Molecular Therapy
Nucleic Acids
Review

Dual effects of N^6-methyladenosine on cancer progression and immunotherapy

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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, N^6-methyladenosine (m^6A) is the most common internal modification on eukaryotic mRNA, which has attracted increasing attention in recent years. Accumulating studies have shown that aberrant m^6A modifications have profound effects on the characteristics of tumors, which undoubtedly led to a significant breakthrough in cancer treatment. Although m^6A 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 m^6A methylation machinery and mainly summarize the biological mechanism of m^6A in cancer cell death, angiogenesis, epithelial-mesenchymal transition (EMT), and therapeutic resistance. Subsequently, we present the exogenous regulatory factors of m^6A and highlight the role of m^6A on immune cells and cancer immunotherapy. The potential therapeutic strategies of m^6A 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.[1,2] 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.[3] 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.

N^6-methyladenosine (m^6A) occurs in the sixth nitrogen atom of the RNA adenylate and is the most abundant internal modification in eukaryotic mRNAs[4] (Table S1). In 2012, Dominissini et al.[5] compiled the first human and mouse RNA methylomes using the m^6A-seq method and identify over 12,000 m^6A 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 m^6A modification, with an average of 3–5 m^6A sites per transcript.[6] This methylation is mediated by methyltransferases ("writers"), demethylases ("erasers"), and binding proteins ("readers") that are involved in almost all physiological and pathological processes.[7–12] Several studies demonstrated that m^6A can affect the characteristics of cancer cells.[13–24] However, the performance of different phenotypes in a 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 m^6A methylation is of great significance and may become an innovative therapeutic target in the clinic.

In this review, we introduce three kinds of m^6A methylation enzymes and elaborate the dual effect of m^6A 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 m^6A methylation is dynamically regulated by writers, erasers, and readers. They install, remove, and recognize m^6A, respectively.

Writers

The m^6A 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.[25] Although both METTL3 and METTL14 contain conserved catalytic domains, only METTL3 contains the methyl donor, S-adenosylmethionine (SAM),[26] which is mainly localized in the nucleus in a form of speckles.[27] WTAP interacts with the METTL3-METTL14 dimer, leading to the recruitment of the m^6A methyltransferase complex to

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the mRNA target, which affects the methylation efficiency.[27] 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.[28] Moreover, methyltransferase-like 16 (METTL16) can regulate the SAM synthetase intron retention and install m^6A onto the U6 small nuclear RNA.[29,30] In addition, Vir like m^6A methyltransferase associated protein (VIRMA, also known as KIAA1429),[31] zinc finger CCCH domain-containing protein 13 (ZC3H13),[32] and Cbl photo oncogene like 1 (CBLL-1, also known as HAKAI)[33] are also involved in the formation of methyltransferase complex to catalyze the formation of m^6A. Strikingly, methyltransferase-like 5 (METTL5) is an enzyme responsible for the 18S rRNA m^6A modification, and zinc finger CCHC-type containing 4 (ZCCHC4) was confirmed to be a 28S rRNA m^6A modifying enzyme.[34,35] 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.[34] It is confirmed that NOP2/Sun RNA methyltransferase 2 (NSun2) silencing completely blocks PAR2-induced m^6A methylation of pre-miR-125b2, indicating that NSun2 may regulate m^6A modification in an indirect way.[36] However, it has not been reported that NSun2 has a direct effect on m^6A.

Erasers

Fat mass and obesity-associated protein (FTO) is the first eraser that was successfully identified.[37] Subsequently, another eraser, alkB homolog 5 (ALKBH5), a member of the AlkB family along with FTO, was soon discovered.[38] Compared with FTO, ALKBH5 seems to be an m^6A-specific demethylase in mRNA, because FTO is more involved in the demethylation of N6,2′-o-dimethyladenosine (m^6A_m).[39] They show the demethylation process with different foundational mechanisms. FTO follows a traditional oxidative-demethylation pathway to catalyze conversion of m^6A to N6-hydroxymethyladenosine (hm^6A) and then convert the hm^6A to N6-formyladenosine (f^6A). Finally, f^6A is converted to adenosine. In contrast to FTO, ALKBH5 catalyzes a direct m^6A-to-adenosine transformation.[40] In addition, a novel m^6A demethylase, alkB homolog 3 (ALKBH3), has been found to preferentially modify tRNA rather than mRNA or rRNA.[41] The exploitation of erasers showed that m^6A is reversible and that m^6A methylation was dynamically regulated by writers and erasers.

Readers

To perform specific biological functions, specific RNA-binding proteins, readers, are required for m^6A-RNA. At present, m^6A readers include the YT521-B homology (YTH) domain proteins (YTHDF1, YTHDF2, YTHDF3, YTHDC1, and YTHDC2,)[42–46] the heterogeneous nuclear ribonucleoprotein (hnRNPs) family (HNRNPA2B1, HNRNPC, and HNRNPG),[47–49] and the IGF2 mRNA binding protein (IGF2BPs) family (IGF2BP1, IGF2BP2, and IGF2BP3).[50] Structural analysis reveals that YTH domains recognize m^6A modification by forming a conserved aromatic cage.[51,52] Among them, YTHDC1 prefers guanosine at the position preceding the m^6A and preferentially binds to the GG(m^6A)C sequence, which is not observed in other YTH domain proteins.[51] Different from YTH domain, K homology (KH) domains are the RNA-binding domains of IGF2BPs, which directly bind to m^6A-modified mRNAs.[50] hnRNPs may mediate effects of m^6A through an "m^6A switch" mechanism, instead of direct binding to m^6A.[48,49,53] Through the study of cap-independent translation, eukaryotic initiation factor 3 (eIF3), a component of the translation pre-initiation complex, is found to selectively bind m^6A-containing RNA, which confirmed that eIF3 is an m^6A-binding protein.[54] Based on RNA pull-down approaches, fragile X mental retardation 1 (FMR1) is identified as a sequence-context-dependent m^6A reader, and leucine-rich pentatricopeptide repeat-containing (LRPPRC) is also served as a newly recognized reader.[55,56] A recent study manifested that nuclear factor κB (NF-κB)-associated protein (NKAP) is a likely reader of m^6A in pri-miR-25.[57] Remarkably, a novel m^6A binding protein, Proline rich coiled-coil 2 A (Prrc2a), which is widely expressed in various nerve cells and brain regions, was identified.[58] However, more structural and biochemistry data of these proteins need to be further clarified.

m^6A 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^6A through different mechanisms. Here, we briefly summarize m^6A function in cancer cell apoptosis (Figure 1; Table S2) and autophagy (Figure 2).

m^6A and apoptosis

m^6A inhibits apoptosis

m^6A promotes oncogene expression. First, METTL3 is the main regulatory factor in the m^6A-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).[13,59] 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.[60] 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.[61] In glioblastoma multiforme (GBM), METTL3 acts on the oncogene sex determining region Y box 2 (SOX2) by facilitating SOX2 mRNA stability in an m^6A-dependent regulatory manner.[62] Li et al.[63] 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-XS and NCORα expression through promoting the degradation of serine- and arginine-rich splicing factors (SRSFs).[63] 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).[64,65] Moreover,

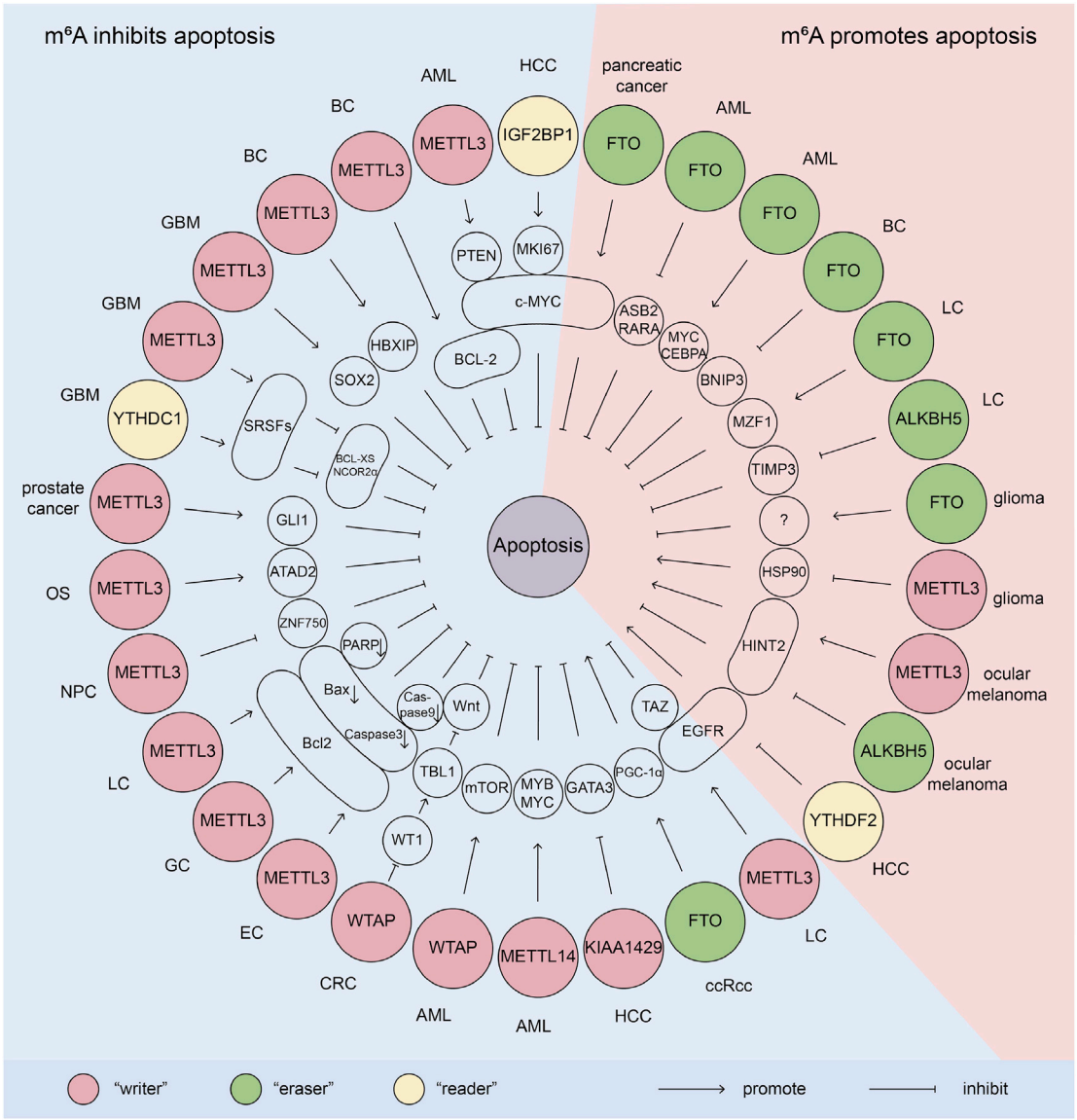

Figure 1. Summary of N^6-methyladenosine (m^{6}A) in cancer apoptosis

ALKBH5, alkB homolog 5; AML, acute myeloid leukemia; ASB2, Ankyrin repeat and SOCS box protein 2; ATAD2, ATPase family AAA domain containing 2; Bax, BCL2 associated X; BC, breast cancer; BCL-2, B cell lymphoma-2; BNIP3, BCL2 interacting protein 3; ccRcc, clear cell renal cell carcinoma; c-Myc, v-myc myelocytomatosis viral oncogene homolog; CRC, colorectal cancer; EC, esophageal cancer; EGFR, epidermal growth factor receptor; FTO, Fat mass and obesity-associated protein; GATA3, GATA Binding Protein 3; GBM, glioblastoma multiforme; GC, gastric cancer; GLI1, GLI family zinc finger 1; HBXIP, hepatitis B X-interacting protein; HCC, hepatocellular carcinoma; HINT2, histidine triad nucleotide-binding protein 2; IGF2BP1, IGF2 mRNA binding protein 1; LC, lung cancer; METTL14, methyltransferase-like 14; METTL3, methyltransferase-like 3; mTOR, mammalian target of rapamycin; MZF1, myeloid zinc finger protein 1; NPC, nasopharyngeal carcinoma; OS, osteosarcoma; PGC-1α, peroxisome proliferator-activated receptor γ coactivator 1α; PTEN, phosphatase and tensin homolog; RARA, Retinoic Acid Receptor Alpha; SOX2, sex determining region Y box 2; SRSFs, serine- and arginine-rich splicing factors; TAZ, tafazzin; TIMP3, TIMP metallopeptidase inhibitor 3; WT1, Wilms' tumor 1; WTAP, Wilms' tumor 1-associated protein; YTHDC1, YTH domain containing protein 1; YTHDF2, YTH domain family protein 2; ZNF750, zinc finger protein 750.

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

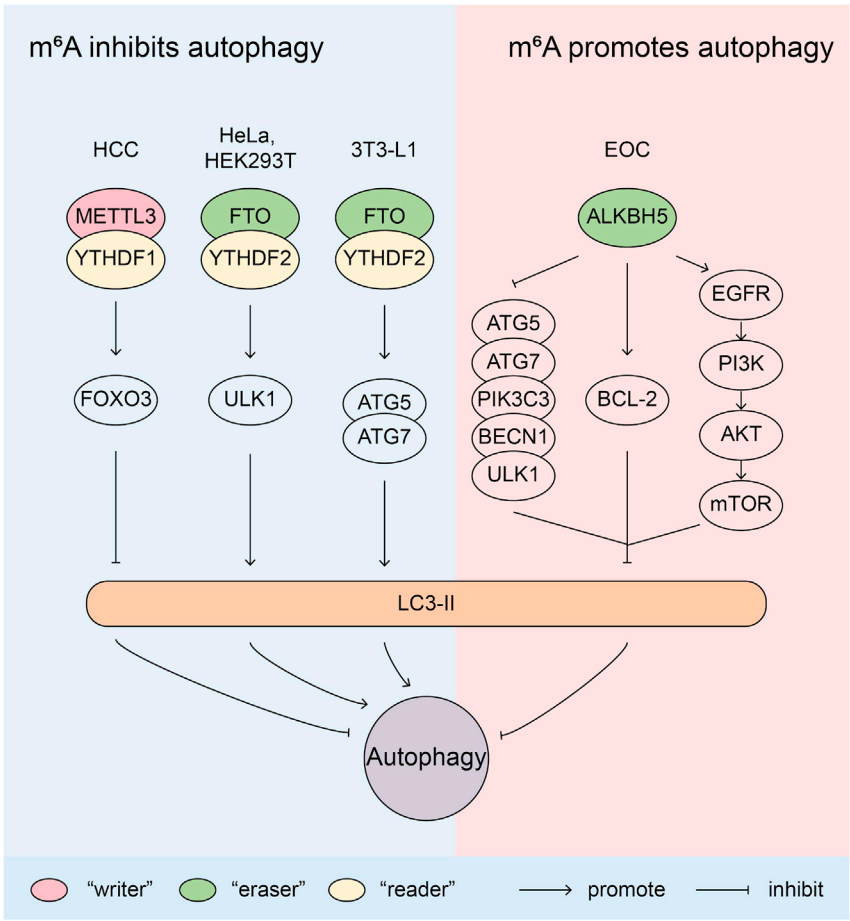

Figure 2. Regulation of m⁶A in cancer autophagy
ATG5, autophagy related 5; ATG7, autophagy related 7; EOC, epithelial ovarian cancer; FOXO3, forkhead box O3; METTL3, methyltransferase-like 3; PIK3C3, phosphatidylinositol 3-kinase catalytic subunit type 3; ULK1, unc-51-like kinase 1; YTHDF1, YTH domain family protein 1.

was associated with the regulation of MYB and MYC target mRNA in m⁶A-mediated manner.[69] In addition, Bansal et al.[70] 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.[71]

m⁶A inhibits anti-oncogene expression.. Similarly, METTL3, WTAP, and KIAA1429 can restrain the apoptotic 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.[73] 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.

In addition to writers, eraser FTO could act as a tumor suppressor to accelerate cell apoptosis, while its knockdown decreases the apoptotic rate in clear cell renal cell carcinoma (ccRcc). Intriguingly, FTO-induced high level of m⁶A that affects the mRNA of the peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) is the critical mechanism of this process.[74]

m⁶A promotes apoptosis

m⁶A inhibits oncogene expression. FTO is a major regulatory factor of m⁶A promoting apoptosis, which is achieved by inhibiting the expression of oncogenes. FTO knockdown accelerates apoptosis via the decreased translation of Myeloid Zinc Finger Protein 1 (MZF1) mRNA in lung squamous cell carcinoma (LUSC), MYC/CEBPA mRNA in AML, and c-MYC mRNA in pancreatic cancer through m⁶A RNA modification.[15,75,76] In glioma, METTL3 low expression restrains glioma cell apoptosis by upregulating HSP90, while FTO high expression inhibits apoptosis in an m⁶A-dependent manner as well.[77]

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 anti-oncogene expression. 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 metallopeptidase inhibitor 3 (TIMP3) mRNA stability via ALKBH5 demethylation.

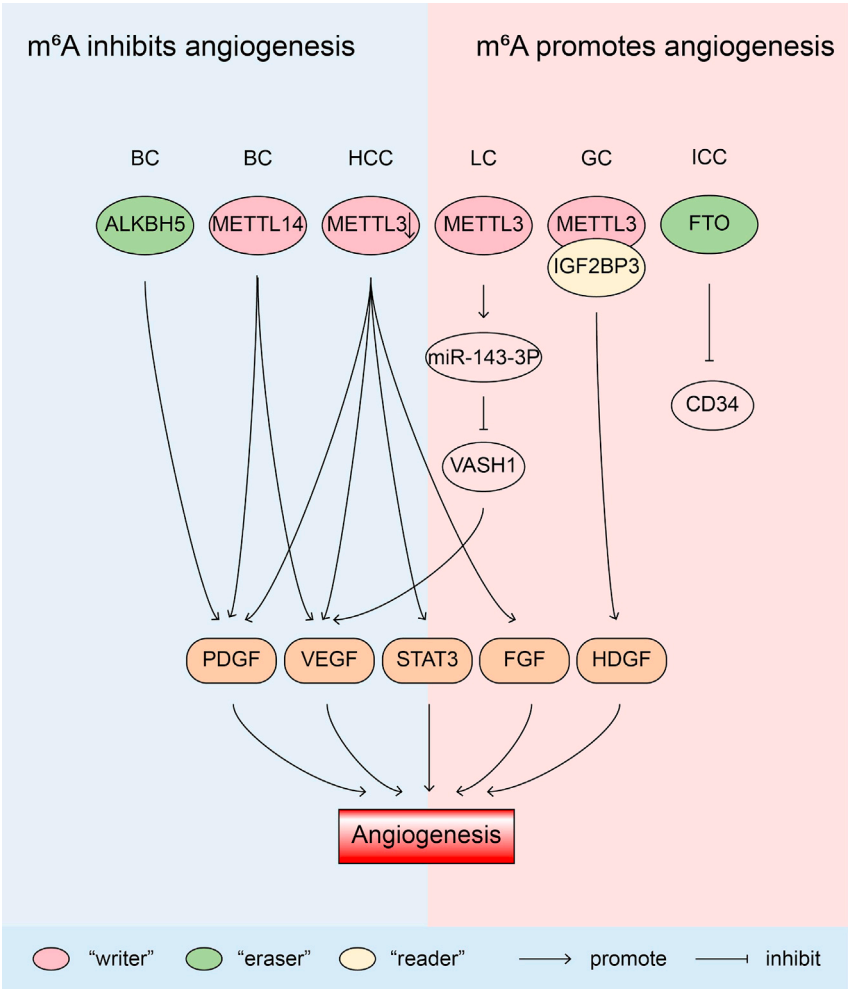

Figure 3. m⁶A function in cancer angiogenesis
ICC, intrahepatic cholangiocarcinoma; IGF2BP3, IGF2 mRNA binding protein 3; VASH1, vasohibin-1.

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.[82] 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.[83] 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^{6}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.[17]

However, METTL14 or ALKBH5 positively regulates some protein markers, such as VEGF and PDGFA, which are angiogenesis-associated indicators in BC cells.[19] These results reveal that aberrant m^{6}A may have a regulatory effect on angiogenesis in cancer; however, the specific mechanism of this event remains to be elucidated.

m^{6}A promotes angiogenesis

In LC, the miR-143-3p/vasohibin-1 (VASH1) axis could be mediated by METTL3 in a m^{6}A-dependent way by activating the expression of VEGFA and promoting the capacity of tube formation.[20] Wang et al.[95] 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^{6}A modification and facilitates angiogenesis by stabilizing HDGF mRNA.[95]

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.[96]

In brief, the current research on m^{6}A involvement angiogenesis mainly focused on METTL3, and further studies need to be carried out in the near future.

m^{6}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.[97,98] Currently, mounting evidence suggested that m^{6}A plays an important role in EMT (Figure 4).

m^{6}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.[21] 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.[99]

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.[100]

m^{6}A promotes EMT

In HCC, METTL3 mutation leads to a low level of m^{6}A in the coding sequence (CDS) and 3′-UTRs of Snail, a key EMT translator. Moreover, YTHDF1 binds to m^{6}A methylated Snail and stimulates its translation, triggering lung metastasis.[22] 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.[101] It is well known that transforming growth factor-β (TGF-β) is an indispensable EMT factor in cancer. Li et al.[102] showed that m^{6}A negatively regulates TGFB1 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.[103] validated that METTL3 deficiency downregulates m^{6}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^{6}A modification, thus driving EMT in LC.[104] Concurrently, the reduction in METTL3 level catalyzes E-cadherin expression and restrains N-cadherin and vimentin expression through downregulating m^{6}A content on JUNB mRNA in LC cells. As expected, JUNB overexpression reverses TGF-β-induced EMT.[105] Additionally, METTL3 could also promote EMT through the miR-143-3p/VASH1 axis in LC.[20] The effect of METTL3 on EMT has also been observed in GC,[106,107] 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.[107] Wu et al.[108] 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^{6}A abundance on YAP mRNA. EMT capacity could be significantly disrupted by YTHDF2-mediated YAP deficiency.[100] In pancreatic cancer, ALKBH5 mediates the demethylation of KCNK15-AS1 and promotes its expression, which accelerates EMT.[109]

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

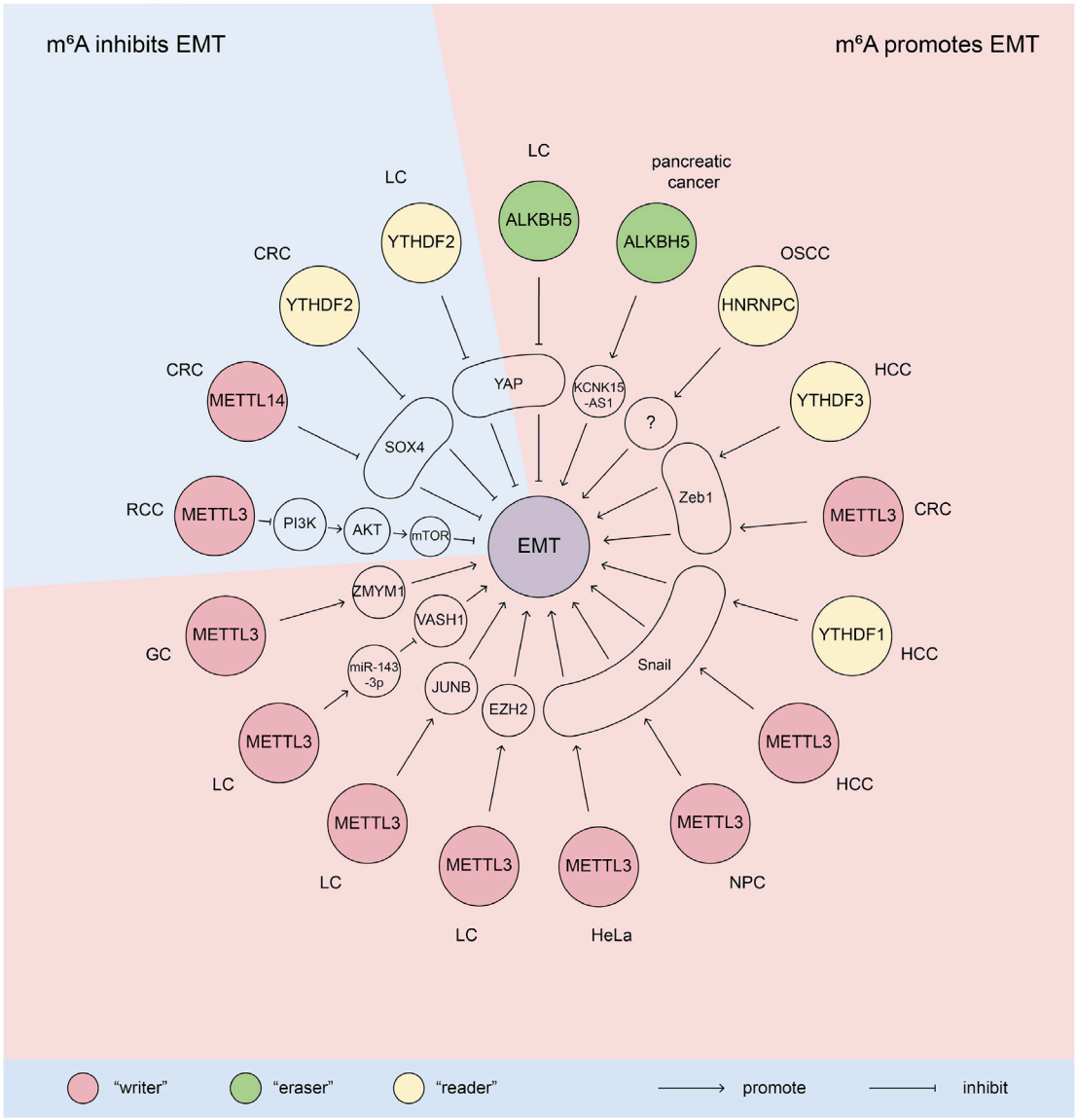

Figure 4. The biological mechanism of m^{6}A in cancer EMT

EMT, epithelial-mesenchymal transition; EZH2, enhancer of zeste homolog 2; HNRNPC, heterogeneous nuclear ribonucleoprotein C; OSCC, oral squamous cell carcinoma; RCC, renal cell carcinoma; SOX4, SRY-related high-mobility-group box 4; YTHDF3, YTH domain family protein 3; ZMYM1, zinc finger MYM-type containing 1.

tumor metastasis via the upregulation of Zeb1. In addition, YTHDF3 can be a reader of m^{6}A-modified Zeb1 and functions as a Zeb1 mRNA stabilizer.[110]

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.[111]

Overall, m^{6}A imposes positive and negative influences on EMT in certain cancer types, and the writers are major regulators of EMT.

m^{6}A IN CANCER THERAPEUTIC RESISTANCE

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)

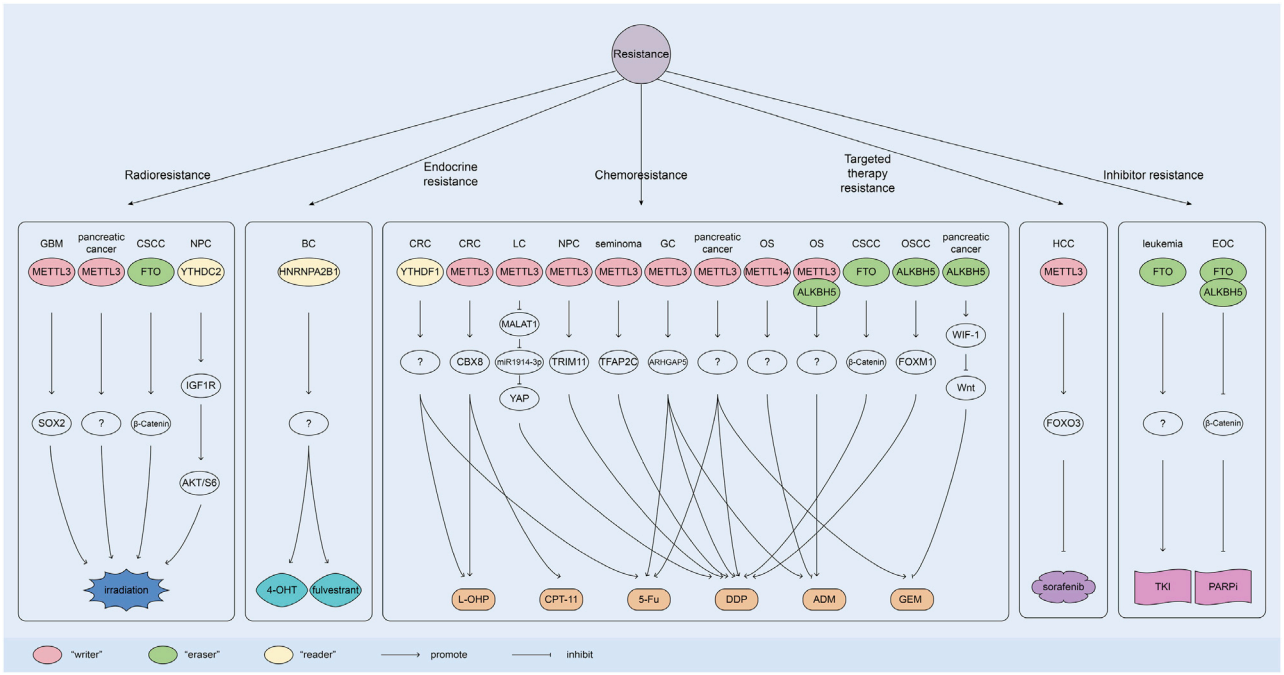

Figure 5. Overview of m^{6}A in cancer therapeutic resistance

An abnormal m^{6}A level leads to chemoresistance, radioresistance, endocrine resistance, resistance to targeted therapy, and resistance to inhibitors in cancer. 4-OHT, 4-hydroxytamoxifen; 5-Fu, 5-fluorouracil; ADM, Adriamycin/doxorubicin; CPT-11, irinotecan; CSCC, cervical squamous cell carcinoma; DDP, cisplatin; FOXM1, Forkhead box protein M1; GEM, gemcitabine; HNRNPA2B1, heterogeneous nuclear ribonucleoprotein A2B1; IGF1R, insulin-like growth factor 1 receptor; L-OHP, oxaliplatin; MALAT1, metastasis-associated lung adenocarcinoma transcript 1; PARPi, poly ADP-ribose polymerase inhibitor; TFAP2C, transcription factor-activating enhancer-binding protein 2C; TKI, tyrosine kinase inhibitor; TRIM11, Tripartite motif containing protein 11; YTHDC2, YTH domain containing protein 2.

m^{6}A 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).[23] In leukemia, the low level of m^{6}A 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 m^{6}A 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 m^{6}A is obviously related to chemoresistance.[114]

m^{6}A 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, m^{6}A methylation that is activated by METTL3 and recognized by IGF2BP2 facilitates the stability of the Tripartite motif containing protein 11 (TRIM11), activates the Dvl-associating pro Tein (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 m^{6}A 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 m^{6}A modification is closely associated with pancreatic cancer GEM resistance.[120] Moreover, both erasers FTO and ALKBH5 are downregulated in resistant EOC cells, and it was shown that m^{6}A concentration on FZD10 mRNA causes resistance to poly ADP-ribose polymerase inhibitor (PARPi) by stimulating the Wnt/β-catenin signaling pathway.[121]

It is worth mentioning that YTHDF1 silencing can promote the CRC sensitization to 5-Fu and L-OHP.[122] However, YTHDC2 knockdown induces a reduction in radioresistance by hampering the insulin-like growth factor 1 receptor (IGF1R)-AKT/S6 signaling pathway in NPC.[123]

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.[124]

In a nutshell, therapeutic resistance caused by m^{6}A occurs in almost all clinical treatments, especially in chemoresistance. By contrast, there exists the phenomenon that m^{6}A enhances drug sensitivity.

m^{6}A IN CANCER TREATMENT

Inhibitors can change the malignant behavior of tumors by targeting m^{6}A 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 m^{6}A

Rhein, the first identified FTO inhibitor, together with FTO competitively recognizes the m^{6}A substrate to raise the methylation of m^{6}A on mRNA.[125] By studying the structure of FTO inhibitors, scholars found that FG-2216/IOX3 and FG-4592/SelleckBio can accurately inhibit FTO.[126] Meclofenamic acid (MA) is a highly specific FTO inhibitor that acts as a nonsteroidal anti-inflammatory drug that effectively competes with FTO binding sites.[127] In particular, the MA ethyl ester form, MA2, also suppresses glioblastoma stem cell (GSC) tumorigenesis through the inhibition of FTO.[128] Fluorescein and its derivatives were shown to selectively and simultaneously inhibit and label FTO.[129] Another FTO inhibitor, MO-I-500, prominently attenuates survival and colony-forming capacity of BC cells under glutamine-free conditions.[130] After a strict screening, He et al.[131] demonstrated that N-CDPCB 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.[132] Interestingly, the FTO inhibitor radicicol and N-CDPCB occupy the same FTO binding position.[133] Huang et al.[134] 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.[135] More recently, two compounds, CS1 (bisantrene) and CS2 (brequinara), 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 m^{6}A-containing RNA.[136] Additionally, Zheng et al.[137] and Toh et al.[138] successively detected a series of unnamed compounds that can inhibit FTO. Notably, citate can bind to both FTO and ALKBH5 and in different modes to serve the function of FTO inhibitor.[139] Furthermore, ALK-04 was identified as a specific ALKBH5 inhibitor.[140] We summarize the half maximal inhibitory concentration (IC_{50}), 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.[141] On the contrary, Selberg et al.[142] 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.

m^{6}A and immune cells

The immune system consists of innate immunity and adaptive immunity. m^{6}A 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.[143] 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).[144] Moreover, RNA-binding motif 4 (RBM4) interacts with YTHDF2 and could inhibit M1 macrophage polarization by the degradation of m^{6}A modified STAT1 mRNA.[145]

The loss of METTL3 suppresses the maturation phenotype of DC as well as decreased T cell response *in vivo* and *in vitro*.[146] Liu et al.[147] indicated that lnc-Dpf3 restrains DC migration, which is mediated by CCR7. At the same time, CCR7 stimulation upregulates lnc-Dpf3 expression by mediating m^{6}A demethylation.[147]

Li et al.[148] 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 m^{6}A in CD4 primary T cells, and the binding of HIV-1 gp120 with CD4 receptor is necessary for the upregulation of m^{6}A.[149] In the exploration of the interaction between YTHDF3 and HIV replication, Jurczyszak et al.[150] 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.[151]

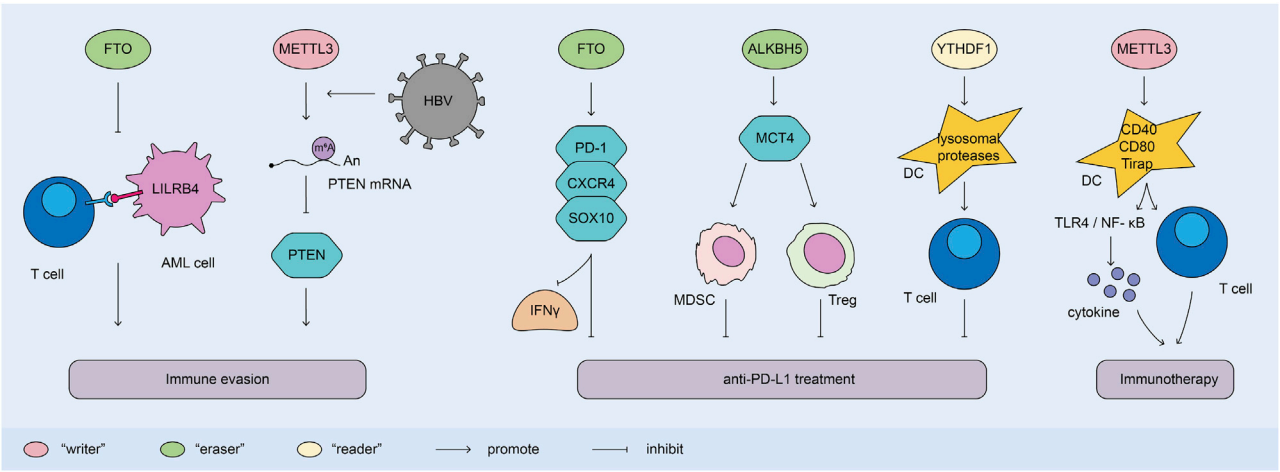

Figure 6. The role of m^{6}A in cancer immunotherapy

Regulatory factors affect immune escape and cancer immunotherapy response through the alteration of the tumor microenvironment. CXCR4, C-X-C chemokine receptor type 4; DC, dendritic cell; HBV, hepatitis B virus; IFNγ, interferon gamma; LILRB4, leukocyte immunoglobulin-like receptor subfamily B4; MDSC, myeloid-derived suppressor cell; PD-1, programmed cell death-1; PD-L1, programmed cell death-ligand 1; SOX10, sex determining region Y box 10; Treg, regulatory T cell.

METTL14 silencing can damage IL-7-induced pro-B cell proliferation, while RNA m^{6}A 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.[152]

m^{6}A and cancer immunotherapy

At present, immunotherapy has become an advanced strategy to conquer cancer, and m^{6}A is of profound importance to immune-checkpoint therapy (Figure 6). Kim et al.[153] demonstrated that the hepatitis B virus (HBV) severely decreases PTEN translation via an METTL3-mediated m^{6}A 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.[153]

Yang et al.[154] 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 m^{6}A 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 lessening of immunotherapy resistance in melanoma.[154] 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 (HMAs). Consequently, FTO inhibition can render AML cells sensitive to T cell toxicity and overcome the HMA-induced immune evasion that was induced through inhibiting the expression of LILRB4.[136]

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).[140] The upregulation of the expression of the PD-ligand 1 (PD-L1) expression by an FTO-catalyzed mRNA demethylation forecast that m^{6}A may make a difference in CRC immunotherapy.[155] Cancer patients are divided into several clusters with diverse m^{6}A regulators, suggesting that m^{6}A methylation has great relevance to PD-L1 expression in head and neck squamous cell carcinoma, BC, and response to anti-PD-1/L1 immunotherapy of GC.[156–158]

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.[159] 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.[146] Collectively, m^{6}A has the potential to become the main immunotherapeutic target.

CONCLUSION

The discovery of m^{6}A methylation enriched the research field of epigenetics. It has been demonstrated that m^{6}A methylation is involved in tumor characteristics, including cell death, angiogenesis, and EMT. However, whether m^{6}A 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 m^6A in cancer cells' therapeutic resistance lays the foundation for its use in combination therapy. The development of m^6A-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 m^6A methylation, which opens up a new approach in improving immunotherapeutic effects.

It is encouraging to note that m^6A contents increase in circulating tumor cells (CTCs) of LC patients after the analysis of RNA methylation by LC-ESI-MS/MS. Therefore, m^6A quantitative assessment in CTCs may be a supplementary evidence for the qualitative and early diagnosis of cancer.[160]

We believe that not all m^6A 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 m^6A regulatory factors and associated mechanisms is required to hew out new possibilities for cancer diagnosis and treatment.

SUPPLEMENTAL INFORMATION

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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.

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