinase activation in cancer. *Mol Cancer*. 2018;17(1):58.
196. Rossari F, Minutolo F, Orciuolo E. Past, present, and future of Bcr-Abl inhibitors: from chemical development to clinical efficacy. *J Hematol Oncol*. 2018;11(1):84.
197. Larrosa-Garcia M, Baer MR. FLT3 Inhibitors in Acute Myeloid Leukemia: Current Status and Future Directions. *Mol Cancer Ther*. 2017;16(6):991-1001.
198. Yan F, Al-Kali A, Zhang Z, Liu J, Pang J, Zhao N, et al. A dynamic N(6)-methyladenosine methylome regulates intrinsic and acquired resistance to tyrosine kinase inhibitors. *Cell Res*. 2018;28(11):1062-1076.
199. Zhang Y, Kang M, Zhang B, Meng F, Song J, Kaneko H, et al. m(6)A modification-mediated CBX8 induction regulates stem-

- ness and chemosensitivity of colon cancer via upregulation of LGR5. *Mol Cancer*. 2019;18(1):185.
200. Yarchoan M, Johnson BA, 3rd, Lutz ER, Laheru DA, Jaffee EM. Targeting neoantigens to augment antitumour immunity. *Nat Rev Cancer*. 2017;17(4):209-222.
201. Sahin U, Derhovnessian E, Miller M, Kloke BP, Simon P, Löwer M, et al. Personalized RNA mutanome vaccines mobilize poly-specific therapeutic immunity against cancer. *Nature*. 2017;547(7662):222-226.
202. Ott PA, Hu Z, Keskin DB, Shukla SA, Sun J, Bozym DJ, et al. An immunogenic personal neoantigen vaccine for patients with melanoma. *Nature*. 2017;547(7662):217-221.
203. Han D, Liu J, Chen C, Dong L, Liu Y, Chang R, et al. Antitumour immunity controlled through mRNA m(6)A methylation and YTHDF1 in dendritic cells. *Nature*. 2019;566(7743):270-274.
204. Yang S, Wei J, Cui YH, Park G, Shah P, Deng Y, et al. m(6)A mRNA demethylase FTO regulates melanoma tumorigenicity and response to anti-PD-1 blockade. *Nat Commun*. 2019;10(1):2782.
205. Chen B, Ye F, Yu L, Jia G, Huang X, Zhang X, et al. Development of cell-active N6-methyladenosine RNA demethylase FTO inhibitor. *J Am Chem Soc*. 2012;134(43):17963-71.
206. Zheng G, Cox T, Tribbey L, Wang GZ, Iacoban P, Booher ME, et al. Synthesis of a FTO inhibitor with anticonvulsant activity. *ACS Chem Neurosci*. 2014;5(8):658-65.
207. Huang Y, Yan J, Li Q, Li J, Gong S, Zhou H, et al. Meclofenamic acid selectively inhibits FTO demethylation of m6A over ALKBH5. *Nucleic Acids Res*. 2015;43(1):373-84.
208. Toh JDW, Sun L, Lau LZM, Tan J, Low JJA, Tang CWQ, et al. A strategy based on nucleotide specificity leads to a subfamily-selective and cell-active inhibitor of N(6)-methyladenosine demethylase FTO. *Chem Sci*. 2015;6(1):112-122.
209. Bedi RK, Huang D, Eberle SA, Wiedmer L, Sledz P, Caflisch A. Small-Molecule Inhibitors of METTL3, the Major Human Epitranscriptomic Writer. *ChemMedChem*. 2020;15(9):744-748.
210. Müller S, Bley N, Busch B, Glaß M, Lederer M, Misiak C, et al. The oncofetal RNA-binding protein IGF2BP1 is a druggable, post-transcriptional super-enhancer of E2F-driven gene expression in cancer. *Nucleic Acids Res*. 2020;48(15):8576-8590.
211. Liu XM, Zhou J, Mao Y, Ji Q, Qian SB. Programmable RNA N(6)-methyladenosine editing by CRISPR-Cas9 conjugates. *Nat Chem Biol*. 2019;15(9):865-871.
212. Li J, Chen Z, Chen F, Xie G, Ling Y, Peng Y, et al. Targeted mRNA demethylation using an engineered dCas13b-ALKBH5 fusion protein. *Nucleic Acids Res*. 2020;48(10):5684-5694.
213. Wilson C, Chen PJ, Miao Z, Liu DR. Programmable m(6)A modification of cellular RNAs with a Cas13-directed methyltransferase. *Nat Biotechnol*. 2020.
214. Chen M, Wei L, Law CT, Tsang FH, Shen J, Cheng CL, et al. RNA N6-methyladenosine methyltransferase-like 3 promotes liver cancer progression through YTHDF2-dependent posttranscriptional silencing of SOCS2. *Hepatology*. 2018;67(6):2254-2270.
215. Li J, Chen F, Peng Y, Lv Z, Lin X, Chen Z, et al. N6-Methyladenosine Regulates the Expression and Secretion of TGFβ1 to Affect the Epithelial-Mesenchymal Transition of Cancer Cells. *Cells*. 2020;9(2).
216. Zhang J, Pi J, Liu Y, Yu J, Feng T. [Knockdown of YTH N(6)-methyladenosine RNA binding protein 2 (YTHDF2) inhibits proliferation and promotes apoptosis in MGC-803 gastric cancer cells]. *Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi*. 2017;33(12):1628-1634.
217. Wang H, Xu B, Shi J. N6-methyladenosine METTL3 promotes the breast cancer progression via targeting Bcl-2. *Gene*. 2020;722:144076.
218. Liu X, Gonzalez G, Dai X, Miao W, Yuan J, Huang M, et al. Adenylate Kinase 4 Modulates the Resistance of Breast Cancer Cells to Tamoxifen through an m(6)A-Based Epitranscriptomic Mechanism. *Mol Ther*. 2020;28(12):2593-2604.
219. Wu L, Wu D, Ning J, Liu W, Zhang D. Changes of N6-methyladenosine modulators promote breast cancer progression. *BMC Cancer*. 2019;19(1):326.

How to cite this article: Dong S, Wu Y, Liu Y, Weng H, Huang H. N⁶-methyladenosine Steers RNA Metabolism and Regulation in Cancer. *Cancer Commun*. 2021;1-22.
<https://doi.org/10.1002/cac2.12161>