The role of RNA N^6-methyladenosine methyltransferase in cancers

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Modification of eukaryotic RNA by methylation of adenosine residues to generate N^6-methyladenosine (m^6A) is a highly prevalent process. m^6A is dynamically regulated during cell metabolism and embryo development, and it is mainly involved in various aspects of RNA metabolism, including RNA splicing, processing, transport from the nucleus, translation, and degradation. Accumulating evidence shows that dynamic changes to m^6A are closely related to the occurrence and development of cancer and that methyltransferases, as key elements in the dynamic regulation of m^6A, play a crucial role in these processes. Therefore, in this review, we describe the role of methyltransferases as m^6A writers in cancer and summarize their potential molecular mechanisms of action.

According to central genetic dogma, genetic information is transferred from DNA to RNA and then from RNA to protein by the transcription and translation processes, respectively. Epigenetic modification mainly involves DNA, RNA, and proteins. During the past few decades, investigation of human epigenetic modification has focused on DNA and proteins, with analysis of chromatin remodeling, gene silencing, DNA methylation, and histone modification. In comparison, RNA-related modification has received less attention, although in recent years, with the continuous development of detection technology, hundreds of different RNA modifications have been discovered, among which N^6-methyladenosine (m^6A) RNA methylation is the most abundant.[1] m^6A in RNA is an epigenetic modification in which a hydrogen atom (–H) connected to the sixth nitrogen atom (N6) on adenine is replaced by a methyl group (–CH3) (Figure S1). With the advent of high-throughput sequencing technology, many m^6A RNA methylation sites have been discovered, which are mainly concentrated in exons and 3′ untranslated regions (UTRs), with the highest concentration near the termination codon. Each transcript contains three to five or more m^6A modification sites, accounting for 0.1%–0.4% of total adenine (m^6A/A).[2] In eukaryotic RNAs, m^6A mostly occurs in the critically conserved motif RRACH (R = G, A, and U; H = U, A, and C) (Figure 1),[3,4] and it is most common in mRNA but is also widely found in long non-coding RNA (lncRNA), tRNA, rRNA, and microRNA (miRNA).

A large number of studies have confirmed that m^6A RNA mainly interacts with three types of proteins, namely methyltransferases, demethylases, and reader proteins. Similar to DNA methylation, RNA methylation is also dynamic and reversible, and the related reactions are mainly catalyzed by methyltransferases and demethylases; methyltransferases catalyze the installation of m^6A on RNA, while the demethylases remove m^6A modifications (Figure 1). The biological function of dynamic RNA methylation is mainly mediated by reader proteins, which act by recognizing and binding to m^6A and reading the biological information, mainly for splicing processing, nucleus to cytoplasm transport, and stabilization, translation, and degradation of RNA (Figure 2). Members of the YT521-B homology (YTH) domain family have been identified as m^6A readers, including YTH domain family protein 1 (YTHDF1), YTH domain family protein 2 (YTHDF2), YTH domain family protein 3 (YTHDF3), YTH domain containing 1 (YTHDC1), and YTH domain containing 2 (YTHDC2), and they have conserved m^6A-binding domains that can bind to the RNA m^6A site. In addition, the heterogeneous nuclear ribonucleoprotein (HnRNP) family, insulin-like growth factor 2 mRNA-binding proteins (IGF2BPs), and eIF3 can be combined with the m^6A site and function as readers. According to relevant reports, YTHDF1,[5] YTHDC2,[6] and eIF3[7] have been shown to promote target mRNA translation, and YTHDF2 participates in RNA degradation,[8] while YTHDF3 plays a synergistic role, interacting with YTHDF1 to promote RNA translation, and binding with YTHDF2 to promote RNA degradation.[9] YTHDC1 facilitates RNA splicing and output,[10] and HnRNP is involved in the splicing of RNA.[11] IGF2BPs enhances the expression of target mRNA by promoting its stability.[12]

As the writers of m^6A RNA methylation, methyltransferases and their components can regulate a variety of cellular physiological processes, including cell cycle, cell growth, cell differentiation, and apoptosis. In addition, methyltransferase components are involved in phenotypic transformation of *Drosophila*[13] and embryonic development of mice.[14] Furthermore, the relationship between methyltransferases and cancers has become an intense area of investigation. Herein, we
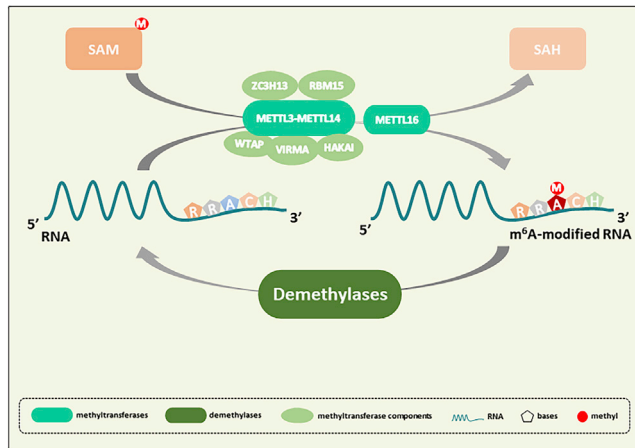
Figure 1. m⁶A methylation is a dynamic and reversible process, installed by methyltransferases, and removed by demethylases

m⁶A methyltransferases include METTL3, METTL14, WTAP, VIRMA, HAKAI, ZC3H13, RBM15, METTL16.
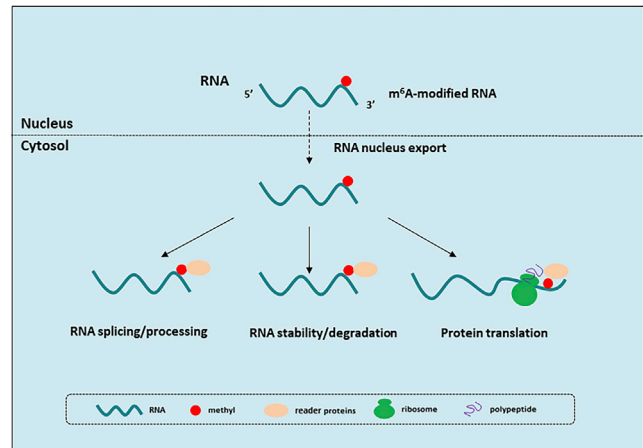

Figure 2. m⁶A is recognized by reader proteins and affects RNA metabolism, including RNA export, splicing, processing, degradation, and protein translation

review the key roles of methyltransferases in human cancers and their mechanisms of tumorigenesis.

RNA m⁶A methyltransferase

In the 1990s, Bokar et al.[15] isolated 200-kDa (MT-A) and 800-kDa (MT-B) nuclear subcomplexes from HeLa cells and identified an m⁶A methyltransferase multi-component complex. This complex utilizes S-adenosylmethionine (SAM) as a methyl donor to provide a methyl group that replaces the hydrogen atom of the sixth nitrogen center of adenine.[15,16] Subsequently, a 70-kDa protein, MT-A70, was extracted from the MT-A complex and was named methyltransferase-like 3 (METTL3).[17] Further investigation led to the discovery of important methyltransferase components, such as methyltransferase-like 14 (METTLl4), Wilms tumor 1-associated protein (WTAP), protein virilizer homolog (VIRMA), E3 ubiquitin-protein ligase Hakai (HAKAI), zinc finger CCCH domain-containing protein 13 (ZC3H13), RNA-binding protein 15 (RBM15), and methyltransferase-like 16 (METTL16) (Table 1; Table S1). These discoveries have greatly advanced our understanding of m⁶A RNA methylation.

Methyltransferases and cancer
METTL3

Accumulating evidence shows that METTL3 can participate in the development of cancer through various mechanisms. For example, METTL3 regulates the level and stability of target gene mRNA by regulating the homeostasis of m⁶A, thereby promoting transcript translation and protein synthesis. METTL3 and METTL14 form stable complexes at a 1:1 ratio.[51] In vitro, the combination of the two can significantly improve methylation efficiency. In vivo, METTL14 recognizes RNA substrates and serves as an RNA-binding platform, and METTL3 has the main methyltransferase catalytic domain. The METTL3-METTL14 complex is also the only known m⁶A methyltransferase heterodimer complex (Figure 1).[52] Furthermore,

METTL3 can recruit the translation initiation factor, eIF3, to the translation initiation complex when combined with certain mRNAs to enhance translation,[7] for example, with epidermal growth factor receptor (EGFR) and bromodomain-containing protein 4 (BRD4).[18] In addition, METTL3 is also a chromatin-based regulatory factor necessary for leukemia status.[19] It locates to the transcription initiation point of SP1 and SP2, induces m⁶A modification of the relevant mRNA coding region, and enhances its translation by alleviating ribosomal stasis. In breast cancer,[20] METTL3 promotes the expression of an oncoprotein, HBXIP, in an m⁶A-dependent manner. HBXIP inhibits the tumor suppressor miRNA let-7g, and because let-7g inhibits METTL3, the positive feedback pathway, HBXIP-let-7g-METTL3-HBXIP, promotes the progression of breast cancer. Similarly, METTL3 upregulates RAB2B expression to promote the growth of cervical cancer cells.[53] In bladder cancer, METTL3 upregulates the expression of the adhesion molecule, ITGA6, to enhance the adhesion, migration, and invasion of tumor cells and to mediate signal output.[54] METTL3 also promotes bladder cancer progression by regulating the AFF4-nuclear factor κB (NF-κB)-MYC multi-level signal network,[55] and the METTL3-m⁶A-CDCP1 axis promotes the growth of chemically transformed cells and bladder cancer cells.[56] In prostate carcinoma, researchers revealed a METTL3-m⁶A-MYC axis.[57] METTL3 enhances MYC expression and plays a carcinogenic role by increasing the level of m⁶A in the MYC transcript.

In colorectal carcinoma (CRC),[58,59] SOX2 and CCNE1 are downstream target genes of METTL3. As an oncogene, METTL3 maintains the expression of SOX2 and CCNE1 in CRCs through an m⁶A-dependent mechanism, maintains the stemness of CRCs, and promotes proliferation and metastasis. Furthermore, in CRC with high glucose metabolism,[21] METTL3 induces CRC in a glycolysis-dependent manner. Mechanistically, METTL3 directly interacts with the mRNA of the glycolysis-related genes HK2 and GLUT1, thereby activating glycolysis in tumor cells. In gastric cancer[22] and hepatocellular

Table 1. Multiple functions exerted by m⁶A RNA methyltransferases in various cancers

Molecule	Role	Cancer	Target	Mechanism	References
METTL3	oncogene	lung cancer	mRNA	METTL3-eIF3 cycle promotes translation of oncogenes	18
	oncogene	AML	mRNA	enhances protein translation	19
	oncogene	breast cancer	mRNA	HBXIP-let-7g-METTL3-HBXIP positive feedback pathway	20
	oncogene	CRC, GC, HCC	mRNA	activates the glycolysis of tumor cells	21–23
	oncogene	HCC	mRNA	regulates sorafenib resistance by mediating autophagy	24
	oncogene	pancreatic cancer, HCC, CRC	miRNA	accelerates the maturation of oncogenic miRNA	25–28
	oncogene	HCC	lncRNA	upregulates the expression of oncogenic LNC00958	29
	suppressor	endometrial cancer	mRNA	inhibits the activation of AKT signaling pathway	30
METTL14	oncogene	AML	mRNA	inhibits myeloid differentiation of AMLs	31
	oncogene	pancreatic cancer	mRNA	activates the signaling pathway	32
	suppressor	RCC	mRNA	inhibits the expression of the oncogene P2RX6	33
	oncogene	breast cancer	miRNA	promotes the maturation of oncogenic miRNA	34
	suppressor	HCC	miRNA	promotes the maturation of cancer suppressor miRNA	35
	suppressor	CRC	lncRNA	downregulates the expression of carcinogenic lncRNA XIST	36
WTAP	oncogene	GC	–	involved in the immune regulation of tumor cells	37
	oncogene	DLBCL	–	stabilizes expression of transcriptional repressor BCL6	38
	oncogene	AML	–	inhibits myeloid differentiation of AMLs	39
VIRMA	oncogene	HCC	mRNA	inhibits the expression of tumor suppressor gene GATA3	40
	oncogene	PCa	lncRNA	upregulates the expression of oncogenic lncRNA CCAT1 and CCAT2	41
	oncogene	breast cancer	mRNA	regulates the expression of CDK1 and regulates the tumor cell cycle	42
HAKAI	oncogene	NSCLC	–	promotes EMT and mediates gefitinib resistance	43
	oncogene	tumor cell line	mRNA	interacts with PSF to stabilize oncogene expression	44,45
ZC3H13	oncogene	CRC	–	MLL1-ZC3H13 gene fusion, affecting mitosis	46
	suppressor	CRC	mRNA	inhibits activation of the Ras signaling pathway	47
RBM15	oncogene	AMKL	–	RBM15-MKL1 gene fusion	48
	oncogene	breast cancer	–	RBM15 gene mutation	49
METTL16	oncogene	CRC	–	METTL16 gene frameshift mutation	50

CRC, colorectal carcinoma; GC, gastric cancer; HCC, hepatocellular carcinoma; RCC, renal cell carcinoma; DLBCL, diffuse large B cell lymphoma; AML, acute myeloid leukemia; PCa, prostate carcinoma; NSCLC, non-small cell lung cancer; AMKL, acute megakaryocytic leukemia.

carcinoma (HCC),[23] METTL3 promotes tumorigenesis by activating glycolysis. METTL3 upregulates the expression of HDGF by increasing the m⁶A modification of HDGF mRNA, and HDGF promotes tumor angiogenesis and glycolysis of cancer cells[22] and can also activate the mTOCR1 pathway to promote glycolysis in cancer cells.[23] It is noteworthy that Liu et al.[60] and Yue et al.[61] confirmed that METTL3 is closely related to the epithelial-mesenchymal transition (EMT) process of gastric cancer. Previous studies have found that METTL3 regulates the EMT process.[62,63] METTL3 first modifies

SNAIL mRNA, a key transcription factor of EMT, triggering the multi-ribosome-mediated translation of SNAIL mRNA. Upregulation of SNAIL then promotes the transforming growth factor β1 (TGFβ1)-induced EMT process. It is noteworthy that METTL3 also mediates sorafenib resistance in HCC by mediating autophagy.[24] Additionally, there is a certain relationship between m⁶A modification and miRNA processing. METTL3 can promote the progression of cancer by accelerating the maturation of primary (pri-)miRNAs, including miR-25-2p in pancreatic cancer,[25] pri-miR221/222 in

HCC,[26] and pri-miR-1246 and miR-6079 in CRC.[27,28] Furthermore, METTL3 also upregulates the expression of oncogenic LINC00958 in HCC.[29]

METTL3 not only promotes cancer progression as an oncogene, but it also plays an anticancer role in some tumors. In glioblastoma, METTL3 inhibits the growth and self-renewal of tumor stem cells.[64] In endometrial cancer,[30] a low level of m[6]A promotes the proliferation of tumor cells, and low METTL3 expression activates the AKT pathway, leading to increased proliferation and tumorigenicity of endometrial cancer cells. In CRC,[65] METTL3 inhibits the proliferation, migration, and invasion of tumor cells by inhibiting the phosphorylation of p38 and extracellular signal-regulated kinase (ERK).

METTL14

As an important methyltransferase component, METTL14 may play multiple roles in the occurrence and progression of cancer. For example, in HCC,[66] METTL14 not only promotes cancer progression, but it also acts as a tumor suppressor to inhibit tumor development. METTL14 is highly expressed in hematopoietic stem cells (HSPCs) and acute myeloid leukemia (AML) cells.[31] METTL14 inhibits the myeloid differentiation of HSPCs and AML cells by regulating the m[6]A modification of MYB and MYC mRNA, thereby enhancing the self-renewal and proliferation of AML cells, ultimately leading to AML progression. METTL14 is also upregulated in pancreatic cancer tissues,[32] and METTL14 knockdown increases the sensitivity of pancreatic cancer cells to cisplatin and strengthens mTOR pathway-dependent autophagy, thereby accelerating the apoptosis of tumor cells. Remarkably, m[6]A modification can not only stabilize mRNA, but it also downregulates the stability of mRNA. In renal cell carcinoma (RCC),[33] METTL14 regulates the splicing of precursor (pre-)P2RX6 mRNA by regulating its level of m[6]A, thereby inhibiting expression of the P2RX6 mRNA and protein. This is different from the previously reported stabilization of target gene mRNAs and proteins by METTL14. Because P2RX6 increases ATP-dependent Ca^{2+} influx to promote the migration and invasion of RCCs, we suggest that METTL14 plays a tumor suppressor role in RCC. Gu et al.[67] found a low level of m[6]A modification and METTL14 in bladder cancer. As mentioned above for RCC, METTL14-mediated m[6]A modification also suppressed the mRNA stability of the target gene, NOTCH1, which is involved in the occurrence of bladder cancer and the self-renewal of bladder cancer-initiating cells; therefore, METTL14 may inhibit the occurrence and development of bladder cancer by inhibiting NOTCH1 expression.

Yi et al.[34] evaluated m[6]A levels in breast cancer and normal tissues, and they found that the abnormal expression of METTL14 led to a steady-state imbalance of m[6]A modification. Further investigation confirmed that METTL14 promotes the migration and invasion of breast cancer cells by upregulating miR-146a-5p. In HCC,[35] METTL14 interacts with the microprocessor protein, DGCR8, and positively regulates the processing process of pri-miR126 to inhibit HCC metastasis. However, the absence of METTL14 reduces the binding of DOCR8 to pri-miR126, which inhibits the processing of pri-miR126 and decreases miR126 expression, which eventually accelerates HCC metastasis. Notably, METTL14 is also downregulated in CRC,[68] which inhibits CRC progression through miR375-YAP1 and miR375-SP1 pathways. In addition, METTL14 is also involved in the regulation of lncRNA in CRC.[36] Through RNA sequencing (RNA-seq), carcinogenic lncRNA XIST was identified as a downstream target of METTL14. METTL14 downregulated lncRNA XIST in an m[6]A-dependent manner and inhibited the proliferation and invasion of CRC.

WTAP

WTAP is a nuclear protein that is widely expressed in various tissues of the body. As a splicing factor of the WT1 gene, WTAP is involved in regulation of the cell cycle and embryonic development.[69] As a key methyltransferase component, it is involved in the regulation of m[6]A RNA methylation.[70] Although WTAP did not show any methyltransferase activity in vitro, knockdown of WTAP can significantly reduce the m[6]A peak of cellular RNA, even more significantly than knocking down METTL3 or METTL14.[71] This shows that WTAP is an essential component for efficient RNA methylation. In addition, WTAP can interact with the METTL3-METTL14 complex to form a more stable METTL3-METTL14-WTAP complex and improve the catalysis of m[6]A RNA methylation.[72] WTAP also promotes the development of HCC by participating in the regulation of the post-transcriptional inhibition of the tumor suppressor EST1 in an m[6]A-dependent manner.[73] Furthermore, WTAP can also promote pancreatic cancer by combining with FAK mRNA and activating the FAK-phosphatidylinositol 3-kinase (PI3K)-AKT and FAK-SRC-GRB2-ERK axes.[74]

It is noteworthy that high expression of WTAP in gastric cancer is closely related to T lymphocyte infiltration.[37] T regulatory cells and CD4 memory-activated T cells were significantly fewer in high WTAP expression gastric cancer patients than in low WTAP expression gastric cancer patients, indicating that tumor immunoregulation may be an important reason for the poor prognosis of high WTAP expression. In diffuse large B cell lymphoma (DLBCL),[38] overexpression of WTAP can promote the proliferation of cancer cells and enhance their anti-apoptotic ability. In addition, WTAP can form a trimeric complex with heat shock protein 90 (Hsp90) and transcriptional repressor B cell lymphoma 6 in vivo and in vitro, which helps stabilize WTAP and maintain WTAP protein levels. WTAP also plays an important role in the differentiation of bone marrow cells, with increased expression in AML tissues.[39] However, in in vitro experiments, the downregulation of WTAP promoted phorbol 12-myristate 13-acetate (PMA)-induced bone marrow differentiation and increased expression of the bone marrow differentiation markers CD14 and CD11b, indicating that WTAP is involved in the induction of abnormal proliferation and differentiation block in AML cells. Additionally, WTAP is overexpressed in glioblastoma and is involved in regulating the migration and invasion of glioblastoma.[75] In xenograft models, overexpression of WTAP also increases tumorigenicity of tumor cells. Furthermore, WTAP can regulate the activity of EGFR.[71] WTAP is therefore closely related to the progression and poor prognosis of various tumors, including cholangiocarcinoma,[76] CRC,[77] and ovarian cancer.[78]

VIRMA

Schwartz et al.[71] identified a protein network that interacts with METTL3 and showed that VIRMA interacts with WTAP. Knockdown of VIRMA in human A549 cells decreased the peak value of m[6]A by approximately 4-fold compared with the control group. This was a significantly greater decrease than that observed upon knockdown of either METTL3 or METTL14, demonstrating that VIRMA is required for the full methylation program in mammals.[71] VIRMA can be used as a bracket through which WTAP combines with METTL3-METTL14 to guide the enrichment of m[6]A in 3′ UTRs.[79]

In testicular germ cell tumors,[80] the abundance of m[6]A and the expression of VIRMA are different in different subtypes, with the level in seminoma higher than that in non-seminoma. Furthermore, the differences in expression levels of METTL14 and VIRMA can also distinguish seminoma from non-seminoma.[81] Analysis of The Cancer Genome Atlas (TCGA) database shows differences in the expression of VIRMA between HCC and normal liver tissues,[82] which are also observed in in vitro experiments. Through methylated RNA immunoprecipitation sequencing (MeRIP)-PCR analysis, VIRMA will upregulate ID2 mRNA m[6]A, thereby reducing ID2 expression and promoting cancer. VIRMA also regulates GATA3 pre-mRNA in an m[6]A-dependent manner, hindering the binding of HUR to GATA3 pre-mRNA, which in turn reduces GATA3 pre-mRNA expression, resulting in a decrease in GATA3 mRNA expression, eventually promoting HCC progression.[40] In prostate cancer, VIRMA is a key factor for maintaining m[6]A levels,[41] which upregulates the expression of carcinogenic lncRNA CCAT1 and CCAT2 through m[6]A modification, thereby increasing aggressiveness of the cancer. Remarkably, VIRMA can regulate cancer progression independently of m[6]A modified pathways. In breast cancer, VIRMA knockdown did not affect the m[6]A level of its target mRNA, cyclin-dependent kinase 1 (CDK1).[42] In contrast, METTL3 knockdown significantly reduced the m[6]A modification level of CDK1 mRNA. Furthermore, knockdown of METTL3 did not change the amount of CDK1 mRNA interacting with VIRMA, indicating that the m[6]A modification did not interfere with the interaction of VIRMA and CDK1 mRNA and that VIRMA regulates CDK1 mRNA in an m[6]A-independent manner. In addition, in gastric cancer, VIRMA regulates c-JUN expression in an m[6]A-independent way, which ultimately promotes cancer.[83]

HAKAI

HAKAI is a key member of the methyltransferase complex.[84] Knockdown of HAKAI in HeLa cells downregulated the level of m[6]A, which is consistent with the knockdown results of other methyltransferase members.[79] Mammalian HAKAI, also known as Casitas B lineage lymphoma transformation sequence-like protein 1 (CBLL1), is an E3 ubiquitin-ligase that mediates the ubiquitination of E-cadherin to promote the endocytosis of E-cadherin at cell-cell contact. This ultimately leads to its lysosomal pathway degradation. E-cadherin, as a tumor suppressor, is downregulated during EMT, and its loss indicates a poor prognosis.[85]

In CRC,[86] overexpression of HAKAI will drive the conversion of epithelial cells into mesenchymal cells, with the downregulation of E-cadherin and the upregulation of N-cadherin, making tumor cells more aggressive. Therefore, HAKAI is involved in two crucial processes of tumor progression, namely, reducing cell-matrix adhesion and enhancing cell invasion.[85,86] In addition, overexpression of HAKAI can cause lung micrometastasis of tumor cells in vivo. Clinical tumor specimens of non-small cell lung cancer (NSCLC) with acquired gefitinib resistance also showed a decrease in E-cadherin and an increase in HAKAI expression.[43] In vitro experiments confirmed that HAKAI mediates gefitinib resistance by promoting EMT. HAKAI knockout inhibited AKT activity in NSCLCs, making NSCLCs sensitive to cisplatin therapy.[87] Of note, HAKAI can interact with PTB-associated splicing factor (PSF) to promote tumorigenesis.[44,45] HAKAI enhances the binding of PSF to tumor-related protein mRNAs to post-transcriptionally regulate the target mRNA and reverse its tumor suppression effect. Interestingly, in breast cancer,[88] HAKAI acts as a novel estrogen receptor-α core inhibitor to inhibit the proliferation and migration of estrogen-dependent tumor cells, but it does not affect the growth of estrogen-independent cells.

ZC3H13

ZC3H13 is a typical CCCH zinc finger protein involved in regulating RNA m[6]A. In Drosophila, the ZC3H13 homolog, Xio, was identified during female-male transformation studies.[13] Xio colocalizes with the known methyltransferase complex and, importantly, the expression pattern of Xio is similar to that of other members of the m[6]A pathway. Xio also interacts with known methyltransferases, and its deletion causes a reduction in m[6]A levels. After knocking out Zc3h13 in mouse embryonic stem cells, m[6]A levels decreased by approximately 60%–70% compared with wild-type cells, suggesting that ZC3H13 significantly affects m[6]A modification in mice.[89] In addition, knockout of ZC3H13 caused other methyltransferase components to be significantly transferred into the cytoplasm, and the expression levels of METTL3 and METTL14 in the nucleus were decreased. Similarly, knockdown of WTAP, VIRILIZER, or HAKAI also reduced the nuclear accumulation of METTL3 and METTL14, but the inhibition of WTAP, VIRILIZER, or HAKAI did not affect the nuclear localization of ZC3H13. These results indicate that the main role of ZC3H13 is to stabilize the ZC3H13-WTAP-VIRILIZER-HAKAI complex in the nucleus and regulate m[6]A. In addition, in the Zc3h13 mutant mouse, glioblastoma cells were more resistant to temozolomide.[90]

In humans, the fusion mutation MLL1-ZC3H13 disrupts the stability of chromosomes, affects mitosis, and promotes the formation of CRC tumors. It also upregulates the expression of the tumor stem cell marker CD44.[46] In addition, Gewurz et al.[91] found that ZC3H13 is a key upstream factor of NF-κB, which stabilizes NF-κB activation pathway-related mRNA and destabilizes NF-κB inhibitory pathway-related mRNA. Activation of NF-κB signaling accelerates tumor proliferation and invasion; therefore, ZC3H13 may play a role as an oncogene. Remarkably, ZC3H13 inactivates the Ras-ERK signaling pathway to inhibit the proliferation and invasion of CRC, and its reduction is also related to late tumor-node-metastasis

(TNM) staging, indicating that ZC3H13 may be a tumor suppressor gene.[47]

RBM15

RBM15 and its analog RBM15B mediate the formation of m[6]A in mRNA and the lncRNA XIST.[92] RBM15/RBM15B binds to the methyltransferase complex and recruits it to specific sites in RNA, mediating gene silencing in an m[6]A-dependent manner. In addition, RBM15 can also regulate a variety of signaling pathways that participate in the growth and apoptosis of many cells, including blood cells in particular. Some researchers have reported that RBM15 can promote the development of HSPCs and normal megakaryocytes by regulating the target, c-MYC,[93] and can also affect myeloid differentiation by activation of NOTCH-stimulated transcription in HSPCs.[94] In addition, RBM15 is also involved in the development of hematological tumors. The PRMT-RBM15 axis inhibits the terminal differentiation of megakaryocytes in acute megakaryocytic leukemia (AMKL), leading to defects in the formation of mature megakaryocytes.[95] Mechanistically, RBM15 binds to the intron region of pre-mRNAs of important megakaryocytic genes, and it recruits splicing factors to the corresponding binding sites for selective splicing, while overexpression of PRMT1 inhibits the expression of RBM15, resulting in the failure of normal expression of megakaryocyte genes, thereby promoting the development of AMKL. Overexpression of PRMT1 or knockout of RBM15 inhibited the differentiation of CD34^{+} cells into mature megakaryocytes.[96] In addition, the RBM15-MKL1 fusion gene is also associated with AMKL in adults and children.[48]

In addition to its key role in hematological disease, RBM15 is closely associated with other tumors. In pancreatic cancer, the expression of RBM15 has a significant correlation with overall survival.[97] In breast cancer, RBM15 mutations are associated with the incidence of borderline and malignant phyllodes tumors.[49]

METTL16

METTL16 is a methyltransferase newly discovered in HeLa cells and the second catalytically active methyltransferase verified in humans.[52] The METTL16 monomer consists of the N-terminal methyltransferase domain and the C-terminal vertebrate conserved region,[98] which usually exists as a dimer.[99] However, in in vitro experiments, METTL16 can assemble with target RNA in a monomeric form and regulate its m[6]A modification; therefore, the dimer is not necessary for its catalytic activity on RNA. METTL16 is associated with m[6]A of multiple ncRNAs, lncRNAs, and pre-mRNAs.[100] METTL16 catalyzes the m[6]A modification of U6 snRNA at position 43,[98] which post-transcriptionally regulates expression of the SAM synthase gene.[101] This promotes interaction with cancer lncRNA metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) and promotes its expression.[102] In addition, METTL16 is also necessary for the development of mouse embryos.[14] METTL16-deficient mouse 16-cell embryos showed a decreased level of SAM synthase gene transcripts, causing a large number of transcriptome disorders in 64-cell blastocysts, which hindered mouse embryo development. Remark-

ably, a frameshift mutation of METTL16 in CRC can promote the progression of colon cancer.[50]

Conclusions

We have reviewed the role of methyltransferases and their constituent components in different cancers (Table 1; Figure 3). The regulation mechanisms of the various methyltransferases and their components in the m[6]A pathway have many similarities, and the stability and level of protein expression from target genes are regulated through the writing of m[6]A. The regulation can be roughly divided into three categories: first, the same components regulate a particular cancer through different target genes. For example, with regard to METTL3 in gastric cancer, its downstream target genes, HDGF[22] and ZMYM1,[61] are both involved in the progression of cancer. Second, different components regulate the same target gene in different cancers. For example, METTL3 regulates MYC in prostate carcinoma,[57] and METTL14 regulates MYC in AML[31] to promote cancer. Third, different components participate in the regulation of the same cancer through different target genes; for example, METTL3 and MTTL14 regulate the incidence and progression of CRC,[34,58,59] bladder cancer,[55,67] and pancreatic cancer[25,32] through different targets, and METTL3,[23] METTL14,[35] WTAP,[73] and VIRMA[82] jointly regulate the progress of HCC. In addition, methyltransferases and their components can also accelerate the maturation of miRNA and regulate the expression of lncRNA in an m[6]A-dependent manner. Methyltransferases and their components also regulate cancer independently of m[6]A, mainly through two pathways: first, through the regulation of target genes, such as c-JUN,[83] CDK1,[42] and EGFR,[75] and second, through mutations in methyltransferase genes; for example, the fusion mutation of RBM15 promotes the occurrence of AMKL,[95] and the fusion mutation of ZC3H13[46] and the frameshift mutation of METTL16[50] promote the progression of CRC.

Prospects

RNA m[6]A is involved in multiple cellular processes such as RNA maturation, protein translation, and molecular structure, and it is important in gene expression and regulation, especially in the occurrence and development of cancer. As a key regulator of m[6]A, the role that methyltransferases play in tumors cannot be ignored. With the advent of high-throughput sequencing, chemical immunity, and other precise techniques, as well as the emergence of highly specific antibodies, more and more methyltransferases and their components have been discovered, and it has become possible to determine the chemical basis and biological functions of various methyltransferases. Various studies have shown that due to the genetic heterogeneity and specificity of tumors, methyltransferases also exhibit significant tumor specificity, so they can play different roles in cancers. As far as methyltransferases themselves are concerned, different target genes regulated by methyltransferases and their mechanism of action are different in different tumors or the same tumor in different organisms, resulting in diverse results. In addition, in the pathway of m[6]A modification, the overall changes in the expression levels, including methyltransferases, demethylases, and reader proteins, regulate the m[6]A modification levels of target genes, while
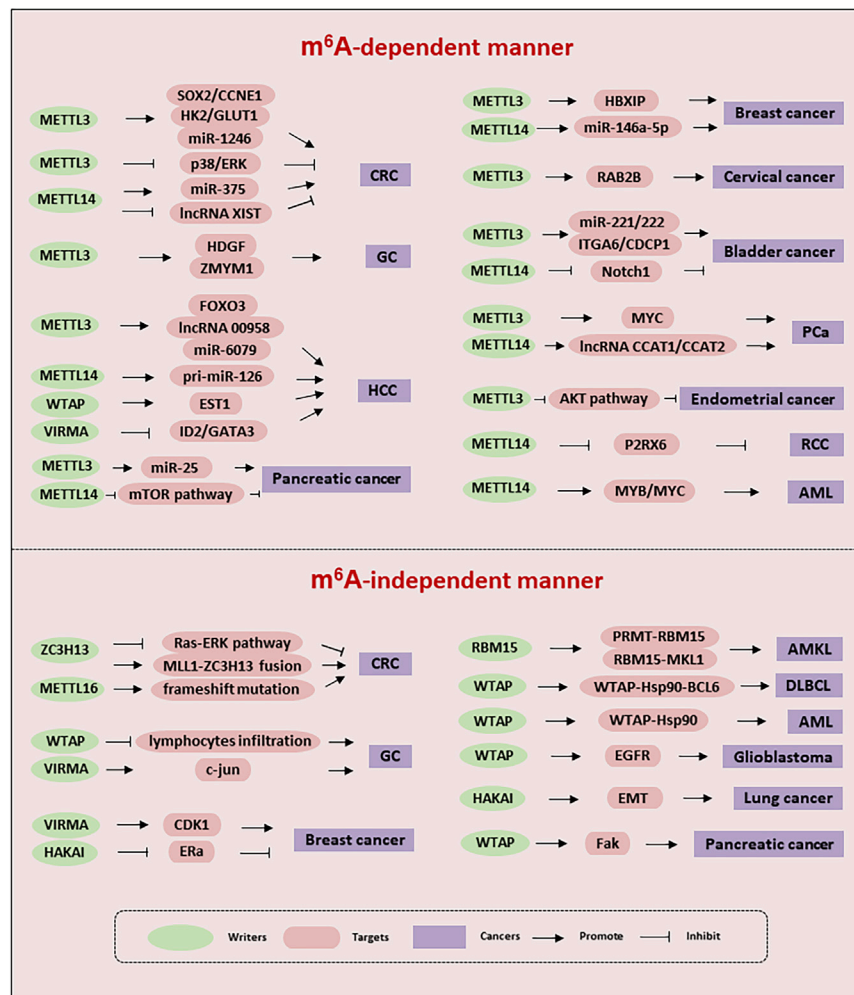
Figure 3. Methyltransferases participate in cancer progression through an m⁶A-dependent manner and m⁶A-independent manner

methyltransferases and demethylases regulate the m^6A modification positions of the target genes, the interaction of the three through influencing m^6A-modified levels and positions, and can also lead to their diverse functions in the course of the tumors. As an activator of cancer, methyltransferases regulate the level of target gene mRNA and protein through transcription and translation pathways, activate multi-level signaling pathways and metabolic pathways, regulate the cell cycle, promote the proliferation and metastasis of tumor cells, and enhance the aggressiveness of tumor cells through the EMT process. In the clinic, methyltransferases are also associated with overall survival rate, TNM stage, and drug resistance. As an inhibitor of cancer, methyltransferases can downregulate downstream oncogene mRNA and protein levels and inhibit the activation of signaling pathways.

Given the prevalence of m^6A modifications in different types of transcripts, further research into m^6A methyltransferases is warranted to further clarify their influence on RNA metabolism and to fully understand their molecular mechanisms. Their biological relevance and

diagnostic value in cancer requires further evaluation and may provide new prognostic markers for cancer and therapeutic targets. The development of m^6A methyltransferase inhibitors and agonists, especially in combination with other therapeutic drugs, is expected to lead to the development of new cancer treatment methods.

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.omtn.2020.12.021.

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Supplemental Information

The role of RNA N^6-methyladenosine

methyltransferase in cancers

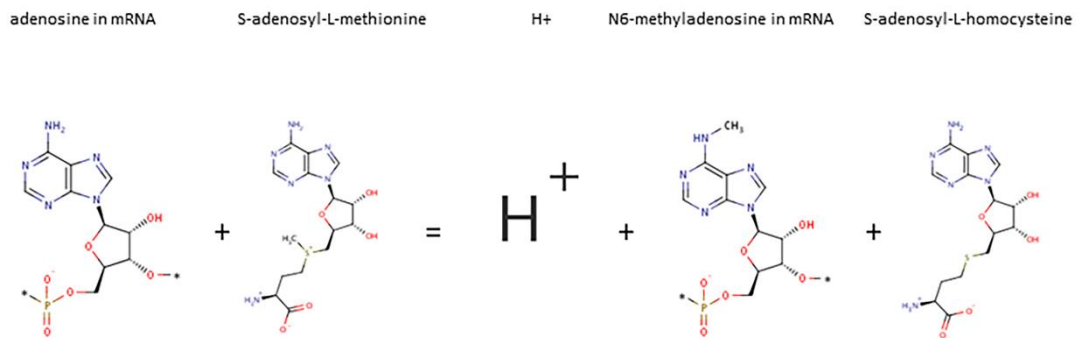**Jiali Huang, Zhenyao Chen, Xin Chen, Jun Chen, Zhixiang Cheng, and Zhaoxia Wang**

Figure S1. m6A RNA methylation reaction(https://www.uniport.org/)

Table S1. Human m⁶A RNA methyltransferase and its corresponding Mouse and Drosophila

m⁶A RNA methyltransferase

Human	Mouse	Drosophila
Gene names（Synonyms）		
METTL3 (MTA70)	Mettl3 (Mta70)	Mettl3 (Ime4)
METTL14 (KIAA1627)	Mettl14 (Kiaa1627)	Mettl14 (KAR4)
WTAP (KIAA0105)	Wtap	fl(2)d (CG6315)
VIRMA (KIAA1429)	Virma (Kiaa1429)	-
HAKAI (CBLL1)	Hakai (Cbll1)	Hakai (CG10263)
ZC3H13 (KIAA0853)	Zc3h13	Flacc (Xio)
RBM15 (OTT)	Rbm15 (Kiaa4257)	-
METTL16 (METT10D)	Mettl16 (Mett10d)	-