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REVIEW ARTICLE

The roles of m^{6}A RNA modifiers in human cancer

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Abstract: Like DNA and proteins, RNA is subject to numerous (over 160) covalent modifications which play critical roles to regulate RNA metabolism. Among these modifications, *N*^{6}-methyladenosine (m^{6}A) is the most prevalent RNA methylation on mRNA which occurs on around 25% of transcripts. The recent studies demonstrated that m^{6}A participates in many aspects of RNA processing, including splicing, nuclear exporting, translation, stabilization, etc. Therefore, it revealed a new layer of regulatory mechanism for gene expression and has been termed "RNA Epigenetics" or "Epitranscriptomics". RNA m^{6}A is regulated and exerts its functions by three groups of "m^{6}A RNA modifiers" including m^{6}A methyltransferases (writers), m^{6}A demethylases (erasers), and m^{6}A binding proteins (readers). In this review, we would summarize and discuss the current understandings of the roles of the conventional m^{6}A RNA modifiers in human cancers.

Keywords: Epitranscriptomics; N^6-methyladenosine; RNA methylation

1. INTRODUCTION

Messenger RNA (mRNA) plays important role in the life process, not only by conveying genetic information from DNA to protein but also by regulating diverse biological processes; the modification on RNA is critical in regulating these processes. RNA modification was first discovered in tRNAs and rRNAs [1]. So far, >160 kinds of RNA modifications with different chemical properties have been found, most of which are found in tRNA and noncoding RNA[2] in different organisms. N^6-methyladenosine (m^{6}A) was first discovered in 1974. Wei et al[3,4] found modified nucleotides such as m^{6}A, m^{7}G, N^4-acetylcytidine (ac^{4}C), and m^{6}Am on the mRNA of HeLa cells. However, due to the characteristics of the mRNA itself and the technology limitation, the field of mRNA modification was slowly progressed.

m^{6}A is the most abundant internal modification in messenger RNA and is the most well-studied type among all RNA modifications. In 2012, Dominissini et al[5] developed a new m^{6}A-seq technology which in turn not only revealed the distribution of m^{6}A modifications at the transcriptome level in both human and mouse but also found >12 000 m^{6}A modification sites on human mRNA. The distribution of the modification sites is highly conserved between human and mouse, mainly distributed near the stop codons, in the 3′-UTR and within internal long exons. Simultaneously, using the similar MeRIP-seq approach, Meyer et al[6] also obtained consistent observation that there are m^{6}A modifications on the mRNA of 7676 mammalian genes, which are mainly enriched in the vicinity of the stop codon and in the 3′-UTR region.

The m^{6}A modification can be catalyzed by m^{6}A "writers" including the conventional complex (METTL3/METTL14/WTAP) and other regulators and removed by m^{6}A "erasers" (FTO and ALKBH5). The function of m^{6}A is extensive depends on its "readers" (YTH domain-containing proteins, IGF2BPs and other newly discovered candidates). These proteins play various roles in different cancers (Table1). At the molecular level, m^{6}A can affect almost all aspects of mRNA metabolisms, including splicing, translation, stability, and miRNA maturation[7–9]. These m^{6}A-mediated mRNA metabolic processes play important roles in regulation of cellular functions, including response to cellular stresses[10,11], modulation of differentiation[12,13], homeostasis of immune cells[14,15], etc. In this review, we systematically introduce the biological functions of m^{6}A RNA modifiers (writers, erasers, and readers) and summarize the current understandings of their roles in human cancers.

2. WRITERS

The components of m^{6}A methyltransferase complex include METTL3,[16] METTL14,[17] WTAP,[18] RBM15,[19] RBM15B,[19] and vir-like m6A methyltransferase associated (VIRMA).[20,21] So far, among these components, METTL3 is the only protein possessing the m^{6}A catalytic activity. Besides the conventional methyltransferase complex, METTL16 is also reported as an RNA m^{6}A methyltransferase which only methylated a limited number of RNAs with specific RNA structure, such as U6 snRNA.

METTL3

After the detection of m^{6}A in 1974,[22,23] scientists attempted to identify the proteins which can catalyze m^{6}A. To identify these proteins, HeLa cell nuclear extracts and artificial synthetic

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mRNAs were used for an *in vitro* methylation assay.[21] [^{3}H]-SAM were used as the methyl group donor to detect the formation of new m^{6}As. As a result, the m^{6}A consensus sequence GGACU was confirmed both in natural RNA and synthetic RNA.[24] And after the nuclear extract was separated into various fractions by column chromatography, a 70-kDa fraction with SAM-binding motif was isolated, purified, and identified. That was MT-A70, which is called METTL3 nowadays.[21] METTL3 turns out to be a member of putative SAM-dependent methyltransferase family, which was highly conserved in yeasts, plants, *Drosophila*, and mammals[25,26]. Deletion of METTL3 in mammalian cells results in huge loss of m^{6}A in mature mRNA[27]. Although METTL3 is the major contributor in the methylation of polyadenylated RNA, the substrates of METTL3 do not include rRNA and snRNA.[28]

WTAP

In 2008, Zhong et al found the MTA-FIP37 complex in *Arabidopsis*; MTA and FIP37 are the METTL3 and WTAP homologs in *Arabidopsis*, respectively.[29] The detailed function of WTAP was then unveiled by Agarwala et al[30] in the yeast model. They noticed that the yeast homolog of METTL3, Ime4, interacted with the yeast WTAP homolog mum2 and showed the crucial role of WTAP for catalyzing m^{6}A. Subsequent studies observed the METTL3-WTAP complex in mammalian cells, which indicates this as an evolutionary conserved complex.[18] WTAP mainly acts as an adaptor for METTL3 and METTL14. It localizes them to nuclear speckles.[18] When WTAP is absent, m^{6}A level in mRNA goes down.[18] It helps METTL3 catalyze m^{6}A in nuclear in a more efficient way.

METTL14

METTL14 is also a member of methyltransferase-like (METTL) proteins, but the SAM-binding domain of it loses its function. As a result, METTL14 does not have the enzymatic activity of m^{6}A generation. METTL14 has strong interaction with METTL3 and crystal structures of the METTL3-METTL14 complex show that it is not METTL14 but METTL3 which transfers a methyl group to RNA.[31–33] It binds to RNA substrate and interacts with METTL3 at the same time, which not only enhances the enzymatic activity of the complex but also positions the methyl group to the targeted site.

VIR-Like m^{6}A Methyltransferase Associated (VIRMA/KIAA1429)

VIRMA is another component of methylation complex. It mainly mediates preferential m^{6}A mRNA methylation in 3′-UTR and near stop codon. Also, it seems to correlate with alternative polyadenylation[34].

RBM15/15B

RBM15 and its paralog, RBM15B, were first noticed through proteomics analysis of WTAP.[19,35] Then it was reported that RBM15 and RBM15B can interact with METTL3 through WTAP.[19] Also, severe loss of m^{6}A was observed in RBM15 and RBM15B knocked down cells, which showed their significance in m^{6}A formation. Further iCLIP studies show that RBM15 and RBM15B bind to U-rich regions in mRNA which are close to m^{6}A sites. Thus, RBM15 and RBM15B seem to work as adaptors which set near m^{6}A motifs and then recruit WTAP/METTL3 complexes to generate new m^{6}As. In 2018, it is revealed that Flacc(Zc3h13) serves as an adaptor between Nito(RBM15B) and Fl(2)d(WTAP). It can stabilize the complex and promote the deposition of m^{6}A on mRNA[36].

METTL16

METTL16 is confirmed as a new m^{6}A "writer" in 2017.[37] It is found to methylate hairpin structures in pre-mRNA of gene MAT2A, which encodes a SAM forming enzyme.[37] Since the sequence of the MAT2A pre-mRNA hairpin is similar to that in U6 snRNA, Conrad et al[37] did the *in vitro* methylation assay and confirmed that METTL16 is also the enzyme which catalyzes m^{6}A formation in U6 RNA. In addition, METTL16 is also reported to associate with non-coding RNAs and pre-mRNAs.[38] A large, positively charged groove is found in METTL16's 3D structure and it is likely to represent the RNA-binding site of this protein.[39]

3. THE ROLE OF m^{6}A WRITERS IN HUMAN CANCERS

METTL3 is mis-regulated in many kinds of cancers such as glioblastoma,[40,41] lung cancer,[42] liver cancer,[43] colon cancer,[42] acute myeloid leukemia,[44,45] pancreatic cancer,[46] bladder cancer,[47] and breast cancer.[48] Its roles differ from cancer to cancer. In glioblastoma, METTL3 can increase SOX2 mRNA stability and expression to enhance the growth of the tumor.[41] But according to Cui et al,[40] knocking down METTL3 and METTL14 in glioblastoma stem cell (GSC) promotes human GSC growth, self-renewal, and tumorigenesis. In liver cancer, METTL3 can reduce SOCS2 mRNA expression and increase tumor cell proliferation, migration, tumorigenicity, and lung metastasis.[43] In lung cancer and colon cancer, METTL3 promotes oncogene translation and leads to cancer cell growth and invasion. In acute myeloid leukemia (AML), METTL3 enhances MYC and BCL2 mRNA translation, inhibits cell differentiation, and accelerates leukemia progression in mice.[42] And in breast cancer, METTL3 increases m^{6}A level in HBXIP mRNA which promotes breast cancer cells proliferation and survival.[48] In endometrial cancer, downregulation of METTL3 causes reduction of m^{6}A methylation and leads to the decreased expression of PHLPP2, the negative AKT regulator and the increased expression of mTORC2, the positive AKT regulator, which results in the promotion in tumor cell proliferation.[49]

In endometrial cancer and GBM, downregulation of METTL14 and METTL3 resulted to increased tumorigenicity.[49] The upregulation of METTL14 in AML can stabilize MYB and MYC through m^{6}A modification, which leads to the development of AML.[50]

WTAP is highly expressed in high-grade serous ovarian carcinoma, and this high expression predicts a shorter overall survival.[51] In liver cancer, WTAP is also upregulated and promotes liver cancer development by guiding the m^{6}A modification on ETS1 mRNA.[52]

4. READERS

YTH domain proteins

YTHDF2 and YTHDF3 were first identified by Dominissini et al[5] in an m^{6}A pull down assay and were named because of their YTH domain. The affinity of YTH domain and m^{6}A methylated mRNA is 10–50 times higher than that of unmethylated mRNA.[53–55] Mammals have two YTH protein families; one family consists of YTHDC1 and YTHDC2, and the other family has three members (YTHDF1/2/3). These genes are conserved across species.

YTHDF1 was originally found to bind to the stop codon and 3′UTRs of m^{6}A-modified transcripts, and its overall distribution is highly consistent with the m^{6}A site distribution. In addition, YTHDF1 binds directly to the eukaryotic translation initiation factor eIF3, promoting the translation efficiency of m^{6}A-modified RNA substrates.[56]

YTHDF2 mediates the degradation of m^{6}A-modified mRNA. Under normal conditions, YTHDF2 co-localizes with the deadenylase complex and the decapping complex proteins and carries the transcripts of its targeted genes into the degrading

Table 1.

Roles of m^{6}A proteins in various cancers

Role in m^{6}A	Molecule	Cancer type	Role in cancer	Effect	References
Writers	Mettl3	GBM	Anti-oncogene	Knockdown of METTL3 or METTL14 dramatically promotes human GSC growth, self-renewal, and tumorigenesis	40
		NPC	Oncogene	Knockdown of METTL3 can stimulate endogenous ZNF750 expression	85
		Lung cancer	Oncogene	Increase the translation of oncogene and promote cancer cell growth, survival, and invasion	42
		Endometrial cancer	Anti-oncogene	Reduced expression of METTL3 increased the proliferation and tumorigenicity of endometrial cancer cells	49
		AML	Oncogene	Enhance the translation of MYC and BCL2 mRNA, inhibit cell differentiation, and induce leukemia progression in mice	45
		Breast cancer	Oncogene	Increase the expression of HBXIP by m^{6}A modification and then induce the proliferation of breast cancer cell	48
		Pancreatic cancer	Oncogene	METTL3-depleted cells showed higher sensitivity to anticancer reagents	46
		HCC	Oncogene	Knockdown of METTL3 drastically reduced HCC cell proliferation, migration, and colony formation *in vitro*, remarkably suppressed HCC tumorigenicity and lung metastasis *in vivo*	43
		Bladder cancer	Oncogene	Knockdown of METTL3 drastically reduced BCa cell proliferation, invasion, and survival	47
	Mettl14	AML	Oncogene	METTL14 depletion promotes myeloid differentiation of HSPCs and AML cells	50
		Endometrial cancer	Anti-oncogene	Loss-of-function of METTL14 increases cell proliferation, anchorage-independent growth, migration, and *in vivo* tumor growth	49
		GBM	Anti-oncogene	Knockdown of METTL3 or METTL14 enhances GSC growth and self-renewal	40
		HCC	Anti-oncogene	METTL14 depletion significantly reduces the expression of tumor suppressor miR126	82
	WTAP	HCC	Oncogene	Facilitate progression of hepatocellular carcinoma via m^{6}A-HuR-dependent epigenetic silencing of ETS1	52
Readers	YTHDF1	NSCLC, adenocarcinoma (ADC)	Oncogene	YTHDF1 depletion can restrain de novo lung ADC progression through regulating the translational efficiency of CDK2, CDK4, and cyclin D1	86
		Colorectal cancer	Oncogene	Knockdown of YTHDF1 can inhibit the spread of cancer and enhance the sensitivity of anticancer drug	69
	YTHDF2	HCC	Oncogene	miR145 modulates m^{6}A levels by targeting the 3′-UTR of YTHDF2 mRNA in HCC cells	70
		Prostate cancer	Oncogene	Promote prostate cancer growth and migration by enhancing m^{6}A-containing mRNA degradation	87
		Pancreatic cancer	Dual effect	Knocking down YTHDF2 induces EMT and inhibits proliferation via AKT/GSK3beta/cyclin D1 pathway	71
	YTHDF3	CRC	Oncogene	Play a key role in YAP signaling by facilitating m^{6}A-modified lncRNA GAS5 degradation, which profile a new insight into CRC progression	88
	YTHDC2	Colon tumor	Oncogene	Contribute to colon tumor metastasis by promoting translation of HIF-1α	72
	IGF2BP1	Liver cancer	Oncogene	Promote the stability and storage of the target mRNAs of m^{6}A modifiers (for example, MYC) in an m^{6}A-dependent manner	68
		Cervical cancer	Oncogene	Enhance an aggressive tumor cell phenotype by antagonizing miRNA-impaired gene expression	89
		HCC, EOC	Oncogene	Promote tumor cell growth by increasing the expression of SRF mRNA	90
Erasers	FTO	AML	Oncogene	Enhance leukemic oncogene-mediated cell transformation and leukemogenesis, inhibit all-trans-retinoic acid-induced AML cell differentiation through regulating expression of targets such as ASB2 and RARA by reducing m^{6}A levels in these mRNA transcripts	77
		AML	Oncogene	Promote AML carcinogenesis by enhancing the stability of MYC and CEBPA mRNA	78
		Cervical cancer	Oncogene	Enhance the resistance of chemo-radiotherapy by reducing the m^{6}A modification of β-catenin mRNA	79
		Glioblastoma	Oncogene	MA2 inhibits FTO catalytic activity which inhibits glioblastoma progression	40
	ALKBH5	Glioblastoma	Oncogene	Enhance FOXM1 expression by demethylating FOXM1 mRNA to maintain tumorigenicity of GSC	80
		Breast cancer	Oncogene	Increase the NANOG mRNA and protein expression by reducing the m^{6}A modification on NANOG mRNA	91
		Pancreatic cancer	Anti-oncogene	Inhibit pancreatic cancer motility by reducing methylation level of lncRNA KCNK15-AS1	81

body.[57] Subsequent studies further showed that YTHDF2 accelerated the degradation of m^{6}A-modified transcripts by recruiting CCR4-NOT adenosine complexes[58].

Based on the PAR-CLIP-seq results, YTHDF3 and YTHDF1 bind to the similar RNA motifs, and the binding sites are mainly located in the 3′UTR. YTHDF3 can promote the translation efficiency of consensus target genes of YTHDF1, suggesting that YTHDF3 and YTHDF1 synergistically regulate mRNA translation efficiency. In addition, YTHDF3 can also mediate mRNA degradation by directly interacting with YTHDF2[56,59].

YTHDC1, locating in the nucleus, interacts with the splicing factors SRSF3 and SRSF10, which suggests that m^{6}A regulates mRNA alternative splicing via YTHDC1[60].

YTHDC2 has the largest molecular weight in the YTH family and also tends to bind to the conserved m^{6}A-modified motifs. It regulates the stability of m^{6}A-modified mRNAs and make interactions between m^{6}A-modified RNAs and the ribosomes to facilitate translation efficiency.[61,62] In addition, YTHDC2 also participates in mouse spermatogenesis.[63,64]

IGF2BPs

Human IGF2BPs were first cloned in 1999 by Nielsen et al[65] who found three of high affinity proteins to IGF-II mRNAs, and these proteins were named IGF-II mRNA binding proteins (IMPs). IGF-II mRNA binding proteins are highly conserved from fish to human[66] and play an important role in regulating

translation, stability, splicing, and intracellular localization of targeted RNAs.[67] IGF2BPs were identified associating with m^{6}A by RNA pull down assays in 2018. They recognize m^{6}A by the KH domains and play oncogenic roles in cancers as m^{6}A readers[68].

5. THE ROLE OF m^{6}A READERS IN HUMAN CANCERS

RNA m^{6}A readers play important but diverse roles in human cancers. For instance, YTHDF1 is reported to be overexpressed in colorectal cancer tissues, and *in vitro* studies shows that knockdown of YTHDF1 can inhibit the spread of cancer and enhance the sensitivity of anticancer drug exposure.[69] YTHDF2 was upregulated in clinical hepatocellular carcinoma (HCC) tissues as an oncogene, and miR145 modulates m^{6}A levels by targeting the 3′-UTR of YTHDF2 mRNA in HCC cells.[70] Interestingly, YTHDF2 has been reported to have dual effects in pancreatic cancer. YTHDF2 is upregulated in mRNA and protein level in pancreatic cancer tissues in which YTHDF2 promotes tumor proliferation by activating AKT/GSK3beta/cyclin D1 but inhibits metastasis by degrading YAP mRNA.[71] RNA helicase YTHDC2 promotes colon tumor metastasis by promoting translation of hypoxia-inducible factor 1α (HIF-1α).[72]

IGF2BPs are shown to be highly expressed in various human cancers. When each of the IGF2BPs is knocked down in HeLa and HepG2 cells, MYC expression is significantly repressed and cell proliferation, colony formation, and migration ability are repressed[68]. Yisraeli et al[73] also demonstrated that in mice and human lung carcinomas, VICKZ1(IGF2BP1) can enhance tumor progression by synergizing with Kras.

6. ERASERS

FTO and ALKBH5

FTO is the first mammalian RNA m^{6}A demethylase.[74] Besides m^{6}A, FTO was also reported to have ability to catalyze the demethylation of the mRNA 5′ cap m6Am.[75] FTO belongs to the AlkB family of nonheme Fe(II)/α-ketoglutarate-dependent dioxygenases.[76] Other members of this family include ALKBH1-8. In mammals, m^{6}A also can be recovered to adenosine through ALKBH5. It has been found that the high expression of ALKBH5 in mouse testis is indispensable for spermatogenesis and mouse reproduction. The level of mRNA m^{6}A increases in the male mice with ALKBH5 deficiency, which affects the apoptosis of spermatocyte in the metaphase of meiosis and consequently leads to impaired fertility.[76]

7. THE ROLE OF m^{6}A ERASERS IN HUMAN CANCERS

In AML, FTO promotes tumorigenesis by reducing m^{6}A on ASB2 and RARA at UTRs and promotes AML carcinogenesis by enhancing the stability of MYC and CEBPA mRNA.[77,78] In cervical cancer, FTO enhances the chemoradiotherapy-resistance by reducing the m^{6}A modification of β-catenin.[79] Treatment with MA2, a chemical inhibitor of FTO, inhibited GSC growth and self-renewal considerably.[40] ALKBH5 also could promote GSC proliferation and tumor progression by reversing m^{6}A methylation on FOXM1 nascent transcripts.[80] Unlike the above studies, the demethylase activity of ALKBH5 acts an anti-oncogene role in pancreatic cancer by reducing lncRNA KCNK15-AS1 methylation[81].

8. DISCUSSION

The m^{6}A modification not only modulates multiple RNA metabolic processes such as RNA splicing, nuclear export, localization, translation, and stability, but also plays crucial roles in many physiological processes including tumorigenesis, self-renewing, and so on. The functions of m^{6}A modification in these processes are mainly determined by the m^{6}A modifiers (writers, erasers, readers). However, in cancer development, the role of these proteins displayed unexpected complexity; the same m^{6}A modifier may have completely opposite effects in different cancers. For example, the high expression of METTL14 contributes to the development of AML[50] but can repress the development of liver cancer[82] due to the different downstream targets of METTL14 in these two cancers. Mechanistically, METTL14 stabilizes ontogenetic mRNA of MYB and MYC in AML[50] but also enhances the level of miR126, which acts as a tumor suppressor in liver cancer.[82] This demonstrated that both overall level and distribution of m^{6}A are important for the regulation of cellular processes and development of cancer.

In addition, the field of Epitranscriptomics is blooming during the recent years. Besides m^{6}A, many new modifications are discovered and investigated continuously, such as RNA 5-methylcytosine (m^{5}C), 2′-O-methylation modification (2′OMe), ac^{4}C, etc. m^{5}C is mostly studied in tRNAs, it helps tRNA to generate the L-shaped three-dimensional structures[83]. 2′OMe was found nearly at the same time as m^{6}A, but its function is still not well-studied[22]. The function of ac^{4}C on mRNA was first described in 2018. Oberdoerffer et al[84] reported that ac^{4}C enhanced substrate translation *in vitro* and *in vivo*. However, the detailed biological functions of these modifications are still unclear and have to be further investigated.

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