

The Critical Role of RNA m⁶A Methylation in Cancer

Qing Lan¹, Pei Y. Liu², Jacob Haase³, Jessica L. Bell³, Stefan Hüttelmaier³, and Tao Liu^{2,4}



Abstract

Since the identification of the first RNA demethylase and the establishment of methylated RNA immunoprecipitation-sequencing methodology 6 to 7 years ago, RNA methylation has emerged as a widespread phenomenon and a critical regulator of transcript expression. This new layer of regulation is termed "epitranscriptomics." The most prevalent RNA methylation, N⁶-methyladenosine (m⁶A), occurs in approximately 25% of transcripts at the genome-wide level and is enriched around stop codons, in 5'- and 3'-untranslated regions, and within long internal exons. RNA m⁶A modification regulates RNA splicing, translocation, stability, and translation into protein. m⁶A is catalyzed by the RNA methyltransferases METTL3, METTL14, and METTL16 (writers), is removed by

the demethylases FTO and ALKBH5 (erasers), and interacts with m⁶A-binding proteins, such as YTHDF1 and IGF2BP1 (readers). RNA methyltransferases, demethylases, and m⁶A-binding proteins are frequently upregulated in human cancer tissues from a variety of organ origins, increasing onco-transcript and oncoprotein expression, cancer cell proliferation, survival, tumor initiation, progression, and metastasis. Although RNA methyltransferase inhibitors are not available yet, FTO inhibitors have shown promising anticancer effects *in vitro* and in animal models of cancer. Further screening for selective and potent RNA methyltransferase, demethylase, or m⁶A-binding protein inhibitors may lead to compounds suitable for future clinical trials in cancer patients.

Introduction

Modified RNA bases were discovered over 60 years ago, and the research field was revived after the identification of the first RNA demethylase, the fat mass and obesity-associated protein (FTO), and realization that RNA methylation is reversible in 2011 (1, 2). The plethora and significance of RNA methylation sites was then further revealed with the establishment of methylated RNA immunoprecipitation-sequencing methodology in 2012 (3, 4). A variety of tRNA, rRNA, long noncoding RNA, and mRNA methylations have been identified and demonstrated to regulate RNA splicing (4–6), stability (7–11), and translation (12–14). This new layer of modifications influencing gene expression and protein translation has been termed "epitranscriptomics" (3, 15, 16).

Common RNA methylation sites include 5-methylcytosine (m⁵C), 7-methylguanosine (m⁷G), m¹G, m²G, m⁶G, N¹-methyladenosine (m¹A) and m⁶A. m⁵C modification promotes splicing and translation (17). In comparison, m¹G, m²G and m¹A modifications, at the first or second codon, repress protein synthesis (18–20), and tRNA m⁷G methylation is required for

mRNA translation into proteins (21). Here, we will focus on m⁶A, the most common mRNA methylation.

Modulation of RNA m⁶A Methylation and Demethylation

m⁶A is considerably enriched around stop codons, in untranslated regions, and within long internal exons

m⁶A immunoprecipitation-sequencing (m⁶A-Seq) enables the first demonstration that approximately 25% of transcripts harbor m⁶A modifications, and that m⁶A modifications are enriched around stop codons, in the 5'- and 3'-untranslated regions (UTR) and within long internal exons (3, 4). m⁶A sites are typically evolutionary conserved, with GAC and AAC consensus motifs being found in approximately 90% of all m⁶A sites (3, 4).

m⁶A methylation by the RNA methyltransferases METTL3, METTL14, and METTL16 and cofactors (m⁶A writers)

m⁶A modification is catalyzed by the methyltransferase complex consisting of the methyltransferase-like 3 and 14 proteins (METTL3 and METTL14) and their cofactors WTAP, RBM15, RBM15B, HAKAI, VIRMA (KIAA1429), and ZC3H13 (Fig. 1A; refs. 22–29). Although METTL3 selectively induces RNA GAC and AAC methylation and METTL14 selectively induces RNA GAC methylation, METTL3 and METTL14 synergistically induce m⁶A (22, 24, 25). By contrast, the other components lack RNA methyltransferase activity. WTAP promotes m⁶A by recruiting METTL3 and METTL14 into nuclear speckles (5). RBM15 and RBM15B bind METTL3 and WTAP and direct the two proteins to specific RNA sites for m⁶A modification (26, 27). VIRMA preferentially mediates mRNA methylation near the 3'-UTR and stop codon regions and associates with alternative polyadenylation (28). ZC3H13, in concert with other cofactors like WTAP, controls nuclear m⁶A methylation (27, 29).

¹Department of Neurosurgery, The Second Affiliated Hospital of Soochow University, Suzhou, Jiangsu P.R. China. ²Children's Cancer Institute Australia, Randwick, Sydney, Australia. ³Institute of Molecular Medicine, Martin Luther University, Halle Saale, Germany. ⁴Centre for Childhood Cancer Research, UNSW Medicine, University of New South Wales, Kensington, Sydney, Australia.

Corresponding Authors: Tao Liu, Children's Cancer Institute Australia, Lowy Cancer Research Centre, UNSW Australia, Randwick, PO Box 81, Kensington, Sydney, NSW 2031, Australia. Phone: 61-2-9385-1935; Fax: 61-2-9662-6584; E-mail: tliu@ccia.unsw.edu.au; and Qing Lan, The Second Affiliated Hospital of Soochow University, E-mail: szliq006@163.com

doi: 10.1158/0008-5472.CAN-18-2965

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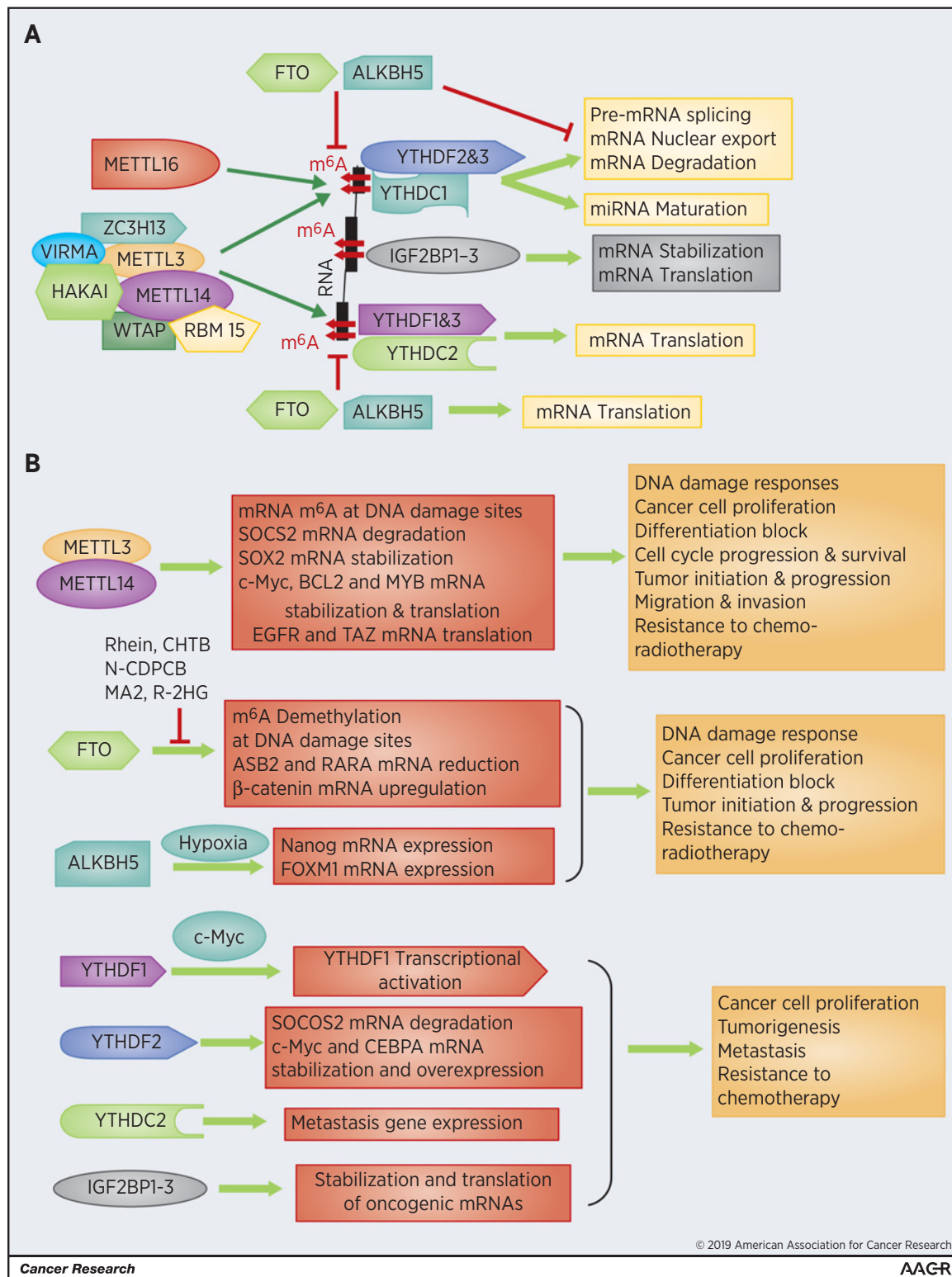


Figure 1.

Functional roles of m⁶A methyltransferases, demethylases, and binding proteins in physiology and cancer. **A**, m⁶A modification is induced by METTL3, METTL14, their cofactors and METTL16 (writers), reversed by FTO and ALKBH5 (erasers), and functionally facilitated by m⁶A binding proteins YTHDF1-3, YTHDC1-2 and IGF2BP1-3 (readers). Red ⊥ indicates inhibition, whereas green arrows represent enhancement. **B**, m⁶A methyltransferases, demethylases, and binding proteins induce onco-transcript expression, cancer cell proliferation, differentiation block, invasion, metastasis, tumorigenesis, and resistance to cancer therapies.

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METTL16 has recently been proposed as an independent RNA methyltransferase (30, 31). METTL16 induces m⁶A modification in the 3'-UTR of mRNAs as well as A43 of the U6 small nuclear RNA that base pairs with 5' splice sites of pre-mRNAs during splicing (Fig. 1A), suggesting that METTL16 plays an important role in mRNA stability and splicing (30, 31).

m⁶A demethylation by the RNA demethylases FTO and ALKBH5 (m⁶A Erasers)

m⁶A modification is reversible. The removal of m⁶A modification relies on demethylases and FTO was the first protein identified to catalyze m⁶A demethylation (1). FTO and ALKBH5, an FTO homologue (32), ensure a balanced equilibrium of m⁶A modification in the transcriptome (Fig. 1A).

m⁶A Regulating proteins modulate RNA splicing, mRNA translocation, degradation and translation, cell proliferation, differentiation, and survival

m⁶A methyltransferases. METTL3-, METTL14- and WTAP-mediated mRNA m⁶A modification leads to mRNA decay, reduces mRNA stability (8–11), facilitates mRNA export from nucleus to cytoplasm (33) and translation of 5'-UTR-methylated mRNAs into proteins (12, 34). METTL3 or METTL14 knockdown upregulates the expression of a number of transcripts such as the pluripotency factor Nanog, and impairs embryonic stem cell differentiation (Fig. 1A; refs. 8, 11, 24, 35). Depletion of ZFP217, which interacts with and sequesters METTL3 protein, globally enhances m⁶A modification, induces degradation of Nanog, Sox2, Klf4, and c-Myc mRNAs, and mitigates embryonic stem cell self-renewal and somatic cell reprogramming (36). In addition, deletion of METTL3 or METTL14 in T cells results in decreased m⁶A modification, slower mRNA decay and increased expression of SOCS family mRNAs, including SOCS1, SOCS3, and CISH, leading to increased SOCS activity and suppressed T-cell homeostatic proliferation and differentiation (37).

RNA methyltransferases also regulate gene expression through modulating microRNA (miRNA) expression. METTL3 methylates pri-miRNAs, marking them for recognition and processing, and enhances global miRNA maturation in a cell type-independent manner (Fig. 1A; refs. 38, 39).

m⁶A demethylases. FTO preferentially binds pre-mRNAs in intronic regions, in the proximity of alternatively spliced exons and poly(A) sites (40), with strict selectivity on its substrates (1). Although FTO demethylates pre-mRNA m⁶A and triggers exon inclusion (41), FTO knockout results in substantial changes in pre-mRNA splicing with prevalence of exon skipping events but upregulation of 3' terminal exons, which is the opposite effects of METTL3 knockdown (41). In addition, by demethylating target mRNAs such as GAP43 mRNA, FTO induces the translation of the de-methylated mRNAs into proteins (Fig. 1A; ref. 42).

ALKBH5 colocalizes with nuclear speckles to regulate mRNA processing factors' assembly/modification, demethylates m⁶A mRNAs, and modulates mRNA export and stability (32). In ALKBH5-deficient cells, cytoplasmic RNA level is significantly increased due to accelerated nuclear RNA export, nascent RNA synthesis is enhanced, global RNA stability is reduced, and spermatocytes die of apoptosis (32, 43). In addition, ALKBH5 knockout increases exon skipping and leads to quick degradation of aberrantly spliced transcripts (Fig. 1A; ref. 44).

m⁶A-binding proteins (readers). m⁶A modification exerts biological functions by binding to the m⁶A "reader" YTH domain-containing proteins, including YTHDC1-2 and the YTH-family proteins YTHDF1-3, as well as insulin-like growth factor 2 mRNA-binding proteins IGF2BP1-3. YTHDC1 binds m⁶A-modified pre-mRNAs, recruits splicing factor SRSF3 but blocks SRSF10 to nuclear speckles, thereby promoting exon inclusion, splicing as well as mRNA export from nucleus to cytoplasm (6, 45, 46). MAT2A m⁶A in the 3'-UTR is read by YTHDC1, which induces the methyl donor S-adenosylmethionine-mediated MAT2A mRNA degradation (31). In comparison, YTHDC2 selectively binds m⁶A at its consensus motif, enhances target mRNA translation efficiency, and decreases target mRNA abundance (Fig. 1A; refs. 47, 48).

Recognition and binding of m⁶A mRNA sites by YTHDF2 and YTHDF3 result in mRNA translocation from translatable pools to mRNA decay sites for mRNA degradation (7, 14). Specifically, YTHDF2 and YTHDF3 C-terminal YTH domains bind m⁶A sites with a conserved G(m⁶A)C core motif, whereas the N-terminal domains are responsible for the protein-mRNA complex to relocate to RNA decay sites, and YTHDF2 and YTHDF3 knock-down leads to considerable increase in m⁶A-modified mRNAs in cells (7, 14).

At the level of mRNA translation, recognition and binding of m⁶A by YTHDF1 and YTHDF3 result in enhanced protein synthesis (13, 14). YTHDF1 increases translation efficiency of m⁶A-modified mRNAs through interacting with translation initiation factors eIF3 and eIF4A3 (13, 14), and YTHDF3 induces m⁶A-modified mRNA translation by binding to YTHDF1 and eIF4A3 (Fig. 1A; ref. 14). In response to cellular stress, m⁶A modification is increased in the 5'-UTRs, and m⁶A residues in the 5'-UTRs induces mRNA translation through direct binding to eIF3, independent of YTHDF1 (12).

Distinct from YTH domain-containing proteins, IGF2BP1/2/3 recognize the consensus GG(m⁶A)C sequence through the K homology domains, and enhance the stability and translation of their target mRNAs in an m⁶A-dependent manner under normal and stress conditions (Fig. 1A; ref. 49).

m⁶A Regulating proteins induce oncogenic protein expression, cancer cell proliferation, survival, tumor initiation and progression

m⁶A methyltransferases. METTL3 and METTL14 have been controversially reported to show tumor-suppressive and oncogenic functions in glioblastoma and hepatocellular carcinoma, but unanimously demonstrated to promote tumorigenesis of other cancer types. In glioblastoma stem cells, knocking down METTL3 or METTL14 reduces m⁶A modification, upregulates the expression of critical oncogenes such as ADAM19, EPHA3, and KLF4, and downregulates the expression of many tumor suppressors, including CDKN2A, BRCA2, and TP53I11, resulting in enhanced glioblastoma stem cell growth, self-renewal and tumorigenesis (50). By contrast, in a later study, METTL3 has been found to be up-regulated in human glioblastoma tissues, and high levels of METTL3 expression in tumor tissues predict poor patient survival (51). METTL3 binds SOX2 mRNA in the 3'-UTR and induces m⁶A modification, and silencing METTL3 reduces SOX2 expression, enhances tumor cell sensitivity to γ -irradiation *in vitro*, inhibits glioblastoma tumor growth and prolongs survival in mice, suggesting an oncogenic role of METTL3 (51). A similar

Table 1. RNA m⁶A methyltransferases, demethylases, and binding proteins are frequently overexpressed in human cancer cells and tissues, and induce oncogenic transcript overexpression, tumor cell proliferation, differentiation block, survival, tumorigenesis, and metastasis

Change in expression	Tumor tissues or cell lines	Functions	References
METTL3 & METTL14	Glioblastoma stem cells	Induces tumor suppressor and reduce oncogene expression and reduce glioblastoma stem cell growth and tumorigenesis	(50)
METTL3	Glioblastoma tissues ↑	Increases SOX2 mRNA stability and expression, enhances glioblastoma growth, and prolongs mouse survival	(51)
METTL14	Liver cancer tissues ↓	Interacts with DGCR8, positively modulates the primary microRNA 126, and suppresses tumor metastasis	(52)
METTL3	Liver cancer tissues ↑	Reduces SOCS2 mRNA expression, increases tumor cell proliferation, migration, tumorigenicity, and lung metastasis	(53)
METTL3	Lung and colon cancer tissues ↑	Promotes oncogene translation and induces cancer cell growth, survival, and invasion	(54)
METTL3	Acute myeloid leukemia cells ↑	Enhances Myc and BCL2 mRNA translation, inhibits cell differentiation, induces leukemia progression in mice	(55, 56)
METTL14	Acute myeloid leukemia cells ↑	Induces MYB and Myc mRNA stability and translation, and induces cell differentiation block, proliferation, and survival	(57)
METTL3	Breast cancer tissues ↑	Increases HBXIP mRNA methylation and expression, induces breast cancer cell proliferation and survival	(58)
FTO	Glioblastoma stem cells	Induces glioblastoma stem cell growth, self-renewal and tumor progression, and prolongs mouse lifespan	(50)
FTO	Acute myeloid leukemia cells ↑	Reduces ASB2 and RARA m ⁶ A, suppresses leukemia cell differentiation, and enhances leukemogenesis	(61)
FTO	Cervical cancer tissues ↑	Increases β-catenin mRNA, increases DNA repair activity, and induces resistance to chemoradiotherapy	(62)
ALKBH5	Hypoxic breast cancer cells ↑	Increases Nanog mRNA and protein expression and induces breast cancer stem cell phenotype and tumor initiation	(63)
ALKBH5	Glioblastoma stem-like cells ↑	Induces FOXM1 expression, glioblastoma stem-like cell proliferation, and tumorigenesis	(64)
YTHDF1	Colon cancer tissues ↑	Induces cancer cell proliferation and metastasis, and renders resistance to the anticancer drugs fluorouracil and oxaliplatin	(65)
YTHDF2	Lung cancer cells	Facilitates METTL3-mediated SOCS2 m ⁶ A modification and degradation and METTL3-induced lung oncogenic effects	(53)
YTHDF2	Leukemia cells	Facilitates c-Myc and CEBPA m ⁶ A modification, stabilization, and overexpression in leukemia cells	(66)
YTHDC2	Colon cancer tissues ↑	Upregulates the expression of metastasis-related proteins and induces colon cancer cell metastasis <i>in vitro</i> and <i>in vivo</i>	(67)
IGF2BP1-3	Liver and cervical cancer tissues ↑	Globally increases oncogene mRNA stability and translation and enhances cancer cell proliferation and colony formation	(49)
IGF2BP1	Ovarian & Liver cancer	Increases SRF mRNA stability in an m ⁶ A-dependent manner and promotes cancer phenotypes <i>in vitro</i> and <i>in vivo</i>	(70)

controversy is reported for hepatocellular carcinoma. One study showed that m⁶A modification is decreased in human hepatocellular carcinoma tissues, that low levels of METTL14, but not METTL3 serve as an adverse prognosis factor for recurrence-free survival and tumor metastasis, and that METTL14 suppresses metastasis (52). In contrast, in another publication, it has been shown that METTL3, but not METTL14, was overexpressed in human hepatocellular carcinoma tissues and that high levels of METTL3 expression was associated with poor patient prognosis (53). Mechanistically, METTL3 induces SOCS2 m⁶A modification and reduces SOCS2 mRNA expression through an YTHDF2-dependent pathway. Suppression of METTL3 or METTL14 considerably reduces hepatocellular carcinoma cell proliferation, migration and colony formation *in vitro*, as well as hepatocellular carcinoma tumorigenicity and lung metastasis *in vivo* (Fig. 1B and Table 1; refs. 53). Although it is very difficult to identify factors responsible for the controversy regarding the roles of METTL3 and METTL14 in glioblastoma and hepatocellular carcinoma, it appears likely that the variable expression of m⁶A readers and the different cell lines and tumor samples used in the studies account for the observed discrepancy and controversy.

RNA methyltransferases have been reported to promote carcinogenesis in other cancers. METTL3, but not METTL14, is higher

expressed in human lung and colon adenocarcinoma tissues, compared with normal tissues (54). METTL3 associates with ribosomes, the translation initiation machinery, and promotes translation of oncogenic mRNAs, including EGFR and the Hippo pathway effector TAZ, leading to lung and colon cancer cell growth, survival, and invasion (54). METTL3 mRNA and protein are overexpressed in acute myeloid leukemia cells, compared with healthy hematopoietic stem/progenitor cells (55, 56). METTL3 associates with chromatin and localizes to the transcriptional start sites of active genes and induces m⁶A modification within the coding region of the associated mRNAs, including c-Myc and BCL2 mRNAs, enhancing their translation by relieving ribosome stalling (56). Consequently, METTL3 inhibits hematopoietic stem/progenitor cell differentiation and increases leukemia cell growth, and METTL3 depletion induces cell-cycle arrest, cell differentiation, and apoptosis and delays leukemia progression in mice (55, 56). In comparison, METTL14 is highly expressed in normal hematopoietic stem/progenitor cells and acute myelogenous leukemia cells carrying t(11q23), t(15;17) or t(8;21) translocation, and increases MYB and c-Myc mRNA stability and translation via m⁶A modification (57). Silencing of METTL14 induces terminal myeloid differentiation, cell growth inhibition and cell death (Fig. 1B and Table 1; ref. 57).

METTL3 is also overexpressed in human breast cancer tissues and promotes breast cancer cell proliferation and survival through increasing HBXIP mRNA methylation and expression (58). In pancreatic cancer cells, METTL3 knockdown does not have an effect on cell proliferation, but increases cell sensitivity to anti-cancer agents such as gemcitabine, 5-fluorouracil, cisplatin, and irradiation (59). In addition, after ultraviolet radiation, PARP recruits METTL3 and METTL14 to DNA damage sites to induce m⁶A modification, resulting in DNA polymerase κ localization to DNA damage sites for nucleotide excision repair, DNA damage repair and cell survival (Fig. 1B and Table 1; ref. 60). These findings support the view that m⁶A-methyltransferases serve oncogenic roles in various cancers.

m⁶A demethylases. After ultraviolet irradiation, FTO accumulates at DNA damage sites, induce m⁶A demethylation, and dynamically regulates cellular DNA damage response in collaboration with METTL3 (60). In glioblastoma stem cells, inhibition of FTO suppresses glioblastoma stem cell growth, self-renewal and tumor progression (50). Similarly, FTO is highly expressed in acute myeloid leukemia with t(11q23)/MLL rearrangements, t(15;17)/PML-RARA fusion, FLT3-ITD or NPM1 mutation. By reducing m⁶A in target mRNAs such as ASB2 and RARA, FTO enhances leukemogenesis and suppresses trans-retinoic acid-mediated leukemia cell differentiation (61). In addition, FTO is over-expressed in human cervical squamous cell carcinoma tissues, and high levels of FTO expression correlates with poor patient prognosis (62). Through reducing β -catenin mRNA m⁶A modification and increasing its expression, FTO induces cervical cancer cell resistance to chemo-radiotherapy and enhances DNA damage responses (Fig. 1B and Table 1; ref. 62).

Under hypoxic conditions, hypoxia-inducible factors 1 α and 2 α induce ALKBH5 expression in breast cancer cells, and ALKBH5 demethylates Nanog mRNA at an m⁶A residue in the 3'-UTR, leading to increased Nanog mRNA and protein expression and breast cancer stem cell phenotype (63). ALKBH5 knockdown in human breast cancer cells significantly reduces the number of breast cancer stem cells and reduces tumor initiation capacity (63). In addition, ALKBH5 is highly expressed in glioblastoma stem-like cells and demethylates FOXM1 nascent transcripts, leading to FOXM1 over-expression, stem-like cell proliferation and tumorigenesis (Fig. 1B and Table 1; ref. 64).

m⁶A-binding proteins. YTHDF1 is highly expressed in human colon cancer tissues, and a high level of YTHDF1 is associated with lymph node metastasis and poor patient prognosis (65). Mechanistically, c-Myc oncoprotein directly induces *YTHDF1* gene expression, and YTHDF1 induces cancer cell proliferation and renders resistance against anticancer drugs, including fluorouracil and oxaliplatin (65). YTHDF2 binds c-Myc and CEBPA mRNAs to facilitate m⁶A modification in the 5'-UTR and coding regions, leading to c-Myc and CEBPA mRNA stabilization and over-expression and leukemia cell proliferation (66). In addition, YTHDF2 directly binds the 3'-end of the *SOCS2* transcript to facilitate m⁶A modification and degradation and lung cancer oncogenesis (Fig. 1B and Table 1; ref. 53).

YTHDC2 upregulates the expression of metastasis-related proteins such as hypoxia-inducible factor 1 α by promoting mRNA translation, and thereby induces colon cancer metastasis (67). In agreement, YTHDC2 is highly expressed in human colon cancer

tissues, and a high level of YTHDC2 correlates with advanced stage and tumor metastasis (Fig. 1B and Table 1; ref. 67).

IGF2BP1/2/3 genes are genetically gained and highly expressed in a variety of human cancer tissues (49, 68). In liver and cervical cancer cells, IGF2BP proteins, in particular IGF2BP1, enhance the stability and translation of oncogenic mRNAs, including c-Myc, promoting cell proliferation and tumorigenesis (Fig. 1B and Table 1; refs. 49, 69). In addition, IGF2BP1 promotes the expression of SRF mRNA in a m⁶A-dependent manner by impairing miR-23a-3p and miR-125a-5p-mediated decay (70).

Small-Molecule RNA Demethylase Inhibitors as Novel Anticancer Agents

Although no small-molecule inhibitors of RNA methyltransferases and their cofactors are currently available, several FTO inhibitors have been discovered by biochemical- or cell-based small-molecule compound library screening, natural product testing or chemical synthesis. The first FTO inhibitors, including rhein, have been identified through structure-based virtual screening and biochemical analyses (71). Rhein effectively suppresses m⁶A demethylation intracellularly by competitively binding the FTO catalytic domain and disrupting FTO from binding m⁶A RNAs (71). CHTB and N-CDPCB have been identified as FTO inhibitors through virtual screening (72, 73). The crystal structure of the CHTB, N-CDPCB, and FTO protein complexes reveals that CHTB binds at the surface area of the FTO active site, and that N-CDPCB is sandwiched between an antiparallel β -sheet and the L1 loop of FTO (72, 73).

Meclofenamic acid 2 (MA2) is an FDA-approved NSAID. MA2 binds at the surface area of the FTO active site, selectively inhibits FTO as compared with other AlkB subfamily proteins such as ALKBH5 and increases m⁶A modification in cells (74). MA2 reduces glioblastoma stem cell growth, self-renewal, and sphere formation *in vitro* and, when injected intratumorally, reduces tumor growth in glioblastoma-bearing mice (50).

The only FTO inhibitor that has been tested through systemic administration in mice is R-2-hydroxyglutarate (R-2HG). R-2HG directly binds FTO protein, inhibits FTO activity and increases global m⁶A modification, which in turn decreases c-Myc and CEBPA m⁶A modification in the 5'-UTR and coding regions and stability, leading to leukemia cell growth inhibition, cell-cycle arrest and apoptosis (66). In a feedback loop, reduction in CEBPA expression after R-2HG treatment decreases *FTO* gene-promoter activity and gene transcription (66). Furthermore, R-2HG in combination with chemotherapy drugs exerts synergistic anticancer effects against leukemia and glioma *in vitro*, and blocks leukemia progression in mice (66).

Implications and Future Directions

m⁶A RNA modifications, methyltransferases, demethylases, and binding proteins are emerging as critical regulators of pre-mRNA splicing, mRNA stability, translation, and cancer (75–77). Remarkably, m⁶A methyltransferases, demethylases, and binding proteins are all frequently overexpressed in human cancer tissues of various organ origins. They induce onco-transcript expression, cancer cell proliferation, differentiation block, tumorigenesis, metastasis, and resistance to cancer therapy through the opposing m⁶A methylation and demethylation mechanisms, respectively.

Currently, it is not clear how the RNA m⁶A methyltransferases and demethylases selectively methylate or demethylate onco-transcripts but eventually all enhance oncoprotein expression. Explanations for this conflict include: distinct m⁶A dynamics and localizations of different onco-transcripts, cellular localizations and target specificity of the m⁶A methyltransferases, demethylases and binding proteins, cellular context and in particular the variable expression of various m⁶A readers. Also remarkably, little is known about whether and how mRNA m⁶A modifying factors have significant effects on tumor-suppressor transcript expression. Future research using m⁶A sequencing, characterizing target mRNA selectivity of m⁶A methyltransferases, demethylases, and binding proteins, is expected to help answer these critical questions.

DNA methylation has been the focus of cancer research for more than three decades, and the DNA methyltransferase inhibitors azacytidine and decitabine have been approved for cancer therapy in patients (78). Although RNA methylation in cancer did not attract research until very recently, small-molecule FTO enzymatic inhibitors, including rhein, MA2 and R-2HG, have been confirmed to induce cancer cell growth inhibition and apoptosis, and combination therapy with R-2HG and other anticancer agents synergistically blocks leukemia progression in mice. Future endeavors should focus on the oncogenic mechanisms of action of the m⁶A methyltransferases METTL3, METTL14, and METTL16 and

their partner proteins, the demethylases FTO and ALKBH5 as well as the m⁶A readers YTHDF1-3, YTHDC1-2, and IGF2BP1-3, so as to provide novel, more specific and effective strategies for therapeutic targeting. Potent and specific small-molecule m⁶A modification inhibitors will then be identified through small-molecule compound library screening and/or developed through chemical synthesis, and their *in vivo* pharmacokinetics, safety profile, and anticancer efficacy will be examined in animal models. Eventually, ideal small-molecule m⁶A modification inhibitors suitable for clinical trials will be established and tested as mono/combination-therapy in patients with cancer tissues characterized by aberrant m⁶A-regulating protein expression.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

This work was partly supported by National Health and Medical Research Council Australia, Cancer Council NSW, and National Institutes of Health grants (R21 CA226959 to T. Liu) and by Deutsche Forschungsgemeinschaft (to S. Hüttelmaier). P. Liu is the recipient of a Cancer Institute New South Wales Fellowship. Children's Cancer Institute Australia is affiliated with UNSW Sydney and Sydney Children's Hospitals Network.

Received September 21, 2018; revised December 10, 2018; accepted January 8, 2019; published first March 20, 2019.

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