Dual effects of N⁶-methyladenosine on cancer progression and immunotherapy

Hui Li, Hao Wu, Qin Wang, Shipeng Ning, Shouping Xu and Da Pang

According to the latest global cancer statistics, cancer has become a major threat to human health, but cancer treatment has encountered many bottlenecks. As an emerging topic in epigenetics, N⁶-methyladenosine (m⁶A) is the most common internal modification on eukaryotic mRNA, which has attracted increasing attention in recent years. Accumulating studies have shown that aberrant m⁶A modifications have profound effects on the characteristics of tumors, which undoubtedly led to a significant breakthrough in cancer treatment. Although m⁶A function as an oncogene or tumor suppressor is not fully revealed, determining its precise function in the development and evolution of malignant tumors is crucial in improving clinical decisions involving targeted therapies. In this review, we briefly introduce the composition of the m⁶A methylation machinery and mainly summarize the biological mechanism of m⁶A in cancer cell death, angiogenesis, epithelial-mesenchymal transition (EMT), and therapeutic resistance. Subsequently, we present the exogenous regulatory factors of m⁶A and highlight the role of m⁶A on immune cells and cancer immunotherapy. The potential therapeutic strategies of m⁶A in human cancer are also discussed, considering research gaps and future applications.

Cancer is a major public health problem worldwide, which is expected to become the leading cause of death in the 21st century. According to the American Cancer Society, 1,806,590 new cancer cases and 606,520 cancer deaths are expected to occur in the United States by 2020. These appalling statistics are forcing researchers to develop advanced treatments against cancer; however, due to the limited current biomedical knowledge, there are still several unknown and urgent issues in cancer research.

N⁶-methyladenosine (m⁶A) occurs in the sixth nitrogen atom of the RNA adenylate and is the most abundant internal modification in eukaryotic mRNAs (Table S1). In 2012, Dominissini et al. compiled the first human and mouse RNA methylomes using the m⁶A-seq method and identify over 12,000 m⁶A sites that are characterized by a typical consensus in the transcripts of more than 7,000 human genes. In mammalian mRNA, approximately 0.1%–0.4% of adenines are exposed to m⁶A modification, with an average of 3–5 m⁶A sites per transcript. This methylation is mediated by methyltransferases (“writers”), demethylases (“erasers”), and binding proteins (“readers”) that are involved in almost all physiological and pathological processes. Several studies demonstrated that m⁶A can affect the characteristics of cancer cells. However, the performance of different phenotypes in the same cancer and the discrepant manifestations of the same phenotype in diverse cancers are not well known. Accordingly, the exploration of tumor characteristics that are based on m⁶A methylation is of great significance and may become an innovative therapeutic target in the clinic.

In this review, we introduce three kinds of m⁶A methylation enzymes and elaborate the dual effect of m⁶A methylation on tumor characteristics of different cancer types. Finally, we discuss its application prospects in cancer therapy, which may become the basis of a thorough research that will provide more options for ensuing clinical treatments.

REGULATORY FACTORS
The m⁶A methylation is dynamically regulated by writers, erasers, and readers. They install, remove, and recognize m⁶A, respectively.

Writers
The m⁶A methylation is catalyzed by a multi-component methyltransferase complex, including methyltransferase-like 3 (METTL3), METTL14, and Wilms’ tumor 1 (WT1)-associated protein (WTAP). METTL3 plays a central role in this complex, where it forms heterodimer complex with METTL14 in the ratio of 1:1. Although both METTL3 and METTL14 contain conserved catalytic domains, only METTL3 contains the methyl donor, S-adenosylmethionine (SAM), which is mainly localized in the nucleus in a form of speckles. WTAP interacts with the METTL3-METTL14 dimer, leading to the recruitment of the m⁶A methyltransferase complex to...
the mRNA target, which affects the methylation efficiency. RNA-binding motif protein 15 (RBM15) and its paralog RBM15B interact with METTL3 in a WTAP-dependent manner and recruit the WTAP-METTL3 complex to specific RNA sites. Moreover, methyltransferase-like 16 (METTL16) can regulate the SAM synthetase intron retention and install m^6^A onto the U6 small nuclear RNA. In addition, Vir like m^6^A methyltransferase associated protein (VIRMA, also known as KIAA1429), zinc finger CCCH domain-containing protein 13 (ZC3H13), and Cbl photo oncogene like 1 (CBLL-1, also known as HAKAI) are also involved in the formation of methyltransferase complex to catalyze the formation of m^6^A. Strikingly, methyltransferase-like 5 (METTL5) is an enzyme responsible for the 18S rRNA m^6^A modification, and zinc finger CCCH-type containing 4 (ZCCHC4) was confirmed to be a 28S rRNA m^6^A modifying enzyme. However, METTL5 is obliged to form a heterodimer complex with the known methyltransferase activator, tRNA methyltransferase subunit 11-2 (TRMT112), to reach a cellular metabolic stability. It is confirmed that NOP2/Sun RNA methyltransferase 2 (NSun2) silencing completely blocks PAR2-induced m^6^A methylation of pre-miR-125b, indicating that NSun2 may regulate m^6^A modification in an indirect way. However, it has not been reported that NSun2 has a direct effect on m^6^A.

### Erasers

Fat mass and obesity-associated protein (FTO) is the first eraser that was successfully identified. Subsequently, another eraser, alkB homolog 5 (ALKBHS), a member of the AlkB family along with FTO, was soon discovered. Compared with FTO, ALKBH5 seems to be an m^6^A-specific demethylase in mRNA, because FTO is more involved in the demethylation of N6,2'-o-dimethyladenosine (m^6^A_d). They show the demethylation process with different foundational mechanisms. FTO follows a traditional oxidative-demethylation pathway to catalyze conversion of m^6^A to N6-hydroxymethyladenosine (hm^6^A) and then convert the hm^6^A to N6-formyladenosine (f^6^A). It is confirmed that NOP2/Sun RNA methyltransferase 2 (NSun2) silencing completely blocks PAR2-induced m^6^A methylation of pre-miR-125b, indicating that NSun2 may regulate m^6^A modification in an indirect way. However, it has not been reported that NSun2 has a direct effect on m^6^A.

### m^6^A IN CANCER CELL DEATH

Cell death plays an important role in the development and maintenance of organisms. It is not only a general process of normal cell metabolism but also a necessary part of cancer cell progression. Several studies have demonstrated that cell death is affected by the aberrant level of m^6^A through different mechanisms. Here, we briefly summarize m^6^A function in cancer cell apoptosis (Figure 1; Table S2) and autophagy (Figure 2).

#### m^6^A and apoptosis

**m^6^A promotes oncogene expression.** First, METTL3 is the main regulatory factor in the m^6^A-mediated apoptosis inhibition. METTL3 level is high in different types of cancers, and it inhibits cancer apoptosis through enhancing the expression of oncogenes. In breast cancer (BC), METTL3 silencing could dramatically trigger the apoptotic capacity of BC cells through targeting B cell lymphoma-2 (BCL-2) or hepatitis B X-interacting protein (HBXIP). In lung adenocarcinoma, both epidermal growth factor receptor (EGFR) and tafazzin (TAZ) play the role of oncogene in the inhibition of apoptosis induced by METTL3.

**m^6^A inhibits apoptosis.** In acute myeloid leukemia (AML), the deficiency of METTL3 can induce apoptosis, which is caused by the increased translation of the v-myc myelocytomatosis viral oncogene homolog (c-MYC), BCL-2, and phosphatase and tensin homolog (PTEN) mRNA. In glioblastoma multiforme (GBM), METTL3 acts on the oncogene sex determining region Y box 2 (SOX2) by facilitating SOX2 mRNA stability in an m^6^A-dependent regulatory manner. Li et al. found that METTL3 knockdown correlates with apoptotic transcripts and the increased ratio of apoptotic GBM cells. Furthermore, YTHDC1 was shown to enhance METTL3-induced oncogenic effect by upregulating BCL-XX and NCOA? expression through promoting the degradation of serine- and arginine-rich splicing factors (SRFSs). In addition, METTL3 depletion suppressed the accumulation of GLI family zinc finger 1 (GLI1) and ATPase family AAA domain containing 2 (ATAD2), leading to apoptosis in prostate cancer and osteosarcoma (OS).

#### m^6^A and metabolic regulation

FTO is a well-known regulator of body fat distribution, growth, and diabetes. It is a key regulator of RNA metabolism. FTO promotes metabolic regulation by controlling a number of processes, including glucose homeostasis, insulin sensitivity, and adiposity. The expression of FTO is upregulated in adipose tissue, leading to the increased expression of genes involved in fat accumulation. Moreover, FTO promotes the metabolism of glucose and fatty acids, leading to the increased energy production. FTO also regulates the expression of genes involved in the metabolism of amino acids, leading to the increased synthesis of proteins.
the expression of the anti-apoptotic protein Bcl2 was decreased in lung cancer (LC), esophageal cancer (EC), and gastric cancer (GC) cells with METTL3 decay, while the expression of the pro-apoptotic proteins, BCL2-associated X (Bax), Caspase 3, Caspase 9, and PARP was increased, resulting in the activation of apoptosis.66–68

Next, the writers METTL14 and WTAP also participate in the apoptosis inhibition of AML and hepatocellular carcinoma (HCC) by upregulating oncogenes MYB, MYC, and the mammalian target of rapamycin (mTOR) pathway. In MM6 and NB4 AML cells, the apoptotic activity is enhanced following METTL14 depletion, which...
was associated with the regulation of MYB and MYC target mRNA in m6A-mediated manner.\(^6\) In addition, Bansal et al.\(^7\) also performed a preliminary study on the role of WTAP in AML and found that WTAP knockdown can promote the apoptosis of leukemia cells following etoposide treatment via the mTOR pathway.

For reader IGF2BP1, its expression is elevated in HCC and its depletion induces apoptosis of liver cancer cells. Mechanically, IGF2BP1 facilitates the translation of the oncogene c-MYC and MKI67 mRNAs, which are effective regulators of cell apoptosis.\(^7\)

m6A inhibits anti-oncogene expression. Similarly, METTL3, WTAP, and KIAA1429 can restrain the autophagic process of nasopharyngeal carcinoma (NPC), colorectal cancer (CRC), and HCC by downregulating the level of anti-oncogenes. Low level of encoding zinc finger protein 750 (ZNF750) maintained by METTL3 upregulation in NPC and the degradation of GATA Binding Protein 3 (GATA3) modulated by KIAA1429 overexpression in HCC ultimately suppresses apoptosis.\(^14,72\) Zhang et al.\(^23\) recently discovered a novel methylated gene, carbonic anhydrase IV (CA4), which is repressed in CRC. CA4 directly modulates WTAP and inhibits the Wnt signaling pathway by activating Wilms’ tumor 1 (WT1), leading to apoptosis.

The role of YTHDF2 and its downstream oncogenes in apoptosis was also studied by Zhong et al.\(^78\) They suggested that hypoxia can cause the down-expression of YTHDF2 through a mechanism involving YTHDF2 that can raise the degradation of EGFR mRNA and accelerate the apoptosis in HCC cells by targeting EGFR 3’-UTR.\(^78\)

m6A promotes apoptosis. In ocular melanoma, BC, AML, and LC, the high level of m6A on anti-oncogenes mediated by low expression of FTO or ALKBH5 activates cancer apoptosis by promoting the accumulation of these genes. The upregulation of FTO engenders a lower m6A level of the mRNA of the pro-apoptotic BCL2 interacting protein 3 (BNIP3) in BC, and Ankyrin repeat and SOCS box protein 2 (ASB2) and Retinoic Acid Receptor Alpha (RARA) in AML, which restrains cancer cell apoptosis.\(^16,79\) In ocular melanoma, low m6A on histidine triad nucleotide-binding protein 2 (HINT2) is mediated by the decrease in METTL3 or the increase in ALKBH5 that results in the downregulation of the expression of the anti-oncogene HINT2, leading to a decrease in apoptosis.\(^80\) In LC samples, Zhu et al.\(^81\) showed that ALKBH5 inhibits the apoptosis through suppressing TIMP metalloproteinase inhibitor 3 (TIMP3) mRNA stability via ALKBH5 demethylation.

Figure 2. Regulation of m6A in cancer autophagy

ATG5, autophagy related 5; ATG7, autophagy related 7; EOC, epithelial ovarian cancer; FOXO3, forkhead box C3; METTL3, methyltransferase-like 3; PIK3C3, phosphatidylinositol 3-kinase catalytic subunit type 3; ULK1, unc-51-like kinase 1; YTHDF1, YTH domain family protein 1.
In general, most of the investigations were focused on METTL3 and FTO, revealing the dual effects of m6A on cancer apoptosis.

**m6A and autophagy**

*m6A inhibits autophagy*

It is well known that light chain 3B (LC3B) is a common membrane marker of autophagy, which can be found in the cytoplasmic matrix and is related to the formation of phagocytic vesicles during early autophagy. Lin et al.\(^{17}\) showed that METTL3 knockdown increases the number of autophagosomes and significantly promotes LC3-II accumulation in HCC through relinquishing the stability of forkhead box O3 (FOXO3) mRNA in a YTHDF1-dependent manner. Jin et al.\(^{83}\) found that FTO silencing inhibits the expression of LC3B II but increases the expression of the autophagy substrate p62. Mechanistically, the authors confirmed that FTO acts on three m6A sites in the 3′-UTR of unc-51-like kinase 1 (ULK1) transcripts, which regulates its protein abundance through demethylation. They also showed that the significant inhibition in the rate of ULK1 mRNA decay is affected by FTO overexpression in a YTHDF2-dependent manner. Conversely, Wang et al.\(^{84}\) proved that FTO can promote autophagy by catalyzing m6A demethylation, but instead of ULK1, autophagy-related 5 (ATG5) and autophagy-related 7 (ATG7) are FTO direct target genes. Moreover, YTHDF2 interacts with ATG5 and ATG7 to decrease their expression by regulating their mRNA stability.\(^{84}\)

**m6A promotes autophagy**

ALKBH5 is another autophagy-related eraser, which is highly expressed in epithelial ovarian cancer (EOC). Although contrary to the results of previous experiments, ALKBH5 has been shown knockout to activate autophagy. Zhu et al.\(^{18}\) demonstrated that ATG5, ATG7, phosphatidylinositol 3-kinase catalytic subunit type 3 (PIK3C3), BECN1, and ULK1 are vital for ALKBH5-induced autophagy. They also confirmed that ALKBH5 regulates cell autophagy through the EGFR-PIK3CA-AKT-mTOR pathway. In addition, ALKBH5 can enhance the stability of BCL-2 mRNA and control autophagy flux of ovarian cancer via m6A RNA modification.\(^{18}\)

Briefly, from a limited number of reports, it seems that m6A does play a crucial role in cancer autophagy, but more research is required to convince this summary.

**m6A IN CANCER ANGIOGENESIS**

Angiogenesis is considered as a symbol of tumor invasiveness. An abundant vascular network provides sufficient oxygen, nutrients, and tumor growth factors for tumor cells. Meanwhile, blood vessels are also vital channels for tumor metastasis.\(^{85-87}\) There is evidence that lactate produced by high glycolysis in hypoxic regions promotes angiogenesis by regulating the AKT pathway in cancer cells and the NF-κB pathway in tumor endothelial cells (TECs).\(^{88-90}\) The effect of m6A on these signaling pathways has been confirmed in multiple cancer types.\(^{61,66-68,91-93}\) In addition, the expression of glycolysis-related gene GLUT1 in TECs is also regulated by m6A.\(^{94}\) Therefore, m6A may modulate cell metabolism under hypoxic conditions, which further leads an impact on cancer angiogenesis. In what follows, we sum up the relationship between m6A and angiogenesis in several cancers (Figure 3).
m<sup>6</sup>A inhibits angiogenesis

In HCC, the knockdown of the core writer METTL3 increases the translation of some angiogenic biomarkers, including FGF, PDGF-B, STAT3, and VEGF-A. The increased formation of tubes by the human umbilical vein endothelial cells (HUVECs) indicates that the absence of METTL3 can stimulate angiogenesis. However, METTL14 or ALKBH5 positively regulates some protein markers, such as VEGF and PDGFA, which are angiogenesis-associated indicators in BC cells. These results reveal that aberrant m<sup>6</sup>A may have a regulatory effect on angiogenesis in cancer; however, the specific mechanism of this event remains to be elucidated.

m<sup>6</sup>A promotes angiogenesis

In LC, the miR-143-3p/vasohibin-1 (VASH1) axis could be mediated by METTL3 in a m<sup>6</sup>A-dependent way by activating the expression of VEGFA and promoting the capacity of tube formation. Wang et al. also observed that METTL3 can promote tube formation in vitro and GC liver metastasis in vivo. Moreover, the increased microvessel density that was evaluated by CD31 staining in METTL3-overexpressing GC tissues could be rescued by HDGF knockdown. In this process, the reader IGF2BP3 recognizes METTL3-induced m<sup>6</sup>A modification and facilitates angiogenesis by stabilizing HDGF mRNA.

Remarkably, the level of FTO is low in intrahepatic cholangiocarcinoma (ICC) cases and cell lines. Patients with low FTO expression are more likely to have positive CD34, a marker that represents microvessel density. Afterward, Gene Ontology (GO) pathway analysis forecasted that FTO is involved in angiogenesis.

In brief, the current research on m<sup>6</sup>A involvement angiogenesis mainly focused on METTL3, and further studies need to be carried out in the near future.

m<sup>6</sup>A IN CANCER EMT

EMT refers to the biological process of epithelial cells transforming into stromal cells through complex procedures. Through EMT, cells acquire higher migration and invasion abilities that promote tumor metastatic dissemination. Currently, mounting evidence suggested that m<sup>6</sup>A plays an important role in EMT (Figure 4).

m<sup>6</sup>A inhibits EMT

METTL14 is known to act as an independent CRC prognostic marker. Specifically, knockdown of METTL14 constrains the recruitment and methylation of downstream SRY-related high-mobility-group box 4 (SOX4), which can be recognized by YTHDF2, thus promoting EMT through a gain in N-cadherin and vimentin expressions and a loss of E-cadherin expression. It is observed that METTL3 silencing elevated the expression of vimentin, β-catenin, and N-cadherin as well as reduced E-cadherin accumulation in renal cell carcinoma (RCC) cells, while overexpression of METTL3 resulted in the opposite change of such protein levels. Additionally, the PI3K-AKT-mTOR pathway may be involved in the potential mechanism.

YTHDF2 is lower expressed in non-small cell lung cancer (NSCLC) compared with matched normal tissues. Low expression of YTHDF2 is associated with high pathological grade and poor overall survival. Moreover, the expression of vimentin is obviously decreased and the expression of E-cadherin is increased when YTHDF2 is upregulated. Correspondingly, expressions of these proteins are reversed when YTHDF2 is downregulated, thus manifesting that YTHDF2 has an inhibitory influence on EMT in NSCLC cells.

m<sup>6</sup>A promotes EMT

In HCC, METTL3 mutation leads to a low level of m<sup>6</sup>A in the coding sequence (CDS) and 3'-UTRs of Snail, a key EMT translator. Moreover, YTHDF1 binds to m<sup>6</sup>A methylated Snail and stimulates its translation, triggering lung metastasis. Similarly, METTL3 knockdown decreases the protein levels of Snail, MMP2, MMP9, and FN1 but increases E-cadherin level, which reveals the impact of METTL3 on EMT of HCC. It is well known that transforming growth factor-β (TGF-β) is an indispensable EMT factor in cancer. Li et al. showed that m<sup>6</sup>A negatively regulates TGFβ1 mRNA stability in HeLa cells, clarifying the pivotal function of METTL3 in TGFβ1-induced EMT, which could be promoted by enhancing the protein level of Snail. Recently, Yu et al. validated that METTL3 deficiency downregulates m<sup>6</sup>A modified Snail, thereby inhibiting EMT of NPC cells by regulating the expression of N-cadherin and E-cadherin proteins. It was reported that enhancer of zeste homolog 2 (EZH2) is relevant to tumor progression in many cancers, where EZH2 expression is elevated via METTL3-installed m<sup>6</sup>A modification, thus driving EMT in LC.

Concurrently, the reduction in METTL3 level catalyzes E-cadherin expression and restrains N-cadherin and vimentin expression through downregulating m<sup>6</sup>A content on JUNB mRNA in LC cells. As expected, JUNB overexpression reverses TGF-β-induced EMT. Additionally, METTL3 could also promote EMT through the mir-143-3p/VASH1 axis in LC. The effect of METTL3 on EMT has also been observed in GC, where METTL3 expression positively correlates with clinical stage and directly interacts with the zinc finger MYM-type containing 1 (ZMYM1). Mechanistically, ZMYM1 targeted and suppressed the promoter of E-cadherin by forming a combination with CtBP/LSD1/CoREST, ultimately provoking EMT and metastasis. Wu et al. uncovered that METTL3 promotes the interaction between LncRNA RP11 and hnRNPA2B1, resulting in metastasis by the upregulation of the EMT activator Zeb1 in CRC cells.

According to the analyses of NSCLC tissues and The Cancer Genome Atlas (TCGA) database, ALKBH5 expression is low in both mRNA and protein level. ALKBH5 exerts an EMT-inhibiting role through mediating m<sup>6</sup>A abundance on YAP mRNA. EMT capacity could be significantly disrupted by YTHDF2-mediated YAP deficiency. In pancreatic cancer, ALKBH5 mediates the demethylation of KCNK15-AS1 and promotes its expression, which accelerates EMT.

Most recently, the overexpression of circ_KIAA1429, a circular RNA (circRNA) that is originated from KIAA1429, leads to the inhibition of E-cadherin and enhancement of N-cadherin and vimentin in HCC. Experimental results showed that circ_KIAA1429 facilitates EMT and...
In oral squamous cell carcinoma (OSCC), immunohistochemistry (IHC) staining showed that HNRNPC expression is upregulated and positively correlates with lymph node metastasis. Furthermore, HNRNPC overexpression enhances EMT through preventing the expression of E-cadherin and promoting the expression of N-cadherin, MMP9, and vimentin. Overall, m^6^A imposes positive and negative influences on EMT in certain cancer types, and the writers are major regulators of EMT.

Inevitable therapeutic resistance has been a major obstacle in overcoming cancer owing to epigenetic alterations. Thus, we introduce the effect of abnormal m^6^A on chemoresistance, radioresistance, endocrine resistance, resistance to targeted therapy, and even resistance to inhibitors in cancer. (Figure 5)
m6A inhibits therapeutic resistance

Sorafenib resistance could occur in METTL3-deficient HCC cells by reducing the stability of FOXO3 mRNA, which is directed by YTHDF1.[17]

In cervical squamous cell carcinoma (CSCC), FTO stirs up chemoradiotherapy resistance by promoting the accumulation of β-catenin due to the activation of the nucleotide excision repair regulator excision repair cross-complementation group 1 (ERCC1). In leukemia, the low level of m6A that is induced by FTO-mediated demethylation promotes the expression of proliferation/survival transcripts, leading to tyrosine kinase inhibitor (TKI) resistance.[112]

DDX3, a RNA helicase, positively modulates Forkhead box protein M1 (FOXM1) and NANOG through ALKBH5-induced low enrichment of m6A on FOXM1 and NANOG nascent transcript in cisplatin (DDP)-resistant OSCC cells.[113] Anomalous expression of METTL3 and ALKBH5 in OS stem cells that is induced by Adriamycin (ADM) resistance indicated that m6A is obviously related to chemoresistance.[114]

m6A promotes therapeutic resistance

In NSCLC, METTL3 upregulation results in DDP resistance via the Metastasis-Associated Lung Adenocarcinoma Transcript 1 (MALAT1)-miR1914-3p-YAP axis.[24] In NPC, m6A methylation that is activated by METTL3 and recognized by IGF2BP2 facilitates the stability of the Tripartite motif containing protein 11 (TRIM11), activates the Dvl-associated protein (Daple)/β-catenin/ABCC9 signaling pathway, and contributes to DDP resistance.[115] Another study showed that the writer METTL3 and the reader IGF2BP1 strengthen DDP resistance by increasing the methylation and stability of m6A on the transcription factor-activating enhancer-binding protein 2C (TFAP2C) mRNA in seminoma.[116] In GC, METTL3 methylation of ARHGAP5 mRNA, which is recruited by lncRNA ARHGAP5-AS1, leads to the translation of ARHGAP5, thereby promoting the resistance to chemotherapeutic drugs, including DDP, doxorubicin (ADM) hydrochloride, and 5-fluorouracil (5-Fu).[117] CBX8, which is highly expressed in oxaliplatin (L-OHP) or irinotecan (CPT-11)-resistant CRC tissues, functions as an oncogene and is stabilized by METTL3 relying on IGF2BP1.[118] Noticeably, METTL3 loss in pancreatic cancer cells enhances radiosensitivity and sensitivity to gemcitabine (GEM), 5-Fu, and DDP.[119] In GBM, elevated levels of METTL3 bind to SOX2 transcripts and maintain their stability, thus promoting radioresistance through increased DNA repair.[62] In addition, METTL14 initiates ADM resistance in OS stem cells.[114]

Apart from methyltransferases, ALKBH5 overexpression sensitizes pancreatic ductal adenocarcinoma (PDAC) cells to chemotherapy...
through upregulating WIF-1 expression, suggesting that m6A modification is closely associated with pancreatic cancer GEM resistance. Moreover, both erasers FTO and ALKBH5 are downregulated in resistant EOC cells, and it was shown that m6A concentration on FZD10 mRNA causes resistance to poly ADP-ribose polymerase inhibitor (PARPi) by stimulating the Wnt/β-catenin signaling pathway.

It is worth mentioning that YTHDF1 silencing can promote the CRC sensitization to 5-Fu and L-OHP. However, YTHDC2 knockdown induces a reduction in radioresistance by hampering the insulin-like growth factor 1 receptor (IGF1R)-AKT/S6 signaling pathway in NPC.

In BC, HNRNPA2B1 overexpression increases the activity of MCF-7 cells that were treated with 4-hydroxytamoxifen (4-OHT) or fulvestrant, revealing a role of HNRNPA2/B1 in BC endocrine resistance.

In a nutshell, therapeutic resistance caused by m6A occurs in almost all clinical treatments, especially in chemoresistance. By contrast, there exists the phenomenon that m6A enhances drug sensitivity.

m6A IN CANCER TREATMENT
Inhibitors can change the malignant behavior of tumors by targeting m6A regulatory factors that can kill tumor cells by regulating immunotherapeutic components. This therapeutic approach points out a novel direction for the treatment of human cancers.

Exogenous regulatory factors of m6A
Rhein, the first identified FTO inhibitor, together with FTO competitively recognizes the m6A substrate to raise the methylation of m6A on mRNA. By studying the structure of FTO inhibitors, scholars found that FG-2216/IOX3 and FG-4592/SelleckBio can accurately inhibit FTO. Meclofenamic acid (MA) is a highly specific FTO inhibitor that acts as a nonsteroidal anti-inflammatory drug that effectively competes with FTO binding sites. In particular, the MA ethyl ester form, MA2, also suppresses glioblastoma stem cell (GSC) tumorigenesis through the inhibition of FTO. Fluorescein and its derivatives were shown to selectively and simultaneously inhibit and label FTO. Another FTO inhibitor, MO-1-500, prominently attenuates survival and colony-forming capacity of BC cells under glutamine-free conditions. After a strict screening, He et al. demonstrated that N-CDCPB has a strong FTO inhibitory activity and provides an absolutely new binding site for FTO inhibitors. Next, CHTB was identified as a novel FTO inhibitor by the same team. Interestingly, the FTO inhibitor radicicol and N-CDCPB occupy the same FTO binding position.

Huang et al. developed new FTO inhibitors, FB23 and its derivative FB23-2, and proved that FB23-2 inhibits the proliferation and accelerates the differentiation and apoptosis of AML cells. A US Food and Drug Administration (FDA)-approved drug, entacapone, was identified as an FTO inhibitor through its direct binding to FTO, which dominantly deprived the activity of the demethylase. More recently, two compounds, CS1 (bisantrene) and CS2 (brequinar), were screened as potent FTO inhibitors. These two small-molecule inhibitors occupy the catalytic pocket of FTO, which blocks the binding of target mRNAs, thus elevating the amount of m6A-containing RNA. Additionally, Zheng et al. and Toh et al. successively detected a series of unnamed compounds that can inhibit FTO. Notably, citrate can bind to both FTO and ALKBH5 and in different modes to serve the function of FTO inhibitor. Furthermore, ALK-04 was identified as a specific ALKBH5 inhibitor. We summarize the half maximal inhibitory concentration (IC50), mechanism, and molecular structure of various FTO inhibitors in Table S3.

S-adenosylhomocysteine (SAH) was proved to be an inhibitor of METTL3-METTL14 heterodimer complex through the measurement of methyltransferase activity. On the contrary, Selberg et al. identified small-molecule ligands that bind to METTL3-METTL14-WTAP and showed strong stimulative effects on METTL3-14-WTAP activity; hence, these compounds were identified as activators of the METTL3-METTL14-WTAP complex.

m6A and immune cells
The immune system consists of innate immunity and adaptive immunity. m6A is involved in the regulation of both innate and acquired immune cells, such as macrophages, dendritic cells (DCs), T cells, and B cells.

METTL3 enhances the stability and expression of STAT1 mRNA via methylation modification, thus driving M1 macrophage polarization. FTO knockout can inhibit the polarization of M1 and M2 macrophages through accelerating the decay of STAT1 and peroxisome proliferation-activated receptor-γ (PPAR-γ). Moreover, RNA-binding motif 4 (RBM4) interacts with YTHDF2 and could inhibit M1 macrophage polarization by the degradation of m6A modified STAT1 mRNA.

The loss of METTL3 suppresses the maturation phenotype of DC as well as decreased T cell response in vivo and in vitro. Liu et al. indicated that Inc-Dpf3 restrains DC migration, which is mediated by CCR7. At the same time, CCR7 stimulation upregulates Inc-Dpf3 expression by mediating m6A demethylation.

Li et al. demonstrated that METTL3 deficiency elevates the expression of SOCS family so that it inhibits interleukin-7 (IL-7)-mediated STAT5 activation and T cell homeostatic proliferation and differentiation. It is shown that HIV-1 infection prominently increases the level of m6A in CD4 primary T cells, and the binding of HIV-1 gp120 with CD4 receptor is necessary for the upregulation of m6A. In the exploration of the interaction between YTHDF3 and HIV replication, Jurczyszak et al. showed that YTHDF3 knockout increases HIV susceptibility in human CD4+ T cells. In regulatory T cells (Tregs), the depletion of METTL3 leads to the increase of SOCS mRNA level, consequently inhibiting the IL-2-STAT5 signaling pathway, which is essential for the function and stability of Tregs.
METTL14 silencing can damage IL-7-induced pro-B cell proliferation, while RNA m6A promotes pro-B cell proliferation via reader YTHDF2. In addition, METTL14 silencing inhibits the transformation of large-pre-B-to-small-pre-B and seriously hinders the development of early B cells.

m6A and cancer immunotherapy

At present, immunotherapy has become an advanced strategy to conquer cancer, and m6A is of profound importance to immune-checkpoint therapy (Figure 6). Kim et al. demonstrated that the hepatitis B virus (HBV) severely decreases PTEN translation via an METTL3-mediated m6A enrichment. Moreover, previous studies have shown that PTEN plays a role in antiviral innate immunity and HCC development. Therefore, these results provide a new insight into the mechanism of HBV-directed immune evasion.

Yang et al. found that demethylase FTO promotes the expression of melanoma-intrinsic programmed cell death-1 (PD-1), C-X-C chemokine receptor type 4 (CXCR4), and sex determining region Y box 10 (SOX10) via m6A modification. Then they verified that FTO decreases the response of interferon gamma (IFNγ) in vitro and anti-PD-1 treatment in vivo, depending on the immune system and the combined application of FTO inhibition and anti-PD-1 blockade, which may lead to the lessering of immunotherapy resistance in melanoma. It was demonstrated that the immune checkpoint gene leukocyte immunoglobulin-like receptor subfamily B4 (LILRB4) is targeted by FTO in AML cells. Immunotherapeutic experiments in mice showed that the inhibition of FTO expression in AML cells can significantly increase the killing effect of T cells on AML cells and synergistically inhibit the progress of AML in mice following treatment with hypomethylating agents (HMA). Consequently, FTO inhibition can render AML cells sensitive to T cell toxicity and overcome the HMA-induced immune evasion that was used through inhibiting the expression of LILRB4.

In another study, ALKBH5 loss or inhibition reduced the translation of MCT4 and inhibited lactate in tumor interstitial fluids, which enhanced the response to anti-PD-1 therapy by deactivating polymorphonuclear myeloid derived suppressor cells (PMN-MDSCs) and Tregs in the tumor microenvironment (TME). The upregulation of the expression of the PD-ligand 1 (PD-L1) expression by an FTO-catalyzed mRNA demethylation forecast that m6A may make a difference in CRC immunotherapy. Cancer patients are divided into several clusters with diverse m6A regulators, suggesting that m6A methylation has great relevance to PD-L1 expression in head and neck squamous cell carcinoma, BC, and response to anti-PD-1/1 immunotherapy of GC.

Remarkably, YTHDF1 knockout results in a significant deceleration of melanoma growth. Mechanistically, YTHDF1 silencing downregulates the translation of lysosomal cathepsins in DCs, thereby facilitating the cross-presentation of tumor antigens and cross-priming of CD8+ T cells. METTL3 facilitates CD40, CD80, and TLR4 Signal adaptor Tirap expression in DCs, which stimulates T cell activation and promotes cytokine production in a TLR4/NF-κB signal-induced mechanism, indicating that METTL3 is helpful in cancer immunotherapy. Collectively, m6A has the potential to become the main immunotherapeutic target.

CONCLUSION

The discovery of m6A methylation enriched the research field of epigenetics. It has been demonstrated that m6A methylation is involved in tumor characteristics, including cell death, angiogenesis, and EMT. However, whether m6A exerts positive or negative impact on cancer progression is still controversial. Strikingly, the influence of
regulatory factors may vary depending on the target genes or pathways being tested, so we list the role of regulatory factors on different characteristics in diverse cancers in Table S4.

On the other hand, the role of m6A in cancer cells' therapeutic resistance lays the foundation for its use in combination therapy. The development of m6A-related small-molecule inhibitors provides support in the realization of cancer-targeted therapies. In addition, cancer immunotherapy, as a frontier method, is also regulated by m6A methylation, which opens up a new approach in improving immunotherapeutic effects.

It is encouraging to note that m6A contents increase in circulating tumor cells (CTCs) of LC patients after the analysis of RNA methylation by LC-ESI-MS/MS. Therefore, m6A quantitative assessment in CTCs may be a supplementary evidence for the qualitative and early diagnosis of cancer.160

We believe that not all m6A regulatory factors have been identified. Compared with writers and erasers, only a small number of readers have been studied in the progression of different cancer types. Consequently, the identification of additional m6A regulatory factors and associated mechanisms is required to hew out new possibilities for cancer diagnosis and treatment.

SUPPLEMENTAL INFORMATION
Supplemental Information can be found online at https://doi.org/10.1016/j.omtn.2021.02.001.

ACKNOWLEDGMENTS
The authors would like to express their gratitude to EditSprings (https://www.editsprings.com/) for the expert linguistic services provided. This work was supported by funding from the Project Nn10 of the Harbin Medical University Cancer Hospital (no. 102017-02); the National Natural Science Foundation of China (nos. 81872149, 81972076, and 82072904); the Outstanding Youth Project of Heilongjiang Provincial Natural Science Foundation (no. YQ2019H027); the Haiyan Fund Project of Harbin Medical University Cancer Hospital (nos. JJQN 2018-06 and JJQN 2018-10); and the Heilongjiang Health and Family Planning Commission Foundation (no. 2019-056).

AUTHOR CONTRIBUTIONS
D.P. and S.X. conceived and designed the review article. H.L., H.W., and Q.W. drafted the manuscript, figures, and tables. H.L. collected the references. D.P. and S.X. provided guidance and reviewed the manuscript. All authors approved the final manuscript.

DECLARATION OF INTERESTS
The authors declare no competing interests.

REFERENCES
1. Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R.L., Torre, L.A., and Jemal, A. (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J. Clin. 68, 394–424.

2. Al-Obthaman, S., Haoudi, A., Alhomboud, S., Alkhienzian, A., Khoja, T., and Al-Zahrani, A. (2015). Tackling cancer control in the Gulf Cooperation Council Countries. Lancet Oncol. 16, e246-e257.

3. Siegel, R.L., Miller, K.D., and Jemal, A. (2020). Cancer statistics, 2020. CA Cancer J. Clin. 70, 7–30.

4. Roundtree, I.A., Evans, M.E., Pan, T., and He, C. (2017). Dynamic RNA Modifications in Gene Expression Regulation. Cell 169, 1187–1200.

5. Dominissini, D., Mohituch-Mohkovic, S., Schwartz, S., Salmon-Divon, M., Ungar, L., Olsenberg, S., Cesarkas, K., Jacob-Hirsch, J., Amariglio, N., Kupiec, M., et al. (2012). Topology of the human and mouse m6A RNA methylomes revealed by m6A-seq. Nature 485, 201–206.

6. Desroisiers, R., Friderici, K., and Rottman, F. (1974). Identification of methylated nucleosides in messenger RNA from Novikoff hepatoma cells. Proc. Natl. Acad. Sci. USA 71, 3971–3975.

7. Zhao, B.S., and He, C. (2015). Fate by RNA methylation: m6a steers stem cell pluripotency. Genome Biol. 16, 43.

8. Shi, H., Zhang, X., Weng, Y.L., Lu, Z., Liu, Y., Lu, Z., Li, J., Hao, P., Zhang, Y., Zhang, F., et al. (2018). m6A facilitates hippocampus-dependent learning and memory through YTHDF1. Nature 563, 249–253.

9. Haussmann, I.U., Bodi, Z., Sanchez-Moran, E., Mongan, N.P., Archer, N., Fray, R.G., and Soller, M. (2016). m6A potentiates Sxl alternative pre-mRNA splicing for robust Drosophila sex determination. Nature 540, 301–304.

10. Fustin, J.M., Kojima, R., Itoh, K., Chang, H.Y., Ye, S., Zhuang, B., Oji, A., Gibo, S., Narasimamurthy, R., Virshup, D., et al. (2018). Two CK16 transcripts regulated by m6A methylation code for two antigenic kinases in the control of the circadian clock. Proc. Natl. Acad. Sci. USA 115, 5980–5985.

11. Berulava, T., Buchholz, E., Elerdashvili, V., Pena, T., Islam, M.R., Lbik, D., Mohamed, B.A., Renner, A., von Lewinski, D., Sacherer, M., et al. (2020). Changes in m6A RNA methylation contribute to heart failure progression by modulating translation. Eur. J. Heart Fail. 22, 54–66.

12. Weng, Y.L., Wang, X., An, R., Cassin, J., Vissers, C., Liu, Y., Liu, Y., Xu, T., Wang, X., Wong, S.Z.H., et al. (2018). Epitranscriptomic m6A Regulation of Axon Regeneration in the Adult Mammalian Nervous System. Neuron 97, 313–325.e6.

13. Wang, H., Xu, B., and Shi, J. (2020). N6-methyladenosine METTL3 promotes the breast cancer progression via targeting βd-2. Gene 722, 144076.

14. Zhang, P., He, Q., Lei, Y., Li, Y., Wen, X., Hong, M., Zhang, J., Ren, X., Wang, Y., Yang, X., et al. (2018). m6A-mediated ZNF570 repression facilitates nasopharyngeal carcinoma progression. Cell Death Dis. 9, 1169.

15. Liu, J., Ren, D., Du, Z., Wang, H., Zhang, H., and Jin, Y. (2018). m6A demethylase FTO facilitates tumor progression in lung squamous cell carcinoma by regulating MZF1 expression. Biochem. Biophys. Res. Commun. 502, 456–464.

16. Niu, Y., Lin, Z., Wan, A., Chen, H., Liang, H., Sun, L., Wang, Y., Li, X., Xiong, X.F., Wei, B., et al. (2019). RNA N6-methyladenosine demethylase FTO promotes breast tumor progression through inhibiting BNIP3. Mol. Cancer 18, 46.

17. Lin, Z., Niu, Y., Wan, A., Chen, D., Liang, H., Chen, X., Sun, L., Zhan, S., Chen, L., Cheng, C., et al. (2020). RNA m6A methylation regulates srsf9 resistance in liver cancer through FOXO3-mediated autophagy. EMBO J. 39, e103181.

18. Zhu, H., Gan, X., Jiang, X., Xiao, S., Wu, H., and Hu, J. (2019). ALKBH5 inhibited autophagy of epithelial ovarian cancer through miR-7 and BCL-2. J. Exp. Clin. Cancer Res. 38, 163.

19. Panneerduos, S., Fedunuri, V.K., Yadav, P., Timilmula, S., Rajamaniickam, S., Viswanadhapalli, S., Abdel fattah, N., Onyegaucha, B.C., Cui, X., Lai, Z., et al. (2018). Cross-talk among writers, readers, and erasers of m6A regulates cancer growth and progression. Sci. Adv. 4, eaar8263.

20. Wang, H., Deng, Q., Lv, Z., Ling, Y., Hou, X., Chen, Z., Dinglin, X., Ma, S., Li, D., Wu, Y., et al. (2019). N6-methyladenosine induced miR-143-3p promotes the brain metastasis of lung cancer via regulation of VASH1. Mol. Cancer 18, 70.

21. Chen, X., Xu, M., Xu, X., Zeng, K., Liu, X., Pan, B., Li, C., Sun, L., Qin, J., Xu, T., et al. (2020). METTL4-mediated N6-methyladenosine modification of SOX4 mRNA inhibits tumor metastasis in colorectal cancer. Mol. Cancer 19, 106.
22. Lin, X., Chai, G., Wu, Y., Li, J., Chen, F., Liu, J., Luo, G., Tauler, J., Du, J., Lin, S., et al. (2019). RNA m^6A methylation regulates the epithelial mesenchymal transition of cancer cells and translation of Snail. Nat. Commun. 10, 2065.

23. Zhou, S., Bai, Z.L., Xia, D., Zhao, Z.J., Zhao, R., Wang, Y.Y., and Zhe, H. (2018). FTO regulates the chemo-radiotherapy resistance of cervical squamous cell carcinoma (CSCC) by targeting β-catenin through mRNA demethylation. Mol. Carcinog. 57, 590–597.

24. Jin, D., Guo, J., Wu, Y., Du, J., Yang, L., Wang, X., Di, W., Hu, B., An, J., Kong, L., et al. (2019). m^6A mRNA methylation initiated by METTL3 directly promotes YAP translation and increases YAP activity by regulating the MALAT1-miR-1914-3p-YAP axis to induce NSCLC drug resistance and metastasis. J. Hematol. Oncol. 12, 135.

25. Wang, X., Feng, J., Yue, Y., Guan, Z., Zhang, D., Liu, Z., Gong, Z., Wang, Q., Huang, J., Tang, C., et al. (2016). Structural basis of N6-adenosine methylation by the METTL3-METTL14 complex. Nature 534, 575–578.

26. Hong, K. (2018). Emerging function of N6-methyladenosine in cancer. Oncol. Lett. 16, 5519–5524.

27. Liu, J., Yue, Y., Han, D., Wang, X., Fu, Y., Zhang, L., Jia, G., Yu, M., Lu, Z., Deng, X., et al. (2014). A METTL3-METTL14 complex mediates mammalian nuclear RNA N6-adenosine methylation. Nat. Chem. Biol. 10, 93–95.

28. Patil, D.P., Chen, C.K., Pickering, B.F., Chow, A., Jackson, C., Guttman, M., and Jaffrey, S.R. (2016). m(6)A RNA methylation promotes XIST-mediated transcriptional repression. Nature 537, 369–373.

29. Pendleton, K.E., Chen, B., Liu, K., Hunter, O.V., Xie, Y., Tu, B.P., and Conrad, N.K. (2017). The U6 snRNA m^6A Methytransferase METTL16 Regulates SAM Synthetase Intron Retention. Cell 169, 824–835.e14.

30. Dゞxtader, K.A., Wang, P., Scarborough, A.M., Seo, D., Conrad, N.K., and Nam, Y. (2018). Structural Basis for Regulation of METTL16, an S-Adenosylmethionine Associated Protein, in Alternative Pre-mRNA Splicing. J. Biol. Chem. 293, 10381–10386.

31. Yue, Y., Liu, J., Cui, X., Cao, J., Luo, G., Zhang, Z., Cheng, T., Gao, M., Shu, X., Ma, H., et al. (2018). VIRMA mediates preferential m^6A modification in 3'UTR and near stop codon and associates with alternative polyadenylation. Cell Discov. 4, 10.

32. van Tran, N., Ernst, F.G.M., Havrely, B.R., Zorbas, C., Ulyncy, N., Hackert, P., Bohnsack, K.E., Bohnsack, M.T., Jaffrey, S.R., Graille, M., and Lafontaine, D.L.J. (2019). The human 18S rRNA m^6A methyltransferase METTL5 is stabilized by TRMT112 and near stop codon and associates with alternative polyadenylation. J. Biol. Chem. 293, 26627–26637.

33. Iσa, G., Fu, Y., Zhao, X., Dai, Q., Zheng, G., Yang, Y., Yi, C., Lindahl, T., Pan, T., Yang, Y.G., and He, C. (2011). N6-methyladenosine in nuclear RNA is a major substrate of the obesity-associated FTO. Nat. Chem. Biol. 7, 885–887.

34. Zheng, G., Daih, J.A., Niu, Y., Fedorczak, P., Huang, C.M., Li, C.J., Vaghi, C.B., Shi, Y., Wang, W.L., Song, S.H., et al. (2013). ALKBH5 is a mammalian RNA demethylase that impacts RNA metabolism and mouse fertility. Mol. Cell 49, 18–29.

35. Mauer, J., Luo, X., Blanqoie, A., Iσa, G., Grozhik, A.V., Patil, D.P., Linder, B., Pickering, B.F., Vasseur, J.J., Chen, Q., et al. (2017). Reversible methylation of m^6A in the 5' cap controls mRNA stability. Nature 541, 371–375.

36. Toh, J.D.W., Crossley, S.W.M., Bruemmeler, K.J., Ge, E.J., He, D., Iovan, D.A., and Chang, C.J. (2020). Distinct RNA N-demethylation pathways catalyzed by nonheme iron ALKBH5 and FTO enzymes enable regulation of formaldehyde release rates. Proc. Natl. Acad. Sci. USA 117, 25284–25292.

37. Ueda, Y., Ooshio, I., Fumasaye, Y., Kitae, K., Kawaguchi, M., I liningushi, K., Hase, H., Harada, K., Hirata, K., and Tsujikawa, K. (2017). ALKB homolog 3-mediated RNA demethylation promotes protein synthesis in cancer cells. Sci. Rep. 7, 42271.

38. Wang, X., Zhao, B.S., Roundtree, I.A., Lu, Z., Han, D., Ma, H., Weng, X., Chen, K., Shi, H., and He, C. (2015). N(6)-methyladenosine Modulates Messenger RNA Translation Efficiency. Cell 161, 1388–1399.

39. Wang, X., Lu, Z., Gomez, A., Hon, G.C., Yue, Y., Han, D., Fu, Y., Parisien, M., Dai, Q., Jia, G., et al. (2014). N6-methyladenosine-dependent regulation of messenger RNA stability. Nature 505, 117–120.

40. Shi, H., Wang, X., Lu, Z., Zhao, B.S., Ma, H., Hsu, P.J., Liu, C., and He, C. (2017). YTHDF3 facilitates translation and decay of N^6-methyladenosine-modified RNA. Cell Res. 27, 315–328.

41. Lence, T., Akhtar, J., Bayer, M., Schmid, K., Spindler, L., Ho, C.H., Kreim, N., Andrade-Navarro, M.A., Poecck, B., Helm, M., and Rigney, J.Y. (2016). m^6A modulates neuronal functions and sex determination in Drosophila. Nature 540, 242–247.

42. Hsu, P.J., Zhu, Y., Ma, H., Gou, Y., Shi, X., Liu, Y., Qi, M., Lu, Z., Shi, H., Wang, J., et al. (2017). Yhd2c is an N^6-methyladenosine binding protein that regulates mammalian spermatogenesis. Cell Res. 27, 1115–1127.

43. Alarcón, C.R., Goodarzi, H., Lee, H., Liu, X., Tavareso, S., and Tavareso, S.F. (2015). HNRNPA2B1 Is a Mediator of a m(6)A-Dependent Nuclear RNA Processing Events. Cell 162, 1299–1308.

44. Liu, N., Dai, Q., Zheng, G., He, C., Parisien, M., and Pan, T. (2015). N^6-methyladenosine-dependent RNA structural switches regulate RNA-protein interactions. Nature 518, 560–564.

45. Liu, N., Zhou, K.L., Parisien, M., Dai, Q., Diatchenko, L., and Pan, T. (2017). N^6-methyladenosine alters RNA structure to regulate binding of a low-complexity protein. Nucleic Acids Res. 45, 6051–6063.

46. Huang, H., Weng, H., Sun, W., Qin, X., Shi, H., Wu, H., Zhao, B.S., Mesquita, A., Liu, C., Yuan, C.L., et al. (2018). Recognition of RNA N^6-methyladenosine by IGF2BP proteins enhances mRNA stability and translation. Nat. Cell Biol. 20, 285–295.

47. Xu, C., Liu, K., Ahmed, H., Loppnau, P., Schapira, M., and Min, J. (2015). Structural Basis for the Discriminative Recognition of N6-Methyladenosine RNA by the Human YTHDC1 YTH Domain. J. Am. Chem. Soc. 137, 1011.e4–1011.e7.

48. Edupuganti, R.R., Geiger, S., Lindeboom, R.G.H., Shi, H., Hsu, P.J., Liu, Z., Wang, S.Y., Balissen, M.P.A., Jansen, P.W.T.C., Rossa, M., et al. (2017). N^6-methyladenosine (m^6A) recruits and repels proteins to regulate mRNA homeostasis. Nat. Struct. Mol. Biol. 24, 870–878.

49. Arguello, A.E., DeLoberto, A.N., and Kleiner, R.E. (2017). RNA Chemical Proteomics Reveals the N^6-Methyladenosine (m^6A)-Regulated Protein-RNA Interactome. J. Am. Chem. Soc. 139, 17249–17252.

50. Zhang, J., Bai, R., Li, M., Ye, H., Wu, C., Wang, C., Li, S., Tan, L., Mai, D., Li, G., et al. (2019). Excessive miR-25-3p maturation via N^6-methyladenosine stimulated by cigarette smoke promotes pancreatic cancer progression. Nat. Commun. 10, 1858.

51. Wu, R., Li, A., Sun, B., Sun, J.G., Zhang, J., Zhang, T., Chen, Y., Xiao, Y., Gao, Y., Zhang, Q., et al. (2019). A novel m^6A reader Prrrc2a controls oligodendroglioc differentiation and myelination. Cell Res. 29, 23–41.

52. Dai, X., Wang, X., Cao, C., Gao, Y., Zhang, S., Yang, Z., Liu, Y., Zhang, X., Zhang, W., and Ye, L. (2018). HBXIP-elevated methytransferase METTL3 promotes the
progression of breast cancer via inhibiting tumor suppressor let-7g. Cancer Lett. 415, 11–19.

60. Lin, S., Choe, J., Du, P., Triboulet, R., and Gregory, R.I. (2016). The m6A methyltransferase METTL3 Promotes Translation in Human Cancer Cells. Mol. Cell 62, 335–345.

61. Yu, L.P., Pickering, B.F., Cheng, Y., Zaccara, S., Nguyen, D., Minuesa, G., Chou, T., Chow, A., Saletore, Y., MacKay, M., et al. (2017). The N6-methyladenosine (m6A)-forming enzyme METTL3 controls myeloid differentiation of normal hematopoietic and leukemia cells. Nat. Med. 23, 1369–1376.

62. Visvanathan, A., Patil, V., Arora, A., Hegde, A.S., Arivazhagan, A., Santosh, V., and Somasundaram, K. (2018). Essential role of METTL3-mediated m6A modification in glioma stem-like cells maintenance and radioresistance. Oncogene 37, 522–533.

63. Li, F., Yi, Y., Miao, Y., Long, W., Long, T., Chen, S., Cheng, W., Zou, C., Zheng, Y., Wu, X., et al. (2019). N6-Methyladenosine Modulates Nonsense-Mediated mRNA Decay in Human Glioblastoma. Cancer Res. 79, 5785–5798.

64. Cai, J., Yang, F., Zhan, H., Situ, J., Li, W., Mao, Y., and Luo, Y. (2019). RNA m6A Methylation of METTL3 Promotes The Growth Of Prostate Cancer By Regulating Hedgehog Pathway. OncoTargets Ther. 12, 9143–9152.

65. Zhou, L., Yang, C., Zhang, N., Zhang, X., Zhao, T., and Yu, J. (2020). Silencing METTL3 inhibits the proliferation and invasion of osteosarcoma by regulating ATAD2. Biomed Pharmacother. 125, 109964.

66. Wei, W., Huo, R., and Shi, X. (2019). m6R-800 inhibits lung cancer via downregulating the expression of METTL3. Cancer Manag. Res. 11, 1177–1187.

67. Hou, H., Zhao, H., Yu, X., Cong, P., Zhou, Y., Jiang, Y., and Cheng, Y. (2020). METTL3 promotes the proliferation and invasion of esophageal cancer cells partly through AKT signaling pathway. Pathol. Res. Pract. 216, 153087.

68. Lin, S., Liu, J., Jiang, W., Wang, P., Sun, C., Wang, X., Chen, Y., and Wang, H. (2019). METTL3 Promotes the Proliferation and Mobility of Gastric Cancer Cells. Open Med. (Wars.) 14, 25–31.

69. Weng, H., Huang, H., Wu, H., Qin, X., Zhao, B.S., Dong, L., Shi, H., Skibbe, J., Shen, C., Hu, C., et al. (2018). METTL14 Inhibits Hematopoietic Stem/Progenitor Differentiation and Promotes Leukemogenesis via mRNA m6A Modification. Cell Stem Cell 22, 191–205.e9.

70. Bansal, H., Yihua, Q., Iyer, S.P., Ganapathy, S., Proia, D.A., Penalva, L.O., Uren, P.J., Suresh, U., Carew, J.S., Karnad, A.B., et al. (2014). WTAP is a novel oncopgenic protein in acute myeloid leukemia. Leukemia 28, 1171–1174.

71. Gutschner, T., Hammerle, M., Patau, S., Bley, N., Fiskin, E., Uckelmann, H., Heim, A., Grof, M., Hofmann, N., Gelfers, R., et al. (2014). Insulin-like growth factor 2 mRNA-binding protein 1 (IGF2BP1) is an important protumorigenic factor in breast cancer. Oncogene 33, 3345–3356.

72. Ren, J., and Cui, J. (2018). m6A mRNA modiﬁcation controls autophagy through up-regulating ULK1 protein abundance. Cell Res. 28, 955–957.

73. Zhu, Z., Qian, Q., Zhao, X., Ma, L., and Chen, P. (2020). N6-Methyladenosine ALKBH5 promotes non-small cell lung cancer progression by regulating TIMP3 stability. Gene 731, 144348.

74. Hancock, M.K., Hermanson, S.B., and Dolman, N.J. (2012). A quantitative TR-FRET plate reader immunoassay for measuring autophagy. Autophagy 8, 1227–1244.

75. Goel, S., Duda, D.G., Xu, L., Munn, L.L., Boucher, Y., Fukumura, D., and Jain, R.K. (2011). Normalization of the vasculature for treatment of cancer and other diseases. Physiol. Rev. 91, 1071–1121.

76. Sonveaux, P., Végran, F., Schroeder, T., Wergin, M.C., Verrax, J., Rabbani, Z.N., De Saedeleer, C.J., Kennedy, K.M., Diepart, C., Jordan, B.F., et al. (2008). Targeting lactate-fueled respiration selectively kills hypoxic tumor cells in mice. J. Clin. Invest. 118, 3930–3942.

77. Ruan, G.X., and Kazlauka, A. (2013). Lactate engages receptor tyrosine kinases Axl, Tie2, and vascular endothelial growth factor receptor 2 to activate phosphoinositide 3-kinase/Akt and promote angiogenesis. J. Biol. Chem. 288, 2126–21172.

78. Goel, S., Duda, D.G., Xu, L., Munn, L.L., Boucher, Y., Fukumura, D., and Jain, R.K. (2011). Normalization of the vasculature for treatment of cancer and other diseases. Physiol. Rev. 91, 1071–1121.

79. Végran, F., Boidot, R., Michels, C., Sonveaux, P., and Feron, O. (2011). Lactate influx through the endothelial cell monocarboxylate transporter MCT1 supports an NF-kB/JAK-8 pathway that drives tumor angiogenesis. Cancer Res. 71, 2550–2560.

80. Liu, J., Eckett, M.A., Harada, B.T., Liu, S.M., Lu, Z., Yu, K., Tienda, S.M., Chryplewicz, A., Zhu, A.C., Yang, Y., et al. (2018). m6A mRNA methylation regulates AKT activity to promote the proliferation and tumorigenicity of endometrial cancer. Nat. Cell Biol. 20, 1074–1083.

81. Liu, J., Shen, L., Liu, Y., Ming, H., Zhu, X., Chu, M., and Lin, J. (2020). The m6A methyltransferase METTL3 cooperates with demethylase ALKBH5 to regulate osteogenic differentiation through NF-κB signaling. Mol. Cell. Biochem. 463, 203–210.

82. Cheng, M., Sheng, L., Gao, Q., Xiong, Q., Zhang, H., Wu, M., Liang, Y., Zhu, F., Zhang, Y., Zhang, X., et al. (2019). The m6A methyltransferase METTL3 promotes bladder cancer progression via AFF4/NF-κB signaling network. Oncogene 38, 3667–3680.

83. Yue, W.L., Lin, C.L., and Fu, W.M. (2008). Enhancement of glucose transporter expression of brain endothelial cells by vascular endothelial growth factor derived from glioma exposed to hypoxia. Mol. Pharmacol. 73, 170–177.

84. Wang, Q., Chen, C., Ding, Q., Zhao, Y., Wang, Z., Chen, J., Jiang, Z., Zhang, Y., Xu, G., Zhang, J., et al. (2020). METTL3-mediated m6A modification of HDGF mRNA promotes gastric cancer progression and has prognostic significance. Gut 69, 1193–1205.

85. Rong, Z.X., Li, Z., He, J.J., Liu, L.Y., Ren, X.X., Gao, J., Mu, Y., Yuan, G.D., Duan, Y.M., Zhang, X.P., et al. (2019). Downregulation of Fat Mass and Obesity Associated (FTO) Promotes the Progression of Intrahepatic Cholangiocarcinoma. Front. Oncol. 9, 369.

86. Nieto, M.A., Huang, R.Y., Jackson, R.A., and Thiery, J.P. (2016). EMT: 2016. Cell 166, 21–45.

87. Goel, S., Duda, D.G., Xu, L., Munn, L.L., Boucher, Y., Fukumura, D., and Jain, R.K. (2011). Normalization of the vasculature for treatment of cancer and other diseases. Physiol. Rev. 91, 1071–1121.

88. Végran, F., Boidot, R., Michels, C., Sonveaux, P., and Feron, O. (2011). Lactate influx through the endothelial cell monocarboxylate transporter MCT1 supports an NF-kB/JAK-8 pathway that drives tumor angiogenesis. Cancer Res. 71, 2550–2560.
98. Brabletz, T., Kalluri, R., Nieto, M.A., and Weinberg, R.A. (2018). EMT in cancer. Nat. Rev. Cancer 18, 128–134.
99. Li, X., Tang, J., Huang, W., Wang, F., Li, P., Qin, C., Qin, Z., Zou, Q., Wei, J., Hua, L., et al. (2017). The M6A methyltransferase METTL3: acting as a tumor suppressor in renal cell carcinoma. Oncotarget 8, 96103–96116.
100. Jin, D., Guo, J., Wu, Y., Yang, L., Wang, X., Du, J., Dai, J., Chen, W., Gong, K., Miao, S., et al. (2020). m6A demethylase ALKBHS inhibits tumor growth and metastasis by reducing YTHDF-mediated YAP expression and inhibiting miR-107/LATS2-mediated YAP activity in NSCLC. Mol. Cancer 19, 40.
101. Xu, H., Wang, H., Zhao, W., Fu, S., Li, Y., Ni, W., Xin, Y., Li, W., Yang, C., Bai, Y., et al. (2020). SUMO1 modulation of methyltransferase-like 3 promotes tumor progression by regulating Stat1 mRNA homeostasis in hepatocellular carcinoma. Theranostics 10, 5671–5686.
102. Li, J., Chen, F., Peng, Y., Lv, Z., Lin, X., Chen, Z., and Wang, H. (2020). N6-Methyladenosine Regulates the Expression and Secretion of TGFβ1 to Affect the Epithelial-Mesenchymal Transition of Cancer Cells. Cancers 11, 296.
103. Yu, X., Zhao, H., and Cao, Z. (2020). The m6a methyltransferase METTL3 aggravates the progression of nasopharyngeal carcinoma through inducing EMT by m6a-modified Stat1 mRNA. Minerva Med. Published online June 5, 2020. https://doi.org/10.23736/S0026-4806.20.06653-7.
104. Wang, Y., Zeng, L., Liang, C., Zan, R., Ji, W., Zhang, Z., Wei, Y., Tu, S., and Dong, Y. (2019). Identiﬁcation of a Novel Small-Molecule Binding Site of the Fat Mass and Obesity Associated Protein (FTO). J. Med. Chem. 62, 9430–9439.
105. Cui, Q., Shi, H., Ye, P., Li, L., Xu, X., Sun, Z., Sun, G., Li, Z., Huang, W., Zhang, Z., and Cai, Y. (2017). m6A RNA Methylation Regulates the Self-Renewal and Tumorigenesis of Glioblastoma Stem Cells. Cell Rep. 19, 128.
106. Wei, J., Yin, Y., Zhou, J., Chen, H., Peng, J., Yang, J., and Tang, Y. (2020). METTL3 potentiates resistance to cisplatin through m6A modification of TAFAP2C in seminoma. J. Cell. Mol. Med. 24, 11366–11380.
107. Zhu, L., Zhu, Y., Han, S., Chen, M., Song, P., Dai, D., Xu, W., Jiang, T., Feng, L., SHin, V.Y., et al. (2019). Impaired autophagic degradation of IncRNA ARHGAP5-A51 promotes chemoresistance in gastric cancer. Cell Death Dis. 10, 383.
108. Zhang, Y., Kang, M., Zhang, B., Meng, F., Song, J., Kaneko, H., Shimamoto, F., and Tang, B. (2019). m6A modification-mediated CBX8 induction regulates stemness and chemosensitivity of colon cancer via upregulation of LGR5. Mol. Cancer 18, 185.
109. Taketo, K., Konno, M., Asai, A., Koski, J., Toratani, M., Satoh, T., Doki, Y., Mori, M., Ishii, H., and Ogawa, K. (2018). The epitranscriptome m6a writer METTL3 promotes chemo- and radiosensitivity in pancreatic cancer cells. Int. J. Oncol. 52, 621–629.
110. Tang, B., Yang, Y., Kang, M., Wang, X., Wang, Y., Bi, Y., He, S., and Shimamoto, F. (2020). m6A demethylase ALKBHS inhibits pancreatic cancer tumorigenesis by decreasing WIF-1 RNA methylation and mediating Wnt signaling. Mol. Cancer 19, 3.
111. Fukushima, T., Zhu, H., Nacarelli, T., Karakashev, S., Fatkhiutdinov, N., Wu, S., Liu, P., Kossenkov, A.V., Showe, L.C., Jean, S., et al. (2019). N6-Methylation of Adenosine of FZZ10 mRNA Contributes to PARP Inhibitor Resistance. Cancer Res. 79, 2812–2820.
112. Nishizawa, Y., Konno, M., Asai, A., Koski, J., Kawamoto, K., Miyoshi, N., Takahashi, H., Nishida, N., Haraguchi, N., Sakai, D., et al. (2017). Oncogene c-Myc promotes epitranscriptome m6A reader YTHDF1 expression in colorectal cancer. Oncotarget 9, 7476–7486.
113. He, J.J., Li, Z., Rong, Z.X., Gao, J., Mu, Y., Guan, Y.D., Ren, X.X., Zi, Y.Y., Liu, L.Y., Fan, Q., et al. (2020). m6A Reader YTHDC2 Promotes Radiotherapy Resistance of Nasopharyngeal Carcinoma via Activating IGFIR/akt5/6 Signaling Axis. Front. Oncol. 10, 1166.
114. Klinge, C.M., Piell, K.M., Tooley, C.S., and Rouchka, E.C. (2019). HNRNPA2/B1 is upregulated in endocrine-resistant LCC9 breast cancer cells and alters the mRNA transcriptome when overexpressed in MCF-7 cells. Sci. Rep. 9, 9430.
115. Chen, B., Ye, F., Yu, L., Jia, G., Huang, X., Zhang, X., Peng, S., Chen, K., Wang, M., and Song, S. (2012). Development of cell active N6-methyladenosine RNA demethylase FTO inhibitor. J. Am. Chem. Soc. 134, 17963–17971.
116. Aik, W., Demetriades, M., Hamdan, M.K., Bagg, E.A., Yeoh, K.K., Leujeune, C., Zhang, Z., McDonough, M.A., and Schofield, C.J. (2013). Structural basis for inhibition of the fat mass and obesity associated protein (FTO). J. Med. Chem. 56, 3680–3688.
117. Huang, Y., Yan, J., Li, Q., Li, J., Gong, S., Zhou, H., Gan, J., Jiang, J., Jia, G.F., Luo, C., and Yang, C.G. (2015). Medofenacid acid selectively inhibits FTO demethylation of m6A over ALKBH5. Nucleic Acids Res. 43, 373–384.
118. Cui, Q., Shi, H., Ye, P., Li, L., Xu, X., Sun, G., Sun, G., Li, Z., Huang, W., and Cai, Y. (2017). m6A RNA Methylation Regulates the Self-Renewal and Tumorigenesis of Coliblastoma Stem Cells. Cell Rep. 18, 2622–2634.
119. Wang, T., Hong, T., Huang, Y., Su, H., Wu, F., Chen, Y., Wei, L., Huang, W., Xia, Y., et al. (2015). Fluorescein Derivatives as Bifunctional Molecules for the Simultaneous Inhibiting and Labeling of FTO Protein. J. Am. Chem. Soc. 137, 13736–13739.
120. Singh, B., Kinne, H.E., Milligan, R.D., Washburn, L.J., Olsen, M., and Lucci, A. (2016). Important Role of FTO in the Survival of Rare Pancreatic Triple-Negative Inflammatory Breast Cancer Cells Facing a Severe Metabolic Challenge. PLoS ONE 11, e0159072.
121. He, W., Zhou, B., Liu, W., Zhang, M., Shen, Z., Han, Z., Jiang, Q., Yang, S., and Wang, R., et al. (2015). Identification of A Novel Small-Molecule Binding Site of the Fat Mass and Obesity Associated Protein (FTO). J. Med. Chem. 58, 7341–7348.
122. Qiao, Y., Zhou, B., Zhang, M., Liu, W., Han, Z., Song, C., Wu, Y., Yang, Q., Wang, R., and Wang, S., et al. (2016). A Novel Inhibitor of the Obesity-Related Protein FTO. Biochemistry 55, 1516–1522.
123. Wang, R., Han, Z., Liu, B., Zhou, B., Wang, N., Jiang, Q., Qiao, Y., Song, C., Chai, J., and Chang, J. (2018). Identification of Natural Compound Radicicol as a Potent FTO Inhibitor. Mol. Pharm. 15, 4092–4098.

www.moleculartherapy.org

Review
