REVIEW

Aberrant expression of enzymes regulating m⁶A mRNA methylation: implication in cancer

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ABSTRACT	N⁶-methyladenosine (m⁶A) is an essential RNA modification that regulates key cellular processes, including stem cell renewal, cellular differentiation, and response to DNA damage. Unsurprisingly, aberrant m⁶A methylation has been implicated in the development and maintenance of diverse human cancers. Altered m⁶A levels affect RNA processing, mRNA degradation, and translation of mRNAs into proteins, thereby disrupting gene expression regulation and promoting tumorigenesis. Recent studies have reported that the abnormal expression of m⁶A regulatory enzymes affects m⁶A abundance and consequently dysregulates the expression of tumor suppressor genes and oncogenes, including *MYC*, *SOCS2*, *ADAM19*, and *PTEN*. In this review, we discuss the specific roles of m⁶A "writers", "erasers", and "readers" in normal physiology and how their altered expression promotes tumorigenesis. We also describe the potential of exploiting the aberrant expression of these enzymes for cancer diagnosis, prognosis, and the development of novel therapies.
KEYWORDS	RNA modification; N⁶-methyladenosine (m⁶A); cancer; tumor suppressor; oncogene

Introduction

RNA modifications have recently been shown to play important roles in normal and disease biology. Over 170 different types of post-transcriptional modifications have been identified in RNA, many of which have unknown functions. Among all RNA modifications, N⁶-methyladenosine (m⁶A) is the most abundant internal modification on eukaryotic mRNAs. m⁶A occurs predominantly at the consensus RRACH motif and is enriched near the 5' or 3' untranslated regions (UTRs) of mRNAs[1,2]. m⁶A is now established as a key modification that influences virtually all aspects of mRNA fate and metabolism, including mRNA stability[3,4], splicing[1,3,5], transport and localization[4], translation[6-8], microRNA (miRNA) processing[9], and RNA-protein interactions[10]. Advances in characterizing m⁶A were made possible through the recent development of m⁶A immunoprecipitation and sequencing techniques[1,2] and the discovery of enzymes that regulate m⁶A modification[4,11-14]. Enzymes that regulate m⁶A deposition on

mRNAs and their consequent transcriptional outcomes include RNA specific methylases (writers), demethylases (erasers), and reader proteins (**Figure 1**).

Together, the tightly-regulated functions of m⁶A writers, erasers, and readers are critical in maintaining the integrity of m⁶A RNA modification in cells. Recent studies have indicated that the aberrant expression of these enzymes is involved in human diseases, including cancer. In this review, we describe the roles of known writers, erasers, and readers of m⁶A mRNA methylation in diverse biological processes. We summarize the role of the overexpression and/or downregulation of m⁶A regulatory enzymes in promoting cancer initiation and progression (**Figure 2**, **Table 1**). We also discuss the potential of targeting m⁶A regulatory enzymes with aberrant expression for cancer therapy.

m⁶A mRNA methylation occurs *via* a methyltransferase complex

m⁶A RNA methylation is mediated by a core methyltransferase complex composed of the methyltransferase-like 3 and 14 (METTL3 and METTL14) proteins and their cofactor, Wilms' tumor 1-associated protein (WTAP)[12,13,15,16]. Structural studies indicate that METTL3 is the catalytic component that forms a heterodimer with METTL14 to facilitate the correct

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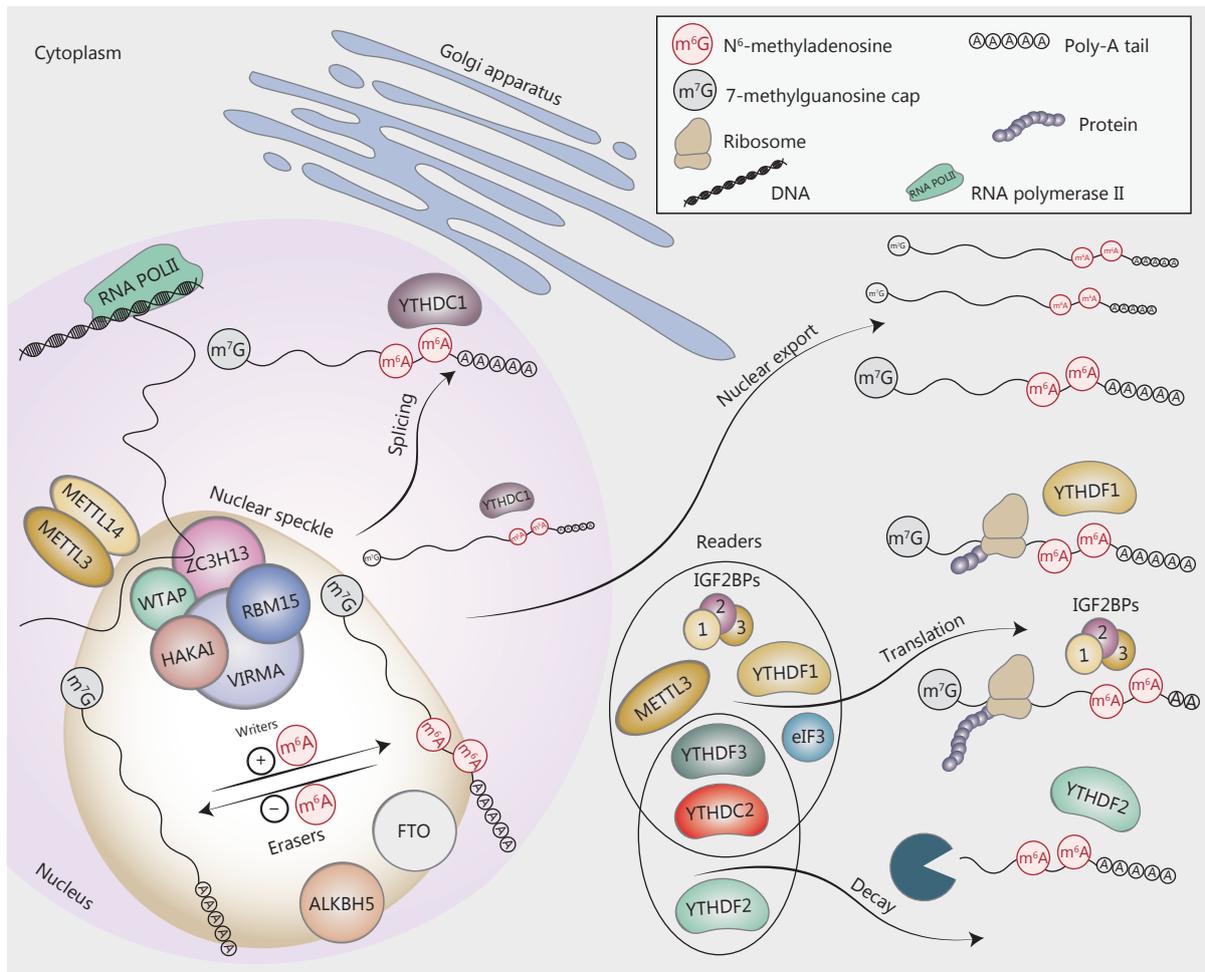E-mail: j.wong@centenary.org.au

Figure 1 The m⁶A regulatory enzymes. N⁶-Methyladenosine (m⁶A) RNA methylation is a dynamic and reversible process coordinated by a battery of enzymes with defined roles. The methyltransferase complex, made up of m⁶A 'writers' including METTL3, METTL14, WTAP, ZC3H13, CBLL1, VIRMA and RBM15, localizes to the nuclear speckles where it methylates target RNA transcripts. Conversely, FTO and ALKBH5 (m⁶A 'erasers') remove m⁶A from select transcripts, establishing a dynamic equilibrium between methylated and unmethylated RNA transcripts within the cell. Finally, proteins like YTHDF1-3, YTHDC1, IGF2BPs and eIF3 (m⁶A 'readers') recognize and bind m⁶A to direct essential molecular functions such as RNA splicing, nuclear export, translation and RNA decay.

interactions with its target mRNAs[15]. WTAP ensures the localization of the METTL3/METTL14 heterodimer to nuclear speckles where m⁶A RNA methylation occurs[16].

Ancillary to this METTL3/METTL14/WTAP core machinery are several m⁶A regulatory proteins necessary for the full methylation program, including virilizer like m⁶A methyltransferase associated protein (VIRMA), zinc-finger CCCH-type containing 13 (ZC3H13), Cb1 proto-oncogene like 1 (CBLL1), and RNA-binding motif protein 15 (RBM15)[7,13,14,17,18]. Knockdown of each of these proteins leads to a significant decrease in m⁶A deposition[7,13,14]. In addition, VIRMA was recently shown to mediate the recruitment of the methyltransferase core subunits to the 3'

UTRs and stop codons for region-selective RNA methylation[17]. Depletion of VIRMA was shown to result in the lengthening of the 3' UTR in hundreds of mRNAs with significant overlap in target transcripts[17]. As a large 202 kD protein, VIRMA has also been proposed to be a scaffold upon which all other subunits attach. RBM15, an RNA structure recognizing protein, is involved in complex recruitment to specific consensus sequences for m⁶A methylation[7]. This protein interacts with WTAP *via* ZC3H13[18]. ZC3H13 also regulates the nuclear localization of the complex that comprises WTAP, VIRMA, and CBLL1[14]. Upon ZC3H13 depletion, methyltransferase complexes form in the cytoplasm, which may be counterproductive for m⁶A
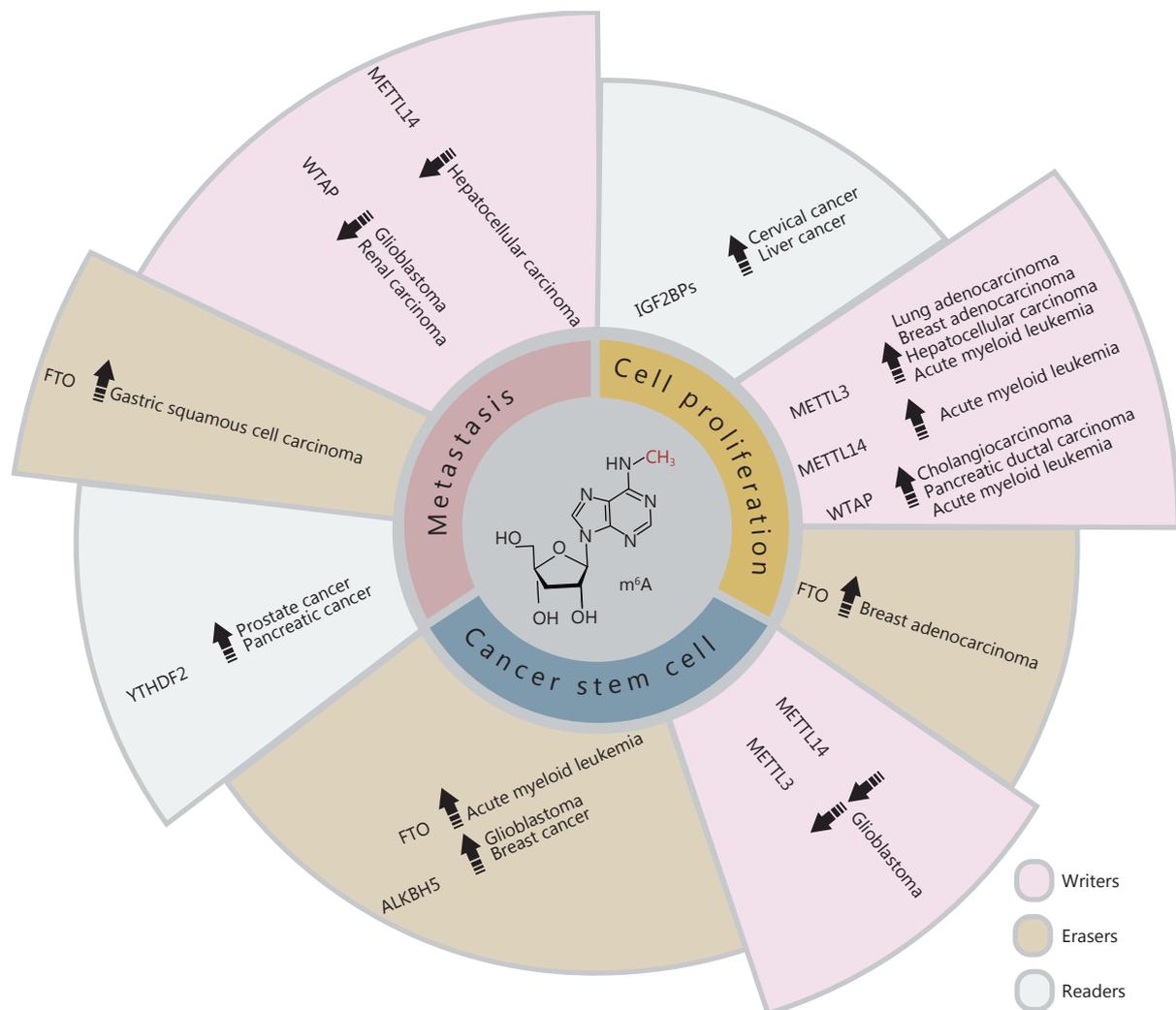
Figure 2 m6A regulators and the hallmarks of cancer. Abnormal expression of m6A writers, erasers and readers has been associated with various types of cancer affecting at least three of the hallmarks of cancer: metastasis, cell proliferation and the cancer stem cell phenotype.

methylation because it occurs primarily in the nucleus[14]. Other proteins, including TRIM28 and HNRNPH, have also been identified as components of the m6A methyltransferase complex although their specific roles remain unclear[17]. Co-immunoprecipitation studies using different antibodies have identified 26 core interacting factors among hundreds of WTAP-binding proteins, while more than 100 proteins may bind METTL3 or METTL14[19,20]. Thus, there may be other components of the m6A methyltransferase complex that have not yet been identified.

m6A mRNA methylation is removed *via* specific demethylases

Unlike the large multi-subunit m6A methyltransferase

complex, only two m6A demethylases have been identified: the fat-mass and obesity-associated protein (FTO) and AlkB homolog 5 (ALKBH5)[4,11]. Both FTO and ALKBH5 are members of the AlkB family of Fe(II)/α-ketoglutarate-dependent dioxygenases with a well-conserved catalytic domain. The knockdown and overexpression of both enzymes have been shown to affect functions regulated by m6A methylation, including splicing, RNA stability, and translation[3,21,22]. While the function of each of these enzymes is to remove m6A, they also appear to act on select subsets of mRNA targets, which is consistent with their enrichment in different tissues and subcellular compartments[4,23]. A recent study demonstrated that target recognition by FTO and ALKBH5 is not dependent on the consensus m6A sequence motif but instead the structure and conformation of target

Table 1 Roles of aberrant m⁶A writers, erasers and readers in human cancers

m⁶A-related function	Enzyme	Cancer type	Aberrant expression in cancer	Role in cancer	Reference
Writers	METTL3	Lung adenocarcinoma	Overexpressed	Promotes growth, survival and proliferation Enhances translation of *EGFR*, *TAZ*, *MK2*, *DNMT3* and *BRD4*	[6,30,31]
		Breast adenocarcinoma	Overexpressed	Promotes proliferation *via* HBXIP/let-7g/METTL3/HBXIP positive feedback loop	[32]
		Hepatocellular carcinoma	Overexpressed	Targets the tumor suppressor gene, *SOCS2*, for m⁶A-mediated degradation by YTHDF2	[33]
		Glioblastoma	Downregulated	Promotes GSC growth and self-renewal Knockdown results in upregulation of *ADAM19*, *EPHA3* and *KLF4* (oncogenes) and downregulation of *CDKN2A*, *BRCA2*, and *TP53I11* (tumor suppressor genes)	[34]
			Overexpressed	Confers poor survival	[35]
		Endometrial carcinoma	Downregulated	Promotes cell proliferation by altering AKT signaling	[43]
		AML	Overexpressed	Promotes cell survival and proliferation by promoting m⁶A-mediated translation of c-*MYC*, *BCL2* and *PTEN*	[36,37]
	METTL14	Hepatocellular carcinoma	Downregulated	Depletion promotes metastasis Depletion reduces levels of tumor suppressor miR-126	[39]
		AML	Overexpressed	Enhances translation of oncogenes *MYC* and *MYB*	[42]
		Glioblastoma	Downregulated	Promotes GSC growth and self-renewal Knockdown causes upregulation of *ADAM19*, *EPHA3* and *KLF4* (oncogenes) and downregulation of *CDKN2A*, *BRCA2*, and *TP53I11* (tumor suppressors)	[34]
		Breast adenocarcinoma	Downregulated	Enhances tumor growth, angiogenesis and cancer progression	[68]
		Endometrial carcinoma	Loss due to mutation	Promotes cell proliferation by altering AKT signaling	[43]
	WTAP	Glioblastoma	Overexpressed	Regulates migration and invasion *via* EGF signaling	[44]
		Cholangiocarcinoma	Overexpressed	Oncogenic	[45]
		AML	Overexpressed	Promotes proliferation and clonogenicity Inhibits differentiation	[46]
		Renal carcinoma	Overexpressed	Promotes cell proliferation (by enhancing *CDK2* expression), cell migration *in vitro* and tumorigenesis *in vivo*	[3]
		Malignant glioma	Overexpressed	Oncogenic	[47]
		Pancreatic ductal carcinoma	Overexpressed	Oncogenic	[48]
Erasers	FTO	AML	Overexpressed	Promotes survival, proliferation and clonogenicity Reduced m⁶A affects *ASB2* and *RARA* stability to block myeloid differentiation	[21]
		Gastric squamous cell carcinoma	Overexpressed	Promotes proliferation and invasiveness of cancer cells	[54]
		Breast adenocarcinoma	Overexpressed	May increase glycolysis *via* PI3K/AKT signaling activity	[55]

m⁶A-related function	Enzyme	Cancer type	Aberrant expression in cancer	Role in cancer	Reference
	ALKBH5	Glioblastoma	Overexpressed	Maintenance and proliferation of glioblastoma stem-like cell and critical for brain tumorigenesis Enhances *FOXM1* expression by increasing m⁶A levels	[22]
		Breast cancer	Overexpressed	Promotes *NANOG* overexpression	[56]
		AML	Downregulated	Putative tumor suppressor Copy number loss associated with poorer survival and *TP53* mutation	[57]
Readers	YTHDF1	Hepatocellular carcinoma	Overexpressed	Associated with poor prognosis	[58]
		Colorectal cancer	Overexpressed	Associated with poor prognosis Overexpression is driven by *c-MYC*	[59]
	YTHDF2	Prostate cancer	Overexpressed	Promotes cell migration	[60]
		Pancreatic cancer	Overexpressed	Expression levels increased with cancer stage Inhibits epithelial-mesenchymal transition by suppressing YAP signaling	[61]
	IGF2BPs	Cervical cancer Liver cancer	Overexpressed	Promotes stability and translation of m⁶A-modified oncogenes including *MYC*	[29]

mRNAs that results from m⁶A deposition itself[24]. However, mRNA targets of FTO and ALKBH5 cannot be distinguished by RNA structure and conformation alone[24]. The specificity of mRNA substrate recognition by these enzymes remains to be determined.

m⁶A mRNA methylation is recognized by reader proteins to confer specific phenotypic outcomes

The most well-studied readers of m⁶A are the YT521-B homology (YTH) family of proteins. Structurally, this family of proteins shares a 100–150 amino acid residue YTH domain, forming a hydrophobic aromatic cage to accommodate and recognize m⁶A modifications[25]. This cage-like structure has high selectivity for the N⁶-modified adenosine within the RRACH consensus motif. Of these proteins, YTHDF1-3 are cytoplasmic m⁶A readers that have a variety of functions. YTHDF1 has been implicated in ribosome loading for transcript translation, while YTHDF2 regulates mRNA stability and translation[26,27]. In contrast, YTHDC1 is a nuclear m⁶A reader reported to assist in the localization of modified transcripts and the formation of focal sites of transcription called YT bodies adjacent to nuclear speckles[28]. YTHDC1 further interacts with several splicing factors, including SRSF3 and SRSF10, to regulate splicing[28].

The insulin-like growth factor-2 mRNA-binding proteins 1–3 (IGF2BP1-3) have also been reported to be m⁶A readers[29]. They possess K homology domains necessary for interaction with m⁶A-modified RNA. In contrast to YTHDF2, which destabilizes mRNA, IGF2BP1-3 proteins promote transcript stability by inhibiting degradation, enhancing storage, and facilitating translation of their target mRNAs[29].

Expression of m⁶A writers is altered in diverse cancers

METTL3

The role of aberrant METTL3 expression in cancers is well characterized. In lung adenocarcinoma, METTL3 overexpression promotes growth, survival, and invasion of cancer cells[6], whereas the repression of METTL3 expression in non-small cell lung cancer inhibits cell proliferation[30]. Increased expression of METTL3 enhances the translation of oncogenes, including *EGFR*, *TAZ*, *MK2*, and *DNMT3*, *via* interaction with the translation initiation complex[6]. Thus, besides introducing m⁶A in transcripts, METTL3 might also function as an m⁶A reader in the cytoplasm through interaction with the translation machinery[6]. Indeed, METTL3 has recently been shown to localize to the polyribosomes and interact directly with the eukaryotic translation initiation factor 3 subunit h (eIF3h) in lung cancer[31]. This interaction induces mRNA circularization,

thereby enhancing the translation of oncogenes, including the bromodomain-containing protein 4[31].

In breast adenocarcinoma, METTL3 expression is regulated by an oncoprotein, the hepatitis B X-interacting protein (HBXIP)[32]. HBXIP suppresses a miRNA, let-7g, which typically targets METTL3[32]. The consequent increase in METTL3 expression further enhances HBXIP expression *via* m6A modification of transcripts encoding this oncogene. This positive feedback loop involving HBXIP/let-7g/METTL3/HBXIP drives the proliferation of breast cancer cells[32].

METTL3 overexpression has been observed in hepatocellular carcinoma (HCC) and is associated with poorer survival[33]. Depletion of METTL3 reduces cell proliferation, migration, and colony formation *in vitro* and also decreases HCC tumorigenicity and metastasis capacity *in vivo*[33]. METTL3 directly targets the tumor suppressor gene *SOCS2* to facilitate its degradation *via* an m6A-dependent YTHDF2-mediated mechanism[33].

The association between METTL3 levels and glioblastoma development and maintenance remains unclear. One study showed that reduced METTL3 levels enhance glioblastoma stem-like cell (GSC) growth, self-renewal, and tumorigenesis[34]. Knockdown of METTL3 and METTL14 was shown to lead to the upregulation of oncogenes, including *ADAM19*, *EPHA3*, and *KLF4*[34], and downregulation of a number of tumor suppressor genes such as *CDKN2A*, *BRCA2*, and *TP53I11*[34]. However, another study showed that METTL3 was overexpressed in GSCs and glioblastoma samples[35]. Importantly, elevated METTL3 levels are associated with poorer survival in patients with glioblastoma[35]. METTL3 depletion was found to reduce tumor growth and prolong survival in mice, indicating that a decrease in METTL3 is unfavorable for the maintenance of glioblastoma[35]. Despite these discrepancies, it is clear that the aberrant expression of METTL3 is involved in this type of brain cancer. It is likely that the expression of m6A RNA methylases needs to be maintained within an optimal range to prevent progression to cancer.

In acute myeloid leukemia (AML), METTL3 expression appears to be a key driver of leukemia maintenance. METTL3 was identified in two independent CRISPR screens as an essential oncogene, and it functions to suppress apoptosis and myeloid differentiation[36,37]. Consistent with this finding, AML exhibited the highest levels of METTL3 expression among all cancers studied by The Cancer Genome Atlas Research Network (TCGA). Depletion of METTL3 in AML cell lines promotes cell differentiation and apoptosis and impairs leukemogenesis in murine leukemia models[36,37].

Mechanistically, METTL3 overexpression drives AML development and maintenance *via* two possible pathways. First, an increase in METTL3 levels promotes the m6A methylation-mediated translation of mRNAs encoding apoptosis repressors and/or oncogenes, including *c-MYC*, *BCL2*, and *PTEN*[36]. Second, METTL3 binds to the promoter of target genes *via* a CCAAT-box binding factor, in a CEBPZ-dependent mechanism, to promote their translation[37]. This process occurs as promoter-bound METTL3 potentiates the m6A methylation of transcripts encoded by these same genes to relieve ribosome stalling[37]. Promoters of key transcription factors, including SP1 and SP2, are among those bound by METTL3[37]. Importantly, *SP1* is a known oncogene that regulates *c-MYC* expression[38]. Thus, when elevated, METTL3, directly or indirectly, targets mRNAs encoding oncogenic proteins to drive leukemogenesis.

METTL14

METTL14 plays a fundamental role in the recognition of RNA substrates for m6A methylation. Therefore, aberrant expression of METTL14 can alter the fate of transcripts regulated by m6A. METTL14 has been reported to alter m6A levels in HCC[39]. It regulates the miRNA biogenesis pathway by facilitating the binding of the microprocessor complex subunit DGCR8 to primary miRNA in an m6A-dependent manner[39]. One prominent example is miR-126, which suppresses metastasis in HCC and breast cancer[40,41]. METTL14 overexpression increases the abundance of m6A methylation on primary miR-126, enhancing its recognition by DGCR8 for processing into mature miRNA[39]. Accordingly, depletion of METTL14 reduces the expression of mature miR-126, while overexpression of METTL14 accelerates miR-126 processing in HCC cells *in vitro*.

Clinically, low METTL14 expression was correlated with metastasis and poorer survival in one cohort of 130 patients with HCC[39]. In contrast, an independent study reported no change in METTL14 expression in the TCGA cohort of 50 HCC samples and showed that the stable repression of METTL14 suppresses proliferation, migration, and colony formation *in vitro*[33]. The discrepancy in the reports by these two groups suggests that METTL14 levels may need to fall within a certain range for normal functions in cells and that uncontrolled regulation, leading to sub-optimal expression, may promote tumorigenesis.

METTL14 is also expressed at high levels in hemopoietic stem and progenitor cells and is downregulated during myeloid cell differentiation[42]; correspondingly, human AML cells bearing one of the abnormal chromosomal

translocations t(11q23), t(15; 17), or t(8; 21) all overexpress METTL14[42]. The *MYC* and *MYB* oncogenes have been identified as METTL14 targets that are post-transcriptionally regulated in an m[6]A-dependent manner[42]. Thus, overexpression of METTL14 may promote leukemogenesis by enhancing the stability of transcripts encoding these oncogenes and their subsequent translation.

Up to 70% of endometrial cancers have been shown to exhibit reduced m[6]A levels, consistent with the presence of a METTL14 mutation and reduced expression of METTL3[43]. These aberrant changes promote cell proliferation. They are associated with decreased levels of a negative regulator of AKT, PHLPP2, and increased expression of mTORC2, which enhances AKT activity[43]. Thus, endometrial cancer may develop *via* altered m[6]A-modulated activity of the AKT signaling pathway[43].

Other components of the m[6]A writer complex

Other subunits of the m[6]A writer complex have previously been implicated in tumorigenesis. WTAP is an oncogene in diverse cancers, including glioblastoma[44], cholangiocarcinoma[45], and AML[46]. Its overexpression is associated with poor prognosis in AML[46], glioma[47], and pancreatic ductal adenocarcinoma[48]. RBM15 was first identified as a fusion oncogene (RBM15-MKL1) involved in the recurrent translocation t(1; 22)(p13; q13) in infant acute megakaryoblastic leukemia[49] and less frequently in other subtypes of AML[50]. RBM15 mutations have also been implicated in the pathogenesis of phyllodes tumors[51]. Nevertheless, the exact molecular mechanisms responsible for the oncogenic properties of WTAP and RBM15 overexpression remain elusive. Whether their aberrant expression leads to downstream disruption of target oncogenes and/or tumor suppressor genes *via* altered m[6]A abundance remains to be determined.

Aberrant expression of m[6]A erasers promotes tumorigenesis

FTO

Aberrant expression of FTO has been shown to be favorable for the survival and expansion of diverse cancer cells. The overexpression of FTO has been reported in subsets of AML bearing mixed lineage leukemia rearrangements, t(15; 17) translocation, FLT3-ITD, and/or NPM1 mutations[21]. Notably, induced overexpression of FTO promotes the survival, proliferation, and transformation of AML cells *in*

vitro and *in vivo*[21]. Reduced m[6]A abundance has been observed in the 3' UTR of mRNA targets, including *ASB2* and *RARA*, consequent to FTO overexpression in AML cell lines[21]. This effect is consistent with increased m[6]A demethylase activity, thereby decreasing the stability of these transcripts encoding myeloid differentiation factors. This aberration blocks myeloid differentiation, which is a hallmark of AML.

However, the consequences of FTO expression in AML are complex when considered in relation to the mutational status of the α-ketoglutarate-dependent dioxygenases IDH1/2[52]. IDH1/2 mutations occur in approximately 20% of AML cases and produce a structurally related metabolite, D-2-hydroxyglutarate, that competitively inhibits α-ketoglutarate-dependent dioxygenases, including FTO. Consistent with this finding, IDH2 mutant cells exhibit higher levels of m[6]A[52]. Knockout of FTO resulted in elevated m[6]A levels in IDH2 WT but not in IDH2 mutants; the latter observation is consistent with the inhibition of FTO demethylase activity *via* D-2-hydroxyglutarate-mediated competitive inhibition[52]. These results indicate the need to interpret the consequences of FTO overexpression in AML in the context of mutations that modulate its demethylase activity[52].

Overexpression of FTO has also been observed in gastric, squamous cell, and breast cancers[53-55]. FTO overexpression promotes the proliferation and invasiveness of gastric cancer cell lines, and the opposite is true when FTO is depleted *in vitro*[54]. Increased expression of FTO is associated with advanced tumor stage and poorer survival in gastric cancer, indicating its potential utility as a prognostic marker[54]. Elevated FTO expression in cervical squamous cell carcinoma has been found to enhance chemo-radiotherapy resistance[53]. In breast cancer cell lines, inhibition of FTO reduces PI3K/AKT signaling activity and increases energy metabolism and glycolysis[55]. However, the functional consequences of FTO overexpression on breast cancer cell proliferation and migration remain to be proven experimentally.

ALKBH5

Overexpression of ALKBH5 has been implicated in the maintenance and proliferation of GSCs, thereby conferring oncogenic potential in glioblastoma[22]. Clinically, elevated ALKBH5 levels predict a poorer outcome in patients with glioblastoma. Inhibition of ALKBH5 in GSC cells perturbs brain tumor formation and proliferation in mice, indicating that ALKBH5 overexpression is critical for the development and maintenance of brain tumorigenesis[22]. ALKBH5 targets nascent transcripts encoding the transcription factor FOXM1

and enhances its expression by decreasing m⁶A levels near the 3' UTR. This process is facilitated by a non-coding antisense RNA, *FOXM1-AS,* that interacts with ALKBH5 and the nascent *FOXM1* transcripts[22]. Notably, overexpression of FOXM1 alone is sufficient to reinstate tumorigenesis in mice following ALKBH5 or *FOXM1-AS* depletion[22], thereby affirming the role of the ALKBH5-FOXM1-*FOXM1-AS* axis in the pathogenesis of glioblastoma.

In breast cancer, the hypoxia-triggered HIF1α and HIF2α proteins have been shown to promote ALKBH5 expression[56]. Consequent to increased ALKBH5 expression, a reduction in m⁶A levels promotes the mRNA stability and expression of transcripts encoding the pluripotency factor *NANOG*[56]. The increase in NANOG expression was shown to result in the accumulation of breast cancer stem cells, which was reversed by depletion of ALKBH5[56]. Overall, ALKBH5 plays a major role in the maintenance of cancer stem cells.

In contrast to the increased expression of ALKBH5 in solid tumors, our own analysis of TCGA data found low levels of *ALKBH5* in AML in conjunction with copy number loss[57]. Notably, copy number loss of ALKBH5 is associated with poorer survival and TP53 mutation in AML[57]. While the functional role of ALKBH5 loss in AML remains to be determined, ALKBH5 may act as a tumor suppressor given its lower expression in this type of aggressive leukemia.

Altered m⁶A reader protein expression is involved in cancer

YTHDF1/2

The aberrant expression of m⁶A readers in cancers is less well documented. Elevated or depleted levels of m⁶A readers may influence the recognition of m⁶A marks on transcripts, thereby affecting mRNA stability, translation efficiency, and splicing. Several studies have reported the association between overexpression of the YTH family proteins and poorer survival in cancer. For example, YTHDF1 overexpression is associated with a poorer prognosis in HCC and colorectal cancers[58,59]. The overexpression of YTHDF1 in colorectal cancer has been shown to be transcriptionally driven by the oncogene *c-MYC*[59]. Notably, *c-MYC* is translationally enhanced *via* METTL3 overexpression in other cancers[59]. It would be interesting to investigate whether increased m⁶A abundance on *c-MYC*-encoding mRNA, consequent to METTL3 overexpression, provides a positive feedback loop to stimulate YTHDF1 expression in cancer.

The overexpression of YTHDF2 has been reported in prostate and pancreatic cancers[60,61]. In pancreatic cancer,

YTHDF2 expression increased in patients at later stages of cancer progression, indicating its potential utility as a prognostic indicator[61]. Inhibition of YTHDF2 expression in prostate and pancreatic cancer cell lines suppresses proliferation and/or colony formation, indicating that YTHDF2 overexpression is oncogenic[60,61]. Surprisingly, YTHDF2 promotes migration in prostate cancer *in vitro*, while the opposite has been observed in the case of pancreatic cancer[60,61]. In pancreatic cancer, YTHDF2 inhibits epithelial-mesenchymal transition by suppressing the YES-associated protein (YAP) signaling pathway[61]. Thus, YTHDF2 plays a dual role by coordinating a proliferation and epithelial-mesenchymal transition dichotomy in pancreatic cancer[61]. Whether or not the same effect of YTHDF2 overexpression occurs in other cancers remains to be investigated.

IGF2BPs

The recently discovered family of m⁶A reader proteins IGF2BP1-3 has been reported to possess oncogenic potential[29]. These proteins are frequently amplified and expressed at high levels in cancers. In support of their oncogenic role, IGF2BP knockdown in cervical and liver cancer cell lines inhibited proliferation, decreased colony formation, and reduced cell migration and invasion abilities[29]. IGF2BPs act as readers that recognize m⁶A methylation on oncogenes, including *MYC*, promoting their stability and translation[29].

Other m⁶A reader proteins

The role of other m⁶A reader proteins in cancer is currently unknown. As YTHDF3 recognizes m⁶A marks on mRNAs to promote translation[62], it may have a role in promoting the translation of oncogenes, similar to IGF2BP proteins. The aberrant expression of YTHDC1 may alter alternative splicing in cancer by elevating the expression of isoforms with oncogenic potential and/or silencing tumor suppressor genes *via* production of isoforms subject to nonsense-mediated decay[28]. Collectively, there remain many undetermined roles of m⁶A readers in cancer, which require further investigation.

Exploiting the aberrant expression of m⁶A regulators for cancer therapy

Recent studies have indicated that the effectiveness of cancer therapy can be improved by modulating the expression of m⁶A regulatory enzymes[21,42]. As METTL3 is overexpressed in

many cancers, inhibition of this enzyme may be a potential strategy for cancer therapy. METTL3 overexpression has been shown to confer radioresistance in GSCs[35]. Therefore, targeting this enzyme in glioblastoma overexpressing METTL3 in combination with radiotherapy may be an effective therapeutic strategy. Knockdown of METTL3 has been shown to enhance sensitivity to chemo- and radiotherapy in pancreatic cancer cells[63]. However, as reviewed elsewhere, the application of an inhibitor of METTL3 in cancer therapy may be complicated because of its additional functions that are independent of its catalytic activity[64].

In AML, inducing differentiation in leukemic stem cells has been an effective strategy in the past. Arsenic and all-trans retinoic acid (ATRA) combination therapy induces terminal differentiation of leukemic blasts in acute promyelocytic leukemia (APL), resulting in the cure of this subtype of AML[65]. Other subtypes of AML do not typically respond to ATRA treatment. However, a recent study demonstrated that inhibition of METTL14 promotes terminal differentiation of myeloid cells *in vitro*[42]. Depletion of METTL14 further sensitizes AML cells, including a non-APL cell line, to ATRA-induced differentiation[42]. It would be interesting to determine whether an inhibitor of METTL14 can be used in combination with ATRA to improve the treatment of AML, particularly those that overexpress METTL14.

Depletion of FTO has also been found to enhance ATRA-induced myeloid differentiation in AML cell lines bearing FTO overexpression[21]. Targeting FTO in combination with ATRA may be a promising approach to treat subsets of AML that overexpress FTO. In addition, FTO has been identified as a target of R-2-hydroxyglutarate (R-2HG), a by-product of mutant IDH1/2 enzymes that is frequently expressed in AML and glioblastoma[66]. R-2HG inhibits the catalytic activity of FTO, thereby increasing m6A abundance in mRNAs encoding key oncogenes, including *MYC*[66]. This leads to the degradation of *MYC* and inhibition of cancer cell proliferation. Thus, R-2HG possesses a previously unrecognized tumor-suppressor role by reducing FTO expression. The application of IDH1/2-mutant inhibitors alone may not be beneficial to the treatment of cancers bearing mutations of these enzymes[66]. These inhibitors may hinder the favorable effect of R-2HG on suppressing the proliferation and viability of cancer cells. Inhibition of FTO in combination with IDH1/2-mutant inhibitors may be more effective in the treatment of cancers with IDH1/2 mutations, particularly those that also express high levels of FTO[66].

Small molecule inhibitors against FTO have been developed. Among these, meclofenamic acid has been found to show high selectivity for FTO with minimal effects on ALKBH5[67]. An ethyl ester derivative of meclofenamic acid, MA2, has recently been reported to successfully inhibit glioblastoma stem cell growth *in vitro* and *in vivo*[34]. Thus, FTO inhibitors could potentially be repurposed for cancer therapy.

Modulating the levels of m6A methylation at specific mRNA targets may ultimately require a full understanding of how m6A writers, erasers, and readers interact to promote tumorigenesis. The complex cross-talk between writers-erasers-readers functions synergistically to establish an m6A threshold that is necessary to maintain the stability of genes controlling cell growth[68]. However, this threshold is also sensitive to oncogenic stimulus. As demonstrated by hypoxia in breast cancer, distorts of the METTL14-ALKBH5-YTHDF3 axis trigger the aberrant expression of specific genes that enhance tumor growth, angiogenesis, and cancer progression[68]. Thus, targeting multiple regulators of m6A simultaneously may be necessary to achieve the best outcome.

Conclusions and future perspective

m6A RNA modification is central to regulating many biological processes by determining the fate of mRNAs. There is substantial evidence that aberrant regulation of this type of RNA methylation is tumorigenic. Furthermore, dysregulated expression of m6A writers, erasers, and readers is widespread in diverse human cancers. Many proteins involved in m6A regulation have been only recently characterized, and some of these, including ZC3H13 and CBLL1, have not been studied in the context of cancer. In AML, there have been confounding observations suggesting that the overexpression of both m6A writers and erasers can promote tumorigenesis. It is plausible that the overexpression of a particular writer or eraser will modulate m6A at different mRNA targets. Additional experiments are required to address the cross-talk among m6A writers, erasers, and readers and determine its role in driving AML and other cancers. To date, how m6A reader proteins recognize specific mRNA targets also remains a mystery.

In summary, further studies on established and potential regulators of m6A and the consequences of their altered expression may provide insights into additional putative roles of m6A in cancer biology. Clinically, the aberrant expression of one or more m6A regulatory proteins may potentially be used as a diagnostic and prognostic biomarker. Targeting m6A regulatory proteins in combination with approved anti-

cancer drugs is a new frontier in epigenetic cancer therapeutics.

There are however many hurdles that need to be overcome before feasible therapeutic approaches that target these proteins can be established. The toxicity and sensitivity of various small molecule inhibitors of m6A writers and erasers remain to be tested *in vivo*. Similar to the application of other drugs in cancer cells, ruling out detrimental off-target effects will be crucial. Achieving these objectives is likely to be more challenging if the inhibition of cancer growth and progression requires concomitant modulation of multiple m6A writers and erasers. Nevertheless, the burgeoning field of RNA modification has no doubt provided new hope in understanding cancer development and opportunities for novel therapies. Further research will clarify whether a deeper understanding of this field can contribute to an improved management of cancer.

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Conflict of interest statement

No potential conflicts of interest are disclosed.

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