Molecular Therapy
Nucleic Acids
Review

N^6-Methyladenosine: A Potential Breakthrough for Human Cancer

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Among more than 100 types of identified RNA modification, N^6-methyladenosine (m^6A) modification is the predominant mRNA modification, which regulates RNA splicing, translocation, stability, and translation. m^6A modification plays critical roles in the growth, differentiation, and metabolism of cells. As a dynamic and reversible modification, m^6A is catalyzed by "writers" (RNA methyltransferases), removed by "erasers" (demethylases), and interacts with "readers" (m^6A-binding proteins). With more advanced technology applied to research, the molecular mechanisms of RNA methyltransferase, demethylase, and m^6A-binding protein have been revealed. An increasing number of studies have implicated the correlation between m^6A modification and human cancers. In this review, we summarize that the occurrence and development of various human cancers are associated with aberrant m^6A modification. We also discuss the progress in research related to m^6A modification, providing novel therapeutic insight and potential breakthrough in anticancer therapy.

BACKGROUND

*N*6-adenosine is the most prevalent epigenetic modification in RNA, originally identified in mRNAs in the 1970s.[1] Similar to the methylation of DNA, N^6-methyladenosine (m^6A) methylation regulates post-transcriptional expression without changing the base sequence. m^6A is the most abundant internal RNA modification, enriched at the RRACH motif in 3′ UTRs, 5′ UTRs, and near stop codons.[2–6] It modulates RNA processing and metabolism, including alternative splicing, transport, translation, and degradation.[7] As a dynamic and reversible modification, m^6A deposition is regulated by methylases and demethylases. m^6A-binding proteins subsequently recognize and bind the m^6A-rich domain, inducing decay or accelerating translation efficiency. With the advancement of methods for detecting m^6A and proteins, many enzymes have been identified and the functions and mechanisms of them have gradually emerged. Some studies looking into the mechanism of alternative splicing regulation have uncovered the function of m^6A. m^6A near splice sites in nascent pre-mRNA mediates heterogeneous nuclear ribonucleoprotein G (hnRNPG) binding. hnRNPG interacts with RNA polymerase II (RNAPII) with Arg-Gly-Gly (RGG) motifs, thereby modulating RNAPII occupancy and alternative splicing.[8] Moreover, the location of m^6A on nascent RNA is likely to modulate splicing kinetics. m^6A co-transcriptionally depositing near splice junctions promotes fast splicing, while m^6A in introns indicates long, slowly processed introns and alternative splicing events.[9] m^6A was also found in precursor mRNA (pre-mRNA), tRNA, microRNAs (miRNAs), and long non-coding RNAs (lncRNAs) later, but the functions of m^6A in circular RNA (circRNA) remain elusive.[10–14]. m^6A in circRNAs are frequently derived from exons that are not methylated in mRNAs, and a single m^6A modification is enough to initiate circRNA translation, although the "writers" and "readers" are the same as those that participate in m^6A modification in mRNA.[15,16] With specific m^6A-related mechanism, circRNA is involved in tumorigenesis, which indicates the important role of circRNA in human cancer.[17]

With burgeoning medical technology, substantial progress has been made in the detection and diagnosis of cancer. Accumulating studies indicate that aberrant m^6A levels closely correlate with carcinogenesis and the progression and metastasis of cancer cells. The dysregulation of writers, "erasers," and readers is proved to be the culprit, activating oncogenes or inhibiting tumor suppressor genes by activating signaling pathways.[18,19] Potential therapeutic targets have also been offered by researching the mechanisms of carcinogenesis. In this review, we discuss the relationships between human cancers and new discoveries in the regulation of m^6A modification.

Regulation of m^6A Modification

Methyltransferases

m^6A writers (methyltransferases) can install the m^6A RNA modification (Figure 1). METTL3 is the first known RNA m^6A methyltransferase. Then, METTL14 was identified, forming a stable METTL3-METTL14 complex that is also called the m^6A-METTL complex

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(MAC).[20] The interface of the heterodimer is formed by strands β4–5 and helix α4 of each methyltransferase domain (MTD), and it contains hydrogen bonds as well as hydrophobic interactions. The hydrophobic region centering on strands β4–5 is protected from solvent exposure by an N-terminal extension of METTL14. In addition, METTL3 and METTL14 each forms a partially disordered loop that can insert an aromatic residue into a hydrophobic pocket in the other subunit. All of these unique structural features enhance the stability and compactness of MAC.[14,21] METTL3 is the only catalytic subunit of MAC, while METTL14 maintains MAC integrity and is likely to mediate RNA binding. Sequence analysis indicated that the METTL3 catalytic site contains a more conserved DPPW motif, whereas the catalytic motif of METTL14 is a divergent EPPL sequence. Moreover, two Cys-Cys-Cys-His (CCCH)-type zinc fingers of METTL3, adjacent to MTD3 and connected by an anti-parallel β sheet, are also necessary for methylation activity. The zinc finger domain (ZFD) specifically recognizes the 5′-GGACU-3′ consensus sequence of RNA, forming an RNA-binding interface. Although METTL14 has the folding configuration similar to METTL13, the cavity where RNA substrates bind is not possessed.[22,23] However, recent research has uncovered the important role of METTL14 in the crosstalk between histone modification and RNA methylation. METTL14 recognizes and directly binds with histone H3 trimethylation at Lys36 (H3K36me3), facilitating MAC to adjacent RNAPII, thereby installing m^{6}A in actively transcribed nascent RNAs co-transcriptionally. This mechanism may suggest how m^{6}A is specifically deposited in the transcriptome.[24]

The discovery of Wilms' tumor 1-associating protein (WTAP) enriched the composition of the m^{6}A methyltransferase complex. WTAP exhibits affinity for the methyltransferase complex, locating the METTL3-METTL14 complex at nuclear speckles and recruiting them to mRNA targets, regulating expression and alternative splicing of genes with the assistance of METTL3.[25] Studies focusing on interaction surfaces within the METTL3-METTL14-WTAP complex unveiled the novel overall architecture. In WTAP, the METTL3-binding surface is within the N-terminal 150 aa, while the WTAP interaction surface on METTL3 is an N-terminal helical structure that is necessary and sufficient for WTAP-METTL3 interaction. Strikingly, METTL3 phosphorylation in the WTAP interaction surface does not affect subcellular localization, WTAP interaction, or catalytic activity. Moreover, the C-terminal arginine-glycine repeats (RGG) of METTL14, contributing to RNA substrate binding, are indispensable for MAC catalytic activity. The finding is consistent with previous conclusions about the function of METTL14.[26]

More WTAP-related proteins and their relevant regulatory factors have been discovered in further studies. Hitherto, we defined the complex consisting of WTAP, VIRMA, Hakai, RBM15 (RNA-binding motif protein 15), and ZC3H13 (a zinc-finger protein) as MACOM (m^{6}A-METTL-associated complex).[11,27–29] Interacting with each other, these factors accumulate around target RNAs and catalyze methylation in specific sites. WTAP plays a central role in MACOM and mediates the nuclear speckle localization of the complex with assistance of BCLAF1 and THRAP3 (arginine/serine-rich domain-containing proteins).[30]

Recently, METTL16 was regarded as another human m^{6}A methyltransferase that targets pre-mRNAs and non-coding RNAs. METTL16 participates in splicing regulation by catalyzing *N*6-methylation in A43, which is in the specific sequence of U6 small nuclear RNA (snRNA). The alteration in A43 can influence the base pairing at 5′ splice sites of pre-mRNAs during splicing.[13] Intriguingly, studies also revealed METTL16 as a regulator that maintains SAM homeostasis by binding with MAT2A mRNA hairpins, implicating its likely important role in early development.[31,32]

Demethylases

Termed as erasers, demethylases can remove m^{6}A in RNA. To date, only two demethylases, fat mass and obesity-associated protein (FTO) and AlkB homolog 5 (ALKBH5), have been identified. FTO is the first identified demethylase, which is highly expressed in brain and muscle.[33] FTO mediates demethylation with its oxidative activity targeting the m^{6}A-rich region in RNA.[34] The mechanism of sequence-specific m^{6}A demethylation was revealed by further studies that demonstrated that FTO and RCas9 can fuse together as an RNA-targeting module. The resulting RCas9-FTO retained demethylation activity and bound to RNA in a sequence-specific manner depending on the single-guide RNA (sgRNA) and PAMmer.[35] Furthermore, consensus motifs GGACU and RRACU were discovered in target sequences. FTO specifically removed m^{6}A from GGACU and RRACU motifs in a concentration-dependent manner.[4] Additionally, FTO also mediates the demethylation of cap *N*6, 2′-*O*-dimethyladenosine (cap m^{6}A$_m$) in snRNA and mRNA. Compared with the demethylation of m^{6}A, FTO act differently in m^{6}A$_m$ demethylation, which can be accounted for by different cellular distribution features of FTO. Experiments conducted in polyadenylated RNAs among different cell lines manifested that FTO is more active with regard to m^{6}A$_m$ (81.9% of m^{6}A$_m$ and 20.3% of m^{6}A were demethylated *in vitro*). m^{6}A is preferentially demethylated by FTO in nucleus, whereas cap m^{6}A$_m$ is a prominent target in cytoplasm.[36] The cap m^{6}A$_m$ at +1 position from 5′ cap in mRNA confers resistance to mRNA-decapping enzyme DCP2 on transcripts, thereby enhancing mRNA stability.[37] FTO-mediated m^{6}A$_m$ demethylation was also detected in snRNAs (U1 RNA and U2 RNA; both the cap and internal m^{6}A$_m$ modifications are more significant in U2 RNA), but m^{6}A demethylation only happened in U6 RNA.[36] Demethylation of m^{6}A$_m$ at the sites adjacent to snRNA cap lead to relatively decreased m^{6}A$_m$-snRNA levels. However, the function of FTO can be inhibited by the oncometabolite-2-hydroxyglutarate, resulting in increased m^{6}A$_m$-snRNA levels, which may change patterns of alternative splicing.[38] The FTO-induced noteworthy changes of snRNAs indicate that FTO is capable of having an influence on mRNA splicing.

ALKBH5 catalyzes demethylation of m^{6}A in RNA and contributes to normal splicing and the formation of longer 3′ UTR mRNAs. Therefore, ALKBH5 also takes part in maintaining stability of transcripts.[33,39] In light of the similar functions of ALKBH5 and FTO,

the underlying mechanism by which the demethylases selectively recognize their target transcripts is intriguing. Some research has indicated that an m^{6}A-induced conformational change on RNA may account for the specificity but that the consensus sequence (GG(m^{6}A)CU) is not indispensable for the specific recognition.[40]

m^{6}A-Binding Proteins

m^{6}A modification exerts biological functions by binding to YTH domain-containing proteins (YTHDC1–2), YTH-family proteins (YTHDF1–3), and other interacting factors. The proteins are collectively defined as readers. In the nucleus, YTHDC1 promotes exon inclusion of targeted mRNAs and regulates mRNA splicing and export from nucleus to cytoplasm by recruiting pre-mRNA splicing factor SFSF3.[41,42] YTHDC1 was also established to participate in the maintenance of intracellular SAM levels, thereby regulating the level of methylation.[32] HNRNPA2B1 is a direct m^{6}A reader which takes part in primary RNA (pri-miRNA) processing and alternative splicing by interacting with the DGCR8 protein.[43] In the cytoplasm, YTHDF1–3 proteins work synergistically to influence RNA metabolism.[41,44,45] YTHDF3 promotes translation initiation of its target mRNA with assistance of YTHDF1 and initiation factor eIF4A3. YTHDF3 also mediates mRNA degradation by cooperating with YTHDF2. Similar to YTHDF3, YTHDC2 also has a dual effect on target mRNA. YTHDC2 enhances translation efficiency of target mRNA, while knockdown of YTHDC2 results in an upregulation of m^{6}A-modified transcripts. This novel function of YTHDC2 is essential for fertility in mammals and ensures the transition from mitosis to meiosis.[46,47] Especially, transcripts with m^{6}A in the 5′ UTR can be translated by directly binding elF3, independent of YTHDF1 and cap-binding factor eIF4E. In addition, diverse cellular stresses selectively result in an increase in mRNAs with 5′ UTR m^{6}A.[5]

As a new class of m^{6}A readers discovered recently, IGF2BP1/2/3 (insulin-like growth factor 2 mRNA-binding proteins 1, 2, and 3) recognize the consensus GG(m^{6}A)C sequence and bind m^{6}A by their K homology domains. In such an m^{6}A-dependent manner, IGF2BP1/2/3 prevent target mRNAs from degradation and promote mRNA translation.[48]

m^{6}A Modification in the Occurrence and Development of Cancers

Acute Myeloid Leukemia (AML)

AML is a malignant disease originating from hematopoietic stem cells or progenitor cells. Chemotherapy and hematopoietic stem cell transplantation are general treatments for AML, but targeted therapy, demethylation therapy, and immunotherapy also show promising therapeutic effects. With high expression of methyltransferases, AML cells feature elevated m^{6}A, which contributes to the maintenance of multilineage differentiation potential and inhibits cell differentiation in AML.[49,50] METTL3 recruited by CEBPZ promotes translation of SP1 by upregulating the m^{6}A level. SP1 subsequently activates the oncogene c-MYC, which can result in the development of AML.[50] Similarly, m^{6}A-modified BCL2 and PTEN induced by elevated METTL3 levels in AML can also lead to AML development caused by downregulation of phosphorylated phosphatidylinositol 3-kinase (PI3K)/AKT.[49] However, elevated non-functional METTL3 also activates the PI3K/AKT pathway, which implicated that there are certain mechanisms independent of m^{6}A modification.[49] Strikingly, similar to m^{6}A readers, METTL3 can also promote the translation of mRNAs by binding m^{6}A-modified regions close to the stop codon and interacting with eIF3h.[51] METTL14 is essential for maintaining self-renewal of AML cells, while knockdown of METTL14 promotes myeloid differentiation. The consistent high expression of METTL14 is attributed to the dysregulation of SPI1.[52] Oncogenes MYB and MYC are targets of METTL14. However, YTHDF proteins are not involved in promoting stability and translation of target mRNAs, while knockdown of METTL14 has an effect on the quantity of MYB and MYC mRNA.[52] METTL16 indirectly regulates the activity of METTL3/MELL14 by regulating the expression of MAT2A, which maintains appropriate SAM levels.[32,53]

WTAP has been found upregulated in AML and to plays an oncogenic role.[54] Increased expression of WTAP in AML cell lines alters alternative splicing, promotes proliferation, and blocks differentiation.[30,54] Studies found that the increased WTAP protein levels result from simultaneous high levels of cytoplasmic METTL3, in agreement with the conclusion that METTL3 and METTL14 are WTAP complex interactors.[55]

The RBM15-MKL1 fusion was originally detected in infants and the transcripts were analyzed for diagnosis of AMKL.[56,57] Interacting with the KMT2G, RBM15-MKL1 fusion protein enhanced cell proliferation.[58] RBM15 can inhibit myeloid differentiation in hematopoietic cells by stimulating Notch signaling via RBPJkappa[59] and contribute to adult hematopoiesis and normal megakaryocyte development mediated at least in part by c-Myc. Therefore, the function of RBM15 in AML is correlated with the activation of Notch signaling and interaction with c-Myc.[60] In addition, overexpression of PRMT1 leads to aberrant alternative splicing and blocks AML cell differentiation by inducing RBM15 ubiquitylation and degradation.[61,62]

FTO is overexpressed in AML with MLL rearrangements, PML-RARA, FLT3-ITD, or NPM1 mutations.[63] FTO can decrease m^{6}A levels in certain mRNA transcripts, including the tumor suppressor ankyrin repeat and ASB2 (SOCS box protein 2) and RARA (retinoic acid receptor alpha), thereby suppressing the expression of ASB2 and RARA, which regulate normal hematopoiesis and all-*trans*-retinoic acid (ATRA)-induced AML cell differentiation. Therefore, overexpressed FTO mediates leukemogenesis and indicates better efficacy of ATRA treatment in AML cells.[63] With the capacity for catalyzing demethylation of m^{6}A$_m$ in mRNA and snRNA and N^1-methyladenosine (m1A) in tRNA,[36] FTO may mediate oncogenesis independent of m^{6}A. Similarly, CAPAM (cap-specific adenosine methyltransferase) mediating m^{6}A modification at the 5′ cap in mRNA may correlate with AML, but the hypothesizes have yet to be identified.[64,65]

Recently, an investigation in leukemia patients indicated that genetic alterations of m^{6}A regulatory genes were associated with p53

mutations in AML. These alterations always predict a poor survival rate.[66] Genetic aberrations, that is, CBFA2T3/GLIS2 and NUP98/KDM5A, may be used for risk group stratification of pediatric AMKL and treatment tailoring.[67]

Colorectal Cancer

YTHDF1 and METTL3 have been identified to play significant roles in colorectal cancer (CRC). YTHDF1 was established to regulate tumorigenesis in CRC cells via the Wnt/β-catenin pathway. Mechanistically, studies found that YTHDF1 induced aberrant activation of Wnt/β-catenin signaling via recognizing and promoting translation of FZD9 and Wnt6 mRNA in an m^{6}A-dependent manner. According to clinical and laboratory data, high YTHDF1 expression in patients signaled poorer overall survival, while silencing of YTHDF1 *in vitro* resulted in poor efficacy of anticancer drugs and suppression of cancer proliferation. However, it is notable that c-Myc was indispensable in the carcinogenic mechanism of YTHDF1, indicating the theory of a c-Myc-driven YTHDF1 axis.[68] Moreover, YTHDF1 gene copy number amplification contributes to YTHDF1 overexpression in CRC.[69] METTL3, acting as a carcinogenic factor, was observed to be overexpressed in CRC metastatic tissues and released a signal of poor prognosis. Confirmed as an m^{6}A methyltransferase (as previously mentioned), METTL3 is capable of methylating SRY (sex determining region Y)-box 2 (SOX2), which is essential for maintaining self-renewal. IGF2BP2 subsequently recognizes the methylated SOX2 transcripts, thereby preventing SOX2 mRNA degradation.[70]

In addition, studies have shown that lncRNA RP11 triggers the dissemination of CRC cells. RP11 induce cell dissemination by post-translationally regulates the stability of Zeb1, an epithelial-mesenchymal transition (EMT)-related transcription factor. An m^{6}A-dependent manner is involved in the accumulation of RP11 in nuclear. Mechanistically, the RP11/HNRNPA2B1/mRNA complex prevented the proteasomal degradation of Zeb1 by promoting the mRNA degradation of Siah1 and Fbxo45.[71]

Gastric Cancer

The molecular mechanism in the occurrence of gastric cancer (GC) remains unclear, but some conclusions have emerged during studies. Some studies have given way to the idea that METTL3 is a carcinogenic factor of GC. METTL3 was observed to be significantly overexpressed in GC tissues, and the expression level of METTL3 was positively related to the tumor/node/metastasis (TNM) stage of GC. m^{6}A methylation level was also upregulated simultaneously. METTL3 leads to inactivation of the AKT signaling pathway, while knockdown of METTL3 activated the apoptotic pathway in GC cells. Downregulation of METTL3 suppressed the proliferation and mobility of GC cells.[72,73] However, another study came to the opposite conclusion that downregulation of m^{6}A contributed to GC cell proliferation and invasiveness by activating Wnt and PI3K/AKT signaling, indicating that the m^{6}A methyltransferases may suppress tumorigenesis.[74] Moreover, compared with adjacent non-tumor tissues, FTO expression in GC tissues featured a high level in protein and mRNA, suggesting that FTO may play a significant role in GC. Overexpression of FTO promotes the proliferation, migration, and invasion of GC cells and is positively related to TNM stage.[75]

Breast Cancer

Aberrant m^{6}A level and dysregulation of METTL3, METTL14, WTAP, and FTO have been observed in breast cancer (BC) cells.[76] Decreased m^{6}A level was confirmed to mediate abnormal expression of certain tumor suppressor genes in a premature polyadenylation (pPA)-dependent manner.[77] Studies have shown that FTO was upregulated in human BC, promoting proliferation, colony formation, and metastasis of BC cells. Mechanistically, FTO inhibits the function of BNIP3, which acts as a tumor suppressor. FTO mediated m^{6}A demethylation at the RRACH motif near the stop codon of BNIP3 mRNA and induced its degradation in an YTHDF2-independent manner.[78] As a reader of the m^{6}A, HNRNPA2/B1 is upregulated in BC cells, reducing sensitivity to antagonists of estrogen receptor by regulating levels of specific miRNAs.[79] Specially, some studies found the dual role of m^{6}A in BC. In immortalized and oncogenically transformed human mammary epithelial cells (HMECs), m^{6}A levels were significantly decreased with a decrease of METTL3 and an increase of ALKBH5. However, increased m^{6}A levels in transformed cells, resulting from overexpression of METTL3 and METTL14, or deletion of ALKBH5, promoted proliferation and migration. Interestingly, m^{6}A levels in the immortalized and transformed cells were found to increase under stress with constant levels of METTL3, METTL14, and ALKBH5, suggesting other underlying pathways for regulation of m^{6}A levels.[80]

Lung Cancer

Deregulation of FTO and METTL3 has been detected in lung cancer, which indicated their significant roles in tumorigenesis and development. Studies established that FTO plays an oncogenic role in NSCLC (non-small-cell lung cancer) and LUSC (lung squamous cell carcinoma). In human NSCLC tissues, overexpressed FTO promoted proliferation and colony formation of cancer cells by increasing USP7 mRNA level and USP7 expression.[81] FTO was also involved in the progression of LUSC. Mechanistically, FTO decreased m^{6}A levels and mRNA stability in the MZF1 mRNA transcript, thereby promoting expression of MZF1 and mediating carcinogenesis.[82] METTL3-eIF3h enhances translation of oncogenic mRNAs in primary lung tumors and leads to oncogenic transformation.[51] METTL3 is also implicated in NSCLC progression, promoting cell growth and survival via the PI3K/Akt pathway.[83] Additionally, METTL16 was found to bind the 3′ terminal triple helix of metastasis-associated lung adenocarcinoma transcript 1 (MALAT1), a cancer-promoting long noncoding RNA, and regulated MALAT1 endocellular accumulation. However, the relationship between tumorigenesis and this mechanism remains unclear.[84] In addition, when researching into the deregulated autophagy in human cancers, aberrant activation of the oncogene UBE2C was noticeable. In NSCLC, upregulated ALKBH5 maintains a lower m^{6}A level in UBE2C mature RNAs and activates UBE2C expression, thereby repressing autophagy. Depletion of UBE2C induces attenuated proliferation, clonogenicity, and invasive growth of NSCLC cells.[85]

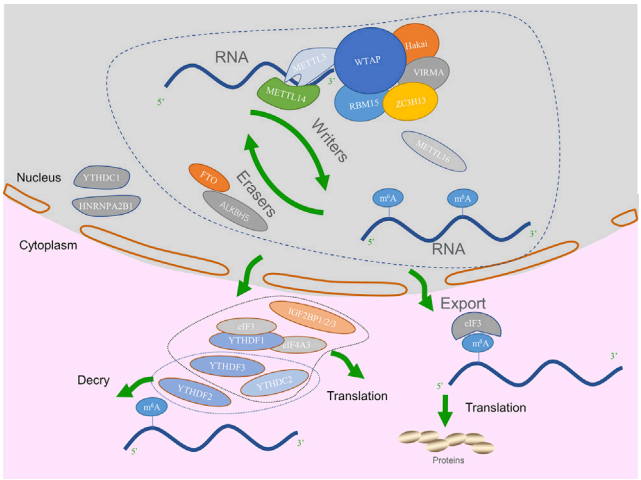

Figure 1. Methyltransferases, Demethylases, and m^{6}A-Binding Proteins in m^{6}A Modification

m^{6}A is catalyzed by writers and removed by erasers (FTO and ALKBH5). MACOM, consisting of WTAP, VIRMA, Hakai, RBM15, and ZC3H13, ensures the location of the METTL3-METTL14 core complex to nuclear speckle. m^{6}A modification exerts biological functions by binding to YTHDC1–2, YTHDF1–3, HNRNPA2B1, IGF2BP1–3, eIF3, and eIF4A3. In the nucleus, YTHDC1 and HNRNPA2B1 bind m^{6}A and perform multiple functions. In the cytoplasm, YTHDC2, YTHDF1–3, IGF2BP1–3, eIF3, and eIF4A3 induce translation or degradation of transcripts. eIF3 can promote translation by directly binding m^{6}A in the 5′ UTR.

Hepatocellular Carcinoma

Overexpressed METTL3, METTL14 downregulation, and upregulated m^{6}A readers have been observed in hepatocellular carcinoma (HCC) cells. Overexpression of METTL3 is a signal of poor prognosis in HCC, and upregulated YTHDF1 is positively correlated with TNM stage, indicating their important roles in HCC cells metastasis and progression.[86–88] With a YTHDF2-dependent mechanism, METTL3 mediated m^{6}A modification of the SOCS2 suppressor, thereby repressing SOCS2 expression and promoting HCC progression.[86] Contrastingly, METTL14 performs a repressing effect in tumor metastasis by interacting with the microprocessor protein DGCR8 and promoting pre-miRNA126 process via m^{6}A modification.[89] VIRMA, another m^{6}A writer, was also identified as an oncogenic factor, facilitating migration, proliferation, and invasion of HCC cells by inducing m^{6}A modification of ID2 mRNA.[90]

Melanoma

Melanoma is a type of malignant cancer that originates from pigment-producing melanocytes. The pathogenesis of melanoma still remains unclear, but some studies have given insight into the mechanism of oncogenesis. FTO and METTL3 are significantly upregulated in human melanoma.[91,92] FTO-mediated m^{6}A mRNA demethylation promotes melanoma tumorigenesis and anti-PD-1 resistance. Knockdown of FTO increases m^{6}A modification in the critical protumorigenic melanoma cell-intrinsic genes, including PD-1, CXCR4, and SOX10, inducing RNA degradation via binding YTHDF2. In mice, knockdown of FTO markedly enhances melanoma cell sensitivity to interferon (IFN)-γ and anti-PD-1 treatment, which suggested a promising anti-cancer therapy.[91] Overexpressed METTL3 enhances colony formation and invasion of melanoma cells via promoting accumulation of MMP2 and N-cadherin.[92]

Glioblastoma (GBM)

Glioblastoma is the most aggressive malignant tumor of central nervous system. Although there are multiple clinical trials for GBM, the recurrence rate is still very high. GB stem cells (GSC) are a subset of GBM cells, closely correlating with tumorigenesis, proliferation, and recurrence. Many studies aiming at GSCs have been performed in recent years. Upregulated m^{6}A mRNA modification was identified to induce the suppression of GSC self-renewal and tumorigenesis. Mechanistically, the changing m^{6}A level altered mRNA expression of certain genes that play critical roles in GSCs. METTL3 and METTL14 suppress GSC growth and self-renewal by downregulating oncogenes (ADAM19, EPHA3, and KLF4) and upregulating tumor suppressors (CDKN2A, BRCA2, and TP53I11).[93] Further studies have shed light on the function of some enzymes. ALKBH5 was overexpressed in GSCs and promoted FOXM1 expression by demethylating its nascent transcripts. Moreover, a lncRNA antisense to FOXM1 (FOXM1-AS) also participated in the ALKBH5-mediated tumorigenesis.[94] Being involved in RNA processing and oncogenic pathways of GSCs, functions of METTL3 are multiple and complex. METTL3 plays a dominant role in m^{6}A modification of GSCs and participates in the expression of GSC-specific actively transcribed genes and alternative splicing. Additionally, METTL3 decreases A-to-I RNA editing by downregulating ADAR and ADARB1, whereas it upregulates editing enzymes APOBEC1 and APOBEC3A to increase C-to-U RNA editing.[95]

Advances in the m^{6}A Modification on Human Cancers

It is becoming increasingly clear that deregulated m^{6}A modification is a key factor in growth, metastasis, and drug resistance of various tumors. Recently, some novel regulatory pathways have been found in cancer cells, which may give insight into potential therapeutic strategies to inhibit cancers. Many enzymes and genes have been identified to play vital roles in the sophisticated system and alter therapeutic efficacy.

METTL3 has been considered to be an oncogenic factor in many human cancers, and studies focusing on blocking METTL3-dependent pathways have been performed. METTL3-depleted pancreatic cancer cells exhibited higher sensitivity to anticancer reagents.[96] miR-600-induced METTL3 suppression can inhibit NSCLC progression.[83] In bladder cancer cells, METTL3 and YTHDF1 promote oncogene CDCP1 translation by binding m^{6}A in the 3′ UTR of CDCP1 mRNA. The METTL3-m^{6}A-CDCP1 axis has been revealed as a target for treating chemical-induced cancers.[97] Moreover, METTL3 participates in the mutagenesis of p53 proteins, which promotes proliferation, metastasis, and drug resistance of cancer cells. METTL3 catalyzes the formation of m^{6}A at the point-mutated codon 273 (G>A) of p53 pre-mRNA, promoting aberrant splicing of p53 pre-mRNA and expression of p53 R273H mutant protein. Furthermore,

Table 1. m6A Methylation-Related Human Cancers

Cancer	Molecule	Function	Mechanism	References
AML	METTL3	oncogenic	(1) indirectly activates the oncogene c-MYC by upregulating m^{6}A modification of SP1 and stimulates its translation (2) induces m^{6}A modification of BCL2 and PTEN and then downregulates apoptosis and the PI3K/AKT pathway (3) promotes the translation of mRNAs by binding m^{6}A-modified regions and interacting with eIF3h	50 49 51
	METTL14	oncogenic	activates the SPI1-METTL14-MYB/MYC signaling pathway	52
	METTL16	oncogenic	regulates the activity of METTL3/MELL14 indirectly	32,53
	FTO	oncogenic	(1) decreases m^{6}A levels in mRNA transcripts of the tumor suppressors ASB2 and RARA, thereby suppressing the expression of them (2) mediates oncogenesis independent of m^{6}A	63 36
	WTAP	oncogenic	promotes proliferation and blocks differentiation of AML cells	30,54
	RBM15	oncogenic	(1) enhances cell proliferation via RBM15-MKL1 fusion (2) inhibits myeloid differentiation by stimulating Notch signaling via RBPJkappa (3) blocks AML cell differentiation via the PRMT1-RBM15 axis	56–58 59 61,62
CRC	YTHDF1	oncogenic	induces aberrant activation of Wnt/β-catenin signaling by recognizing and promoting the translation of m^{6}A-modified FZD9 and Wnt6 mRNA	68
	METTL3	oncogenic	maintains SOX2 expression through an m^{6}A-IGF2BP2-dependent mechanism	70
	RP11	oncogenic	induces cell dissemination via post-translational upregulation of Zeb1	71
GC	METTL3	oncogenic	promotes proliferation and mobility of GC cells by inactivating the AKT signaling pathway	72,73
		suppressive	suppresses proliferation and invasiveness by inactivating Wnt and PI3K-Akt signaling	74
	FTO	oncogenic	unclear	75
BC	FTO	oncogenic	inhibits tumor suppressor BNIP3	78
	HNRNPA2/B1	oncogenic	reduces BC cell sensitivity to antagonists of estrogen receptor	79
Lung cancer	METTL3	oncogenic	(1) promotes growth and survival of NSCLC cells via the PI3K/Akt pathway (2) promotes translation of oncogenic mRNAs in a METTL3-eIF3h manner in primary lung tumors	83 51
	METTL16	unclear	binds the 3′ terminal triple helix of MALAT1	84
	FTO	oncogenic	(1) promotes proliferation and colony formation of NSCLC cells by increasing the expression of USP7 (2) mediates carcinogenesis in LUSC by promoting expression of MZF1	81 82
	ALKBH5	oncogenic	activates oncogene UBE2C expression	85
HCC	METTL3	oncogenic	represses SOCS2 expression in HCC through an m^{6}A-YTHDF2-dependent mechanism	86–88
	METTL14	suppressive	interacts with DGCR8 and promotes primary miRNA126 processing in an m6A-dependent manner	89
	VIRMA	oncogenic	induces m^{6}A modification of ID2 mRNA	90
Melanoma	METTL3	oncogenic	promotes accumulation of MMP2 and N-cadherin and enhances colony formation and invasion	92
	FTO	oncogenic	increases RNA degradation of PD-1, CXCR4, and SOX10 via binding YTHDF2	91
GBM	METTL3/METTL14	suppressor	suppresses GSC growth and self-renewal by downregulating oncogenes (ADAM19, EPHA3, and KLF4) and upregulating tumor suppressors (CDKN2A, BRCA2, and TP53I11)	93
	ALKBH5	oncogenic	promotes FOXM1 expression	94
Endometrial cancer	METTL3/METTL14	suppressor	reduces m^{6}A methylation, upregulates the negative AKT regulator PHLPP2, and downregulates the positive AKT regulator mTORC2, thereby activating the AKT pathway	99

upregulated METTL3 expression is partly attributed to glycosphingolipids, which execute function via activating cSrc and β-catenin signaling.[98] Reduction of m^{6}A methylation caused by abnormal MAC has been found in about 70% of human endometrial cancer samples, resulting in activation of the AKT pathway by upregulating the negative AKT regulator PHLPP2 and downregulating the positive AKT regulator mTORC2. The alteration of MAC can be caused by METTL14 mutation (hotspot R298P mutation) or reduced expression of METTL3.[99]

FTO has been observed significantly upregulated in human cancers, promoting occurrence, proliferation, and migration of cancer cells. These discoveries have conferred an identity as a therapeutic target on FTO. FB23 and FB23-2 are promising small-molecule FTO

inhibitors. FB23-2 negatively regulates proliferation and progression of human AML cell lines by executing inhibition on FTO.[100] In the emerging FTO-m^{6}A-MYC-CEBPA axis, FTO is the target of R-2HG (R-enantiomer of 2-hydroxyglutarate), which has anticancer activity in AML. R-2HG disabled FTO and upregulated m^{6}A level in MYC/CEBPA mRNA, reducing the stability and translation of MYC and CEBPA transcripts. High expression of FTO improved sensitivity to R-2HG, whereas overload of m^{6}A in MYC mRNA weakens the anticancer effect of R-2HG. Similarly, in R-2HG-sensitive leukemia cells, mutant IDH1^{R132H} also induced cell-cycle arrest, apoptosis, and proliferation inhibition.[101] Besides improving sensitivity to cancer suppressors, FTO also enhances resistance to chemoradiotherapy. In cervical squamous cell carcinoma (CSCC) tissues, FTO decreased the m^{6}A level in β-catenin mRNA transcripts and regulated expression of β-catenin, thereby promoting activity of excision repair cross-complementation group 1 (ERCC1).[102] However, FTO is suppressed in clear cell renal cell carcinoma (ccRCC) tissue, and the level of FTO is negatively correlated with tumor severity. This phenomenon is attributed to the FTO-PGC-1alpha axis. With demethylase activity, FTO removes m^{6}A residues in PGC-1alpha mRNA transcripts and increases expression of PGC-1alpha, inducing oxidative stress and impaired tumor growth.[103] In addition, clinical studies have demonstrated the relationship between tumor severity and the expression of FTO and ALKBH1. According to clinical statistics of GC patients, the expression level of ALKBH1 is negatively correlated with tumor sizes and TNM stages, while expression of FTO is associated with better overall survival.[104]

EMT, a crucial step for tumor metastasis, has been confirmed to involve an m^{6}A/YTHDF1-mediated mechanism. YTHDF1 recognizes upregulated m^{6}A in coding sequences of Snail (a key transcription factor of EMT), promoting translation of Snail mRNA.[105] Studies focusing on durable neoantigen-specific immunity also uncovered the critical role of YTHDF1. In classical dendritic cells of mice, YTHDF1 interferes with cross-presentation of tumor antigens and cross-priming of CD8$^+$ T cells. YTHDF1 binds the m^{6}A-modified transcripts, which encode lysosomal proteases, promoting the translation of lysosomal cathepsins. Conversely, knockdown of YTHDF1 induces suppression of cathepsins and dramatically enhances cross-presentation. Furthermore, experiments *in vivo* implicated that YTHDF1 is a potential therapeutic target for enhancing efficacy of PD-L1 checkpoint blockade.[106]

What may bring breakthroughs for targeted therapy is the discovery of the cell specificity of YTHDF2 function. YTHDF2 is not essential for normal hematopoietic stem cell (HSC) function, whereas in leukemic stem cells, it blocks the apoptosis pathway by regulating expression of tumor necrosis factor receptor superfamily 2 (TNFRSF2).[107] Suppression of YTHDF2 is promising for maintenance of HSC self-renewal capacity after transplantation. Knockdown of human YTHDF2 led to a significant increase of HSCs (an average increase of 14.3-fold and 13.6-fold in the *ex vivo* expansion of human umbilical cord blood [hUCB] HSCs, a 5.1-fold increase in colony-forming units [CFU], and more than an 8-fold increase in functional hUCB HSCs in the secondary serial of a limiting dilution transplantation assay), and hematopoietic malignancies were not detected in YTHDF2-knockout mice. Mechanically, YTHDF2 promotes decay of m^{6}A-modified mRNAs, which are critical for stem cell self-renewal.[108] Thus, YTHDF2 is identified as a unique therapeutic target.

Conclusion and Perspective

To summarize, overexpression or suppression of writers and erasers lead to aberrant levels of m^{6}A in the transcripts of oncogenes or tumor suppressor genes, regulating translation efficiency and degradation with full participation of readers (Table 1). These resulting proteins induce activation of signaling pathways (Wnt/β-catenin, PI3K/AKT) and then create cascade reactions. However, we know little about how the enzymes specifically recognize the genes and transcripts.

By detecting the expression levels of the enzymes in cancerous tissues and adjacent non-cancerous tissues, studies have shown the prevalent overexpression of METTL3, METTL14, FTO, and others. However, METTL3 was identified as a suppressor in certain cancers, which is in conflict with previous conclusions. Further studies have indicated that the m^{6}A level was changing with the development of cancer, which may partly explain the contradiction. The exact regulatory mechanism of m^{6}A-related protein expression is not fully clear. m^{6}A modification provides a new idea for cancer therapy. Further studies are needed for weakening drug resistance of cancer cells and improving the accuracy of targeted therapy.

AUTHOR CONTRIBUTIONS

L.L., Y.W., and J.W. wrote and drafted the manuscript and figures. H.F., Z.Q., and J.L., revised the manuscript. All authors read and approved the final manuscript.

CONFLICTS OF INTEREST

The authors declare no competing interests.

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