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Role of *N*^{6}-methyladenosine modification in cancer

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Abstract

As the most abundant internal modification in eukaryotic messenger RNAs identified, *N*^{6}-methyladenosine (m^{6}A) has been shown recently to play essential roles in various normal bioprocesses. Evidence is emerging that m^{6}A modification and its regulatory proteins also play critical roles in various cancers including leukemia, brain tumor, breast cancer and lung cancer, etc. For instance, FTO, the first m^{6}A demethylase identified, has been reported recently to play an oncogenic role in leukemia and glioblastoma. ALKBH5 (another m^{6}A demethylase) has been reported to exert a tumor-promoting function in glioblastoma and breast cancer. METTL3 (a major m^{6}A methyltransferase) likely plays distinct roles between glioblastoma and lung cancer. Here we discuss the recent progress and future prospects in study of m^{6}A machinery in cancer.

Introduction

N^6-methyladenosine (m^{6}A) is the most abundant internal modification in eukaryotic messenger RNAs (mRNAs) that mainly occur at consensus motif of RRm^{6}ACH ([G/A/U][G>A]m^{6}AC[U>A>C] [1,2]. Although m^{6}A was first discovered in 1970s [3,4], functional characteristics and regulatory mechanisms of m^{6}A modification were largely unknown until recent years [1,2]. The identification of the fat mass and obesity-associated protein (FTO) as a *bona fide* demethylase of m^{6}A modification [5] and the development of transcriptome-wide approaches for m^{6}A sequencing [6,7] have indicated that m^{6}A is a reversible and dynamic RNA modification that may affect thousands of mRNAs and non-coding RNAs in a given type of cells. The deposition of m^{6}A is catalyzed by the m^{6}A methyltransferase complex (MTC) composed of methyltransferase-like 3 and 14 (METTL3 and METTL14) (i.e., writers) and their cofactor, Wilms tumor 1-associated protein (WTAP) [8–11]. The

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Conflict of Interest Statement

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removal of m^{6}A is facilitated by FTO and ALKBH5, two m^{6}A demethylase (i.e., erasers) that may target distinct sets of target mRNAs [2,5,12]. YTHDF1, YTHDF2, YTHDF3, and YTHDC1, members of the YT521-B homology (YTH) domain family of proteins, have been identified as m^{6}A direct readers that affect the translation, stability, and/or splicing of target mRNAs [13–17] (see Figure 1). Recent studies have shown that m^{6}A modification in mRNAs or non-coding RNAs plays essential roles in virtually all types of normal bioprocesses including tissue development, self-renewal and differentiation of stem cells, heat shock response, circadian clock control, DNA damage response, and maternal-to-zygotic transition, likely through affecting RNA fate/metabolism and functions such as mRNA stability, splicing, transport, localization, translation, primary microRNA processing, and RNA-protein interactions [6,7,10,12–14,18–26]. While still in the beginning stage, efforts have also been made to investigate the biological impacts of m^{6}A modification in cancer. In this review, we summarize the recent advance in our understanding of the biological functions and underlying molecule mechanisms of m^{6}A regulatory proteins (i.e., writers, erasers and readers) in various types of cancers, and also discuss future prospects.

FTO plays an oncogenic role in leukemia as an m^{6}A demethylase

FTO was first reported to be associated with increased body mass and obesity in humans [27–30]. In line with a link between the single nucleotide polymorphism (SNP) risk genotype and increased FTO expression in human blood cells and fibroblasts [31,32], transgenic mouse model studies have demonstrated a critical role of FTO in regulating fat mass, adipogenesis and body weight [33,34,35•], though IRX3 has also been suggested to be associated with obesity-associated variants within *FTO* [36]. The identification of FTO as the first m^{6}A demethylase suggests that FTO involves in m^{6}A-based post-transcriptional regulation of RNA targets. Through analysis of genome-wide gene expression profiles of several large-cohorts of human primary acute myeloid leukemia (AML) patients, Li Z. et al. found that *FTO* is highly expressed in certain subtypes of AMLs including AMLs carrying t(11q23)/MLL-rearrangements, t(15;17)/PML-RARA, NPM1 mutation (i.e., cytoplasmic localization of NPM1 (NPM1c+)), and/or Fms-like tyrosine kinase 3 with internal tandem duplication (FLT3-ITD) [37••]. More importantly, they provided compelling evidence, based on both in vitro leukemia cell line models and in vivo mouse leukemia models, showing that FTO plays an essential oncogenic role in promoting leukemic cell transformation and AML cell survival/growth and enhancing leukemogenesis, as well as in inhibiting all-trans-retionic acid (ATRA)-induced differentiation of AML cells [37••] (see Figure 2, upper left; Table 1).

Mechanistically, FTO functions as an m^{6}A demethylase that post-transcriptionally regulates expression of its critical target RNAs (such as *ASB2* and *RARA*) in an m^{6}A-dependent manner. *ASB2* and *RARA* have been implicated in leukemia cell growth and drug response, especially in ATRA-induced AML cell differentiation [38–40]. FTO negatively regulates expression of *ASB2* and *RARA* through reducing the m^{6}A abundance of the target RNA transcripts (especially in the 3′ untranslated regions (3′-UTRs)) and thereby decreasing the stability of the RNA transcripts [37••] (see Figure 2, upper left). Notably, in this study, the luciferase-reporter and mutagenesis assays have been introduced into the field of m^{6}A-related research, for the first time, to demonstrate that the putative m^{6}A consensus motif sites on the target RNA transcripts are important for the m^{6}A-based epigenetic regulation of

the target mRNA transcripts [37••]. A recent study suggests that FTO also exhibits demethylation activity towards RNA with N^6,2′-*O*-dimethyladenosine (m^6A_m) in the 5′ cap, as a sub-form of m^6A, leading to enhanced mRNA stability [41]. Nonetheless, roughly ¼ of FTO target mRNAs may contain an A as the first encoded nucleotide adjacent to the 7-methylguanosine (m^7G) cap and there is a pretty low chance that this A is methylated at both N^6 and 2′-O sites; in contrast, on average there are 3–5 internal m^6A sites per mRNA transcript [42]. Thus, the overall abundance of internal m^6A modifications should be much higher than that of the 5′ cap m^6A_m modification in cells. Indeed, analysis of the m^6A-seq data reported in [37••] showed that over 95% of the m^6A peaks with increased abundance upon *FTO* knockdown in AML cells are located in the internal regions (> 150 nucleotides away from the 5′ ends) and thus are impossible 5′ cap m^6A_m. In addition, liquid chromatography-tandem mass spectrometry (LC-MS/MS)-based quantification of m^6A and m^6A_m in human AML cells showed that the internal m^6A abundance is approximately 20–30 times of the near 5′ cap m^6A_m abundance, and the internal m^6A peaks are the main substrates of FTO and represent the major changes in the N^6-methyladenosine abundance when *FTO* is forced expressed or knocked down in AML cells (Su R, et al. unpublished). Moreover, as demonstrated by the luciferase reporter and mutagenesis assays, the internal m^6A sites on *ASB2* and *RARA* transcripts are required for FTO-mediated post-transcriptional regulation of their stability and expression [37••]. Therefore, internal m^6A sites, rather than the rare m^6A_m in the 5′ cap, are responsible for FTO-mediated post-transcription regulation of target mRNAs in cancer cells.

Dysregulation of other m⁶A regulatory proteins in leukemia

WTAP, which was first identified as a partner of Wilms' tumor gene 1 (WT1), has been reported recently to play an oncogenic role in AML [43]. *WTAP* was found to be aberrantly overexpressed in 32% of AML patients, especially in patients carrying *NPM1* mutation and/or *FTL3*-ITD; depletion of expression of *WTAP* significantly inhibited AML cell growth and promoted AML cell differentiation [43] (see Table 1). Further studies are warranted to determine whether WTAP's such oncogenic function in AML is related to its role as a cofactor of METTL3 and METTL14 that form m^6A MTC. Interestingly, the phenomenon that both *WTAP* and *FTO* are highly expressed in AML carrying *NPM1* mutation and/or *FLT3*-ITD [37••,43] suggests that m^6A modification in this subtype of AML is tightly and sophistically controlled by both writer and eraser regulators.

Gene mutations and copy number variations (CNVs) of m^6A regulatory genes, including *METTL3*, *METTL14*, *FTO*, *ALKBH5*, *YTHDF1* and *YTHDF2* have been investigated in leukemia patients through analysis of The Cancer Genome Atlas (TCGA) sequence data [44•]. Around 2.6% (5/191) and 9.7% (18/186) of AML patients were found to carry mutations and CNVs, respectively, in one or more of these genes; the mutations and CNVs are especially enriched in AML patients carrying p53 mutations, but rarely in patients carrying mutations in *NPM1*, *FLT3*, *IDH1* and *IDH2* [44•]. Patients with genetic alterations in these genes are often associated with poor prognosis, largely due to their strong association with p53 mutations [44•]. Notably, among the CNV events of m^6A regulatory genes, copy number loss of *ALKBH5* is the most frequent one (12/191; 6.3%); depletion of *ALKBH5* was significantly associated with poorer cytogenetic risk and the presence of p53

mutations in AML [44•] (see Figure 2, upper left; Table 1). Interestingly, as the second m^{6}A demethylase identified, ALKBH5 was reported to affect mRNA export and RNA metabolism, and *Alkbh5* deficiency leads to aberrant spermatogenesis and apoptosis in mouse testes, likely through regulating genes associated with the p53 network [12]. Consistently, according to The cBioPortal for Cancer Genomics database [45], unlike *METTL3*, *METTL14*, *WTAP*, *FTO*, *YTHDF2* and *YTHDC1* that are expressed at a relatively higher level in AML than in many other types of cancers, *ALKBH5* (as well as *YTHDF1*) is expressed at a low level in AML. Thus, opposite to the oncogenic role of FTO, ALKBH5 may exert a tumor-suppressor function in AML, which warrants further experimental validation.

m^{6}A RNA modification in solid tumors

In brain tumors, Zhang et al. found that *ALKBH5* expression is elevated in glioblastoma (GBM) stem(-like) cells (GSCs) and elevated expression of *ALKBH5* predicts poor prognosis in GBM patients [46••]. ALKBH5 enhances GSC self-renewal proliferation and promotes tumorigensis through demethylating *FOXM1* nascent transcripts, with the aid of the lncRNA antisense (*FOXM1-AS*) that promotes the interaction of *FOXM1* nascent transcripts with ALKBH5, and thereby enhancing *FOXM1* expression [46••] (see Figure 2, lower left; Table 1). Similarly, Cui et al. [47••] reported that m^{6}A levels in GSCs were elevated upon induced differentiation. Accordingly, knockdown of *METTL3* or *METTL14* significantly enhanced GSC growth and self-renewal and promoted tumor progression, and the opposite is true when *METTL3* was overexpressed or FTO function was pharmaceutically inhibited [47••] (see Figure 2, upper right and lower left; Table 1). A number of GSC-associated genes (e.g., *ADAM19*) are likely the targets of m^{6}A modifications in GSCs [47••] (see Figure 2, upper right).

In breast cancer cells, *ALKBH5* expression was found to be up-regulated by hypoxia-stimulated HIF1α and HIF2α, and thereby promoted mRNA stability and expression of *NANOG*, a gene encoding a pluripotency factor, which in turn enhanced breast cancer stem cell (BCSC) enrichment; knockdown of *ALKBH5* inhibited tumor formation and decreased BCSC population in breast tumors [48•] (see Figure 2, lower left; Table 1). ZNF217 may also participate in the hypoxia-induced up-regulation of expression of *NANOG* and *KLF4* (another pluripotency factor gene) in breast cancer cells [49], likely through sequestering METTL3 and thereby inhibiting m^{6}A methylation on *NANOG* and *KLF4* transcripts [50].

METTL3 was observed to be up-regulated in lung adenocarcinoma and played an oncogenic role that promotes growth, survival and invasion of human lung cancer cells [51••]. Notably, METTL3 was shown to promote translation of its target mRNA transcripts (e.g., *EGFR* and *TAZ*) by interaction with translation initiation machinery, in a manner independent of its methytransferase activity [51••] (see Figure 2, lower right; Table 1). However, the biological function of METTL3 in lung cancer cells was determined solely by loss-of-function studies through knockdown of *METTL3* expression, and it is unclear whether METTL3 mutants that lose its methyltransferase activity can exert the same degree of oncogenic effects as wild-type METTL3 in promoting growth, survival and invasion of human lung cancer cells. Thus, systematic gain-of-function studies with both wild-type and mutant METTL3

constructs, under conditions of with or without depletion of endogenous *MELLT3* expression, are necessary to determine the functional importance of METTL3 as an m^6A reader in cytoplasm in the pathogenesis of lung cancer and other types of cancers.

Conclusions & Perspectives

While we are just beginning to understand the function of the m^6A modification machinery in cancer, recent studies have shown that m^6A regulatory proteins likely play essential roles in various types of cancers, including leukemia, brain tumor, breast cancer and lung cancer (see Figure 2 and Table1). Interestingly, some proteins likely play a similar role across different types of cancers, whereas some others may function differently in distinct types of cancers. For instance, FTO has been shown to function as an oncoprotein in both leukemia and GBM [37••,47••]. In contrast, while ALKBH5 functions as an oncoprotein in GBM and breast cancer [46••,48•], it may exert a tumor-suppressor role in AML as implied by its frequent copy number loss [44•] and low expression level in AML. Similarly, METTL3 likely plays an oncogenic role in lung cancer [51••] but a tumor-suppressor role in GBM [47••]. Notably, both *FTO* and *WTAP* are highly expressed and play an oncogenic role in certain subtypes of AMLs. In addition, *METTL3* and *METTL14* are also highly expressed in AML and thus they may also play an oncogenic role in AML. These phenomena suggest that an m^6A eraser (e.g., FTO) does not necessarily function oppositely than a component of the m^6A methyltransferase (writer) complex (e.g., WTAP, METTL3 or METTL14) in the same cancer type. Similarly to this, it was known that both DNMT3A (a DNA methyltransferase) and TET2 (a DNA demethylase) are frequently associated with loss-of-function mutations and both function as tumor-suppressors in myeloid malignancies [52,53], and they may cooperate in repressing lineage differentiation of hematopietic stem cells [54]. Thus, it is possible that a writer and an eraser of the same epigenetic modification could play similar functions in the same cell context, likely through regulating distinct sets of targets. Future systematic studies are warranted to determine the biological function of each individual m^6A regulatory genes in different types of cancers, and to identify their cirtical target genes to reveal the underlying molecule mechansims.

Interestingly, besides its role as the major m^6A methyltransferase in nucleus, METTL3 may also exert some function independent of its catalytic activity, such as promoting translation of target transcripts as an m^6A reader in cytoplasm in lung cancer cells [51••]. Nevertheless, since METTL3's translation-promoting function also relies on the m^6A modification on its target mRNAs, METTL3's catalytic activity is still required for its function in promoting translation of the target transcripts. It would be important to determine how critical its role as an m^6A reader is in its overall pathological function in each type of cancer. It is possible that in some types of cancers, its role as an m^6A reader is dispensible, whereas its m^6A methyltransferase role is still required.

Given the critical roles of the m^6A regulatory proteins in cancers, they (especially the methylatransferase and demethylase proteins with catalytic activies) appear to be good drug targets for cancer therapy. Several FTO small-molecule inhibitors have been identified [55–58]; among them, meclofenamic acid (MA) [56] and MO-I-500 [57] have been shown to be able to effectively inhibit the survival and growth of GBM and breats cancer cells by

inhibition of the catalytic activity of FTO [47••,58]. Development of clinically applicable selective and effective inhibitors for FTO and other m^{6}A regulatory proteins may provide more effetcive novel therapeutic strategies to treat cancers, especially in combination with other therapeutic agents to treat cancers that are resistant to currently available therapies.

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Abbreviations

m^{6}A	N^6-methyladenosine
MTC	methyltransferase complex
FTO	the fat mass and obesity-associated protein
SNP	single nucleotide polymorphism
LC-MS/MS	liquid chromatography coupled with tandem mass spectrometry
METTL3 and METTL14	methyltransferase-like 3 and 14
WTAP	Wilms tumor 1-associated protein
AML	acute myeloid leukemia
FLT3-ITD	Fms-like tyrosine kinase 3 with internal tandem duplication
NPM1c+	NPM1 mutant that causes aberrant cytoplasmic localization of nucleophosmin
ATRA	all-trans-retionic acid
CNV	copy number variation
GBM	glioblastoma
GSC	glioblastoma stem(-like) cell

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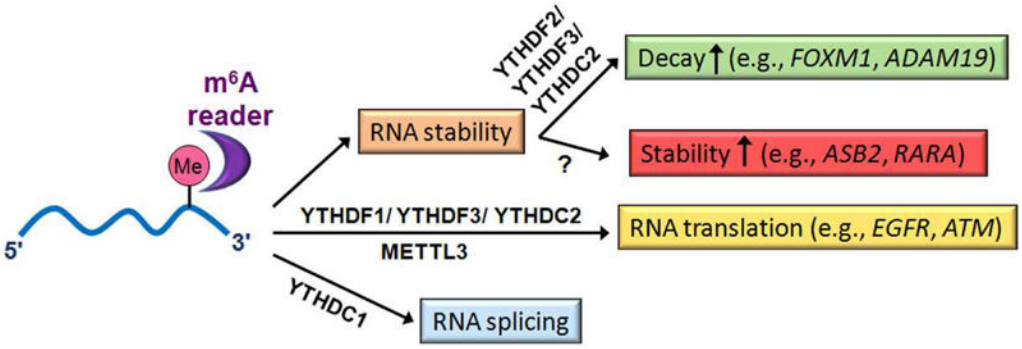

Figure 1. The fates of m^{6}A-modified mRNA transcripts are influenced by different m^{6}A readers

See References [13–17] for more details. The examples of m^{6}A-modification affected target mRNAs shown herein are those that have been reported to be dysregulated in cancer (see Figure 2 and Table 1 for more information). While many m^{6}A-modified mRNA transcripts (e.g., *FOXM1* and *ADAM19*) can be recognized by readers such as YTHDF2, YTHDF3 and/or YTHDC2 that promote mRNA decay, other m^{6}A-modified mRNA transcripts (e.g., *ASB2* and *RARA*) can be recognized by some currently unknown m^{6}A readers that promote mRNA stability. Different m^{6}A readers can also promote translation or affect splicing of m^{6}A-modified target mRNAs. Besides serving as an m^{6}A methyltransferase in nucleus, METTL3 may also serve as an m^{6}A reader in cytoplasm in some scenarios (e.g., Ref. [51]).

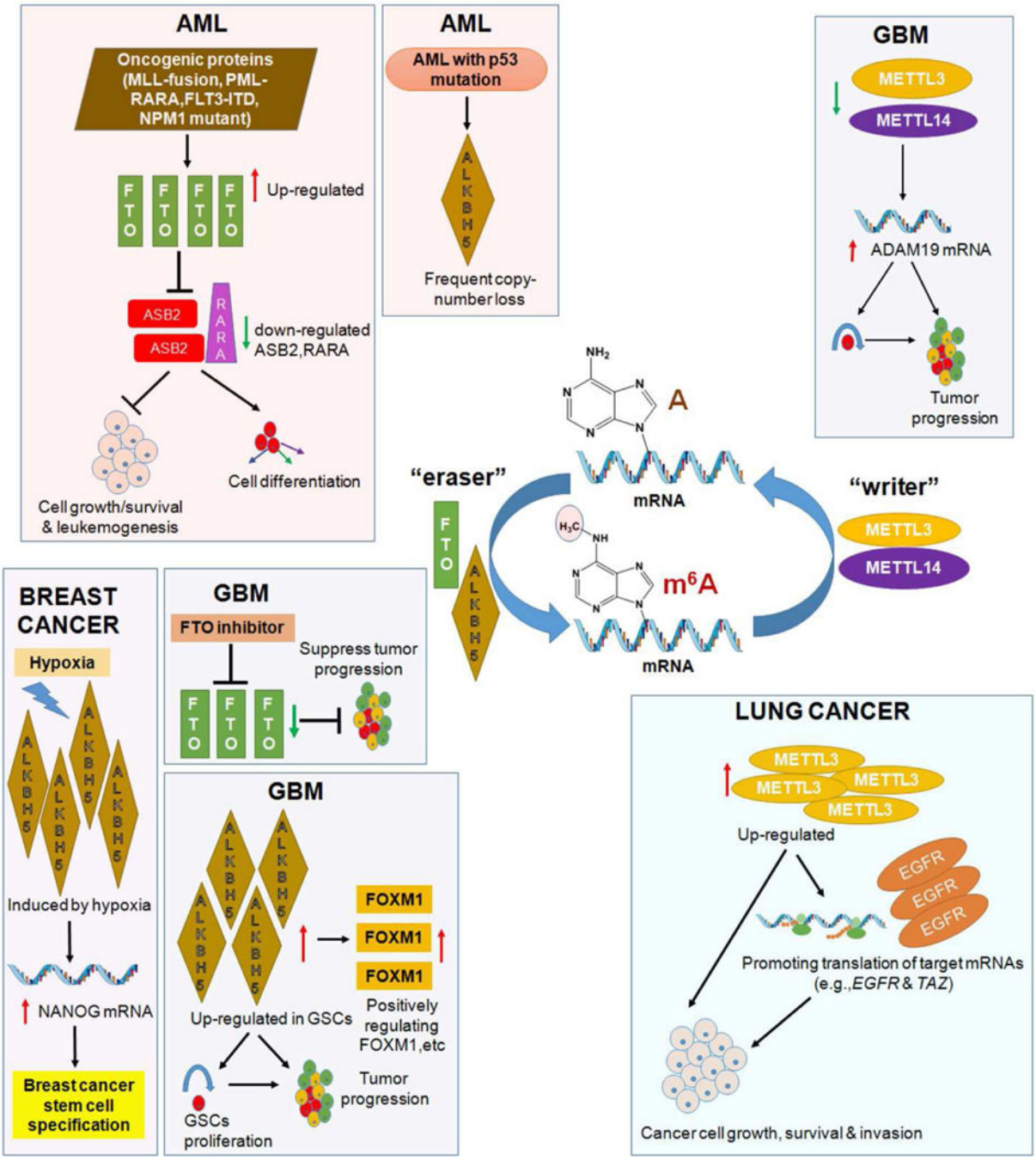

Figure 2. The roles of m^{6}A regulatory proteins in AML, breast cancer, GBM and lung cancer

m^{6}A, N^6 methyladeosine; AML, acute myeloid leukemia; GBM: glioblastoma; GSCs: glioblastoma(-like) cells.

Table 1

m^{6}A regulators in cancer

Protein	Functional classification	m^{6}A-associated function (and underlying mechanism) in cancer	Refs
METTL3	m^{6}A writer component: catalytic subunit of m^{6}A MTC	• Oncogenic role in lung cancer: It is up-regulated in lung cancer; required for the growth, survival and invasion of lung cancer cells; promoting translation of target mRNAs (e.g., *EGFR* and *TAZ*) independent of its catalytic activity. • Tumor-suppressor role in GBM: Its knockdown enhances growth/self-renewal of GSCs and promotes tumor progression; may targets *ADAM19*, etc.	[51••] [47••]
METTL14	m^{6}A writer component: core subunit of m^{6}A MTC	• Tumor-suppressor role in GBM: same as METTL3	[47••]
WTAP	m^{6}A writer component: regulatory subunit of m^{6}A MTC	• Oncogenic role in AML: it is upregulated in >30% AML cases, especially those carrying FLT3-ITD and/or NPM1 mutation; its knockdown inhibits AML cell growth/survival and tumor growth; no m^{6}A-related mechanism was investigated though.	[43]
FTO	m^{6}A eraser (demethylase)	• Oncogenic role in AML: it is upregulated in AMLs carrying t(11q23)/MLL-rearranged, t(15;17)/PML-RARA, FLT3-ITD and/or NPM1 mutation; its knockdown inhibits AML cell growth/survival and leukemogenesis and enhances AML cell differentiation, and the opposite is true when it is forced expressed; negatively regulating *ASB2* and *RARA*, etc. • Oncogenic role in GBM: Catalytic activity inhibition by a small-molecule inhibitor suppresses tumor progression.	[37••] [47••]
ALKBH5	m^{6}A eraser (demethylase)	• Oncogenic role in GBM: It is up-regulated in GSCs; required for GSC proliferation and tumor progression; positively regulating *FOXM1* etc. • Oncogenic role in breast cancer: It is induced by hypoxia in breast cancer cells; required for hypoxic tumor microenvironment; positively regulating *NANOG* etc. • Potential tumor-suppressor role in AML: Its copy-number loss is frequent in AML, especially in AML with p53 mutations.	[46••] [48•] [44•]

m6A, Λ6 methyladeosine; m6A MTC: m6A methyltransferase complex; GBM: glioblastoma; GSCs: glioblastoma(−like) cells; AML, acute myeloid leukemia.