Targeting RNA N⁶-methyladenosine modification: a precise weapon in overcoming tumor immune escape

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Abstract
Immunotherapy, especially immune checkpoint inhibitors (ICIs), has revolutionized the treatment of many types of cancer, particularly advanced-stage cancers. Nevertheless, although a subset of patients experiences dramatic and long-term disease regression in response to ICIs, most patients do not benefit from these treatments. Some may even experience cancer progression. Immune escape by tumor cells may be a key reason for this low response rate. N⁶-methyladenosine (m⁶A) is the most common type of RNA methylation and has been recognized as a critical regulator of tumors and the immune system. Therefore, m⁶A modification and related regulators are promising targets for improving the efficacy of tumor immunotherapy. However, the association between m⁶A modification and tumor immune escape (TIE) has not been comprehensively summarized. Therefore, this review summarizes the existing knowledge regarding m⁶A modifications involved in TIE and their potential mechanisms of action. Moreover, we provide an overview of currently available agents targeting m⁶A regulators that have been tested for their elevated effects on TIE. This review establishes the association between m⁶A modifications and TIE and provides new insights and strategies for maximizing the efficacy of immunotherapy by specifically targeting m⁶A modifications involved in TIE.

Keywords: N⁶-methyladenosine (m⁶A), cancer, Tumor, Immunotherapy, Tumor immune escape (TIE)

Introduction
According to the concept of cancer immunoediting, the immune system has dual roles in preventing and shaping tumors. In immunocompetent hosts, tumor cells obtain three fates: elimination, equilibrium, and escape. Tumor immune escape (TIE) is a process in which the immunologic microenvironment of sculpted tumors can expand in an uncontrolled pathway [1]. Antitumor immunity is mainly mediated by CD8⁺ T cells that specifically recognize antigenic peptides presented by the major histocompatibility complex (MHC, in vertebrates) or class I human leukocyte antigens (HLA-I, in humans) and are activated to kill tumor cells [2]. However, tumor cells can produce inhibitory signals that suppress T-cell function. Immune checkpoint inhibition (ICI) therapy can effectively suppress these signals and reactivate T cells in only a minority of patients. Its limited effectiveness has been demonstrated to be due to reduced antigen presentation, increased immunosuppressive factors, and abnormally activated signaling pathways.

N⁶-methyladenosine (m⁶A) is the most prevalent RNA modification in mammalian cells and was first discovered in 1974 [3]. m⁶A modification sites are evolutionally conserved within a consensus motif DRACH (D = A, G or U; H = A, C, or U), in which A is converted to m⁶A and generally occurs in the coding sequence, 3′ untranslated
region (3′ UTR) proximal to the stop codon, and 5′ untranslated region (5′ UTR) of mRNAs [4–6]. It is a potentially reversible and dynamic post-transcriptional modification of RNA molecules that is regulated by methyltransferases (writers) and demethylases (erasers) and recognized by specific binding proteins (readers). The m6A modification has significant functions in regulating alternative splicing of pre-mRNAs [5], mRNA degradation [7], mRNA stabilization [8], miRNA processing [9], and cap-independent translation [10] (Fig. 1).

Currently, m6A modification involved in TIE is emerging and is expected to be a potential target for improving the limited antitumor immunotherapy response rates. This lack of responsiveness suggests the need to expand the knowledge of m6A modifications in TIE regulation. Furthermore, an in-depth understanding of the underlying mechanism of action will contribute to developing new combination therapies to promote antitumor immunotherapy. This review describes the existing evidence regarding m6A modifications, their influence on TIE, and their underlying molecular regulatory mechanisms. Moreover, we discuss available agents targeting m6A modifications to improve the efficacy of immunotherapy. We hope this review will broaden our understanding of the association between m6A modifications and TIE and provide new insights into therapeutic strategies targeting m6A involvement in TIE.

**N6-methyladenosine (m6A) regulators**

**Writers**

The deposition of m6A is mainly catalyzed by a methyltransferase complex (MTC) comprising numerous components. As a core component of MTC, methyltransferase-like protein 3 (METTL3) is an S-adenosyl methionine (SAM)-binding protein that catalyzes the transfer of methyl groups in SAM to adenine bases in RNA, METTL14 stabilizes the structure of MTC and identifies the consensus motif DRACH, and Wilms tumor 1-associated protein (WTAP) promotes the recruitment of METTL3 and METTL14 [11, 12].

Fig. 1 The molecular mechanism of N6-methyladenosine (m6A) modification. m6A modification is a dynamic and reversible epigenetic modification that is regulated by ‘writers’ and ‘erasers.’ It is primarily catalyzed by the m6A methyltransferase complex comprising the main components METTL3/METTL14/WTAP and other regulatory proteins (RBM15/15B, KIAA1429, ZC3H13, CBLL1, and VIRMA). In addition, METTL16, METTL5, ZCCHC4, and PCIF1 are methyltransferases that directly catalyze m6A modifications in RNA molecules. The erasers mainly consist of FTO, ALKBH5, and ALKBH3. The ‘readers’ are binding proteins that recognize m6A modifications in the RNA. m6A modification can affect alternative splicing of pre-mRNA, mRNA degradation, mRNA stabilization, mRNA processing, and translation.
MTC, several other enzymes act as independent methyltransferases: METTL16 catalyzes m^6^A on U6 snRNA, ncRNAs, and pre-RNAs [13], zinc finger CCHC-type containing 4 (ZCCHC4) deposits m^6^A on 28S rRNA [14]. METTL5 adds m^6^A to 18S rRNA [15, 16] and phosphorylated CTD interacting factor 1 (PCIF1) catalyzes both m^6^A and a different RNA modification: N^6^,2'-O-dimethyladenosine (m^6^Am) [17–19]. However, with the advent of m^6^A-Crosslinking-Exonuclease-sequencing (m^6^ACE-seq), a newly developed technique for quantitative single-base-resolution sequencing of m^6^A and m^6^Am, which quantitatively map precise locations of transcriptome-wide m^6^A/m^6^Am in cells [20]. This technique can compensate for the shortcomings of past techniques, such as the poor resolution (~150 nt) of methylated RNA immunoprecipitation sequencing (MeRIP-seq), the time-consuming and inconvenient procedures (such as radioactive gel electrophoresis) of m^6^A individual-nucleotide-resolution cross-linking and immunoprecipitation (miCLIP), and their inability to detect m^6^A between different samples. It is argued that METTL16 does not methylate m^6^A directly at its specific ‘UACm^6^AGAGAA’ motif but controls intracellular SAM levels [20]. Knockdown of METTL16 resulted in the loss of m^6^A in the transcriptome, except for METTL16 directly methylated sites. Motif analysis also indicated that sequences located at the METTL16-dependent m^6^A sites delineate the METTL3-dependent “DRm^6^ACH” motif. METTL16 knockdown also reduced m^6^A at all 5 ‘UACAGAGAA’ sites and ‘UACAGAAAA’ sites within the 3’ UTR of Mat2a transcript, which encodes a SAM synthetase. All these results suggest that METTL16 may catalyze m^6^A through an indirect mechanism, likely via regulating MAT2A expression to control intracellular SAM levels.

**Erasers**

Fat mass and obesity-associated protein (FTO) is the first demethylase discovered in 2011, and another demethylase, AlkB homolog 5 (ALKBH5), was discovered 2 years later [21, 22]. Recent studies in 2017 have identified ALKBH3 as a demethylase responsible for removing m^6^A on transfer RNA (tRNA) [23]. All three demethylases belong to the alpha-ketoglutarate-dependent dioxygenase family and catalyze m^6^A demethylation in an Fe (II)- and α-ketoglutaric acid-dependent manner. With the discovery of these demethylases, m^6^A is considered a dynamic reversible process; however, many studies have argued that the physiological target of FTO is not m^6^A but m^6^Am [25]. First, several studies found that the m^6^A is deposited, it cannot be removed [26–28]. Second, the reaction rates of FTO toward m^6^A are low [21]. Third, the m^6^A demethylation function of FTO is not sequence-specific [29–33]. Lastly, transcriptome-wide analysis of FTO knockdown does not show a robust increase in m^6^A [34]. A detailed analysis of m^6^A peak intensities in wild-type and FTO knockdown mice to investigate the reasons for the lack of the global increase in m^6^A levels when FTO was knocked down. The results showed that the m^6^A peak exhibits a 5' UTR increase which is the site of m^6^Am. Further study also proved that FTO had nearly 100 times higher catalytic activity against m^6^Am compared to m^6^A, suggesting m^6^Am as the indeed substrate [25, 35]. Furthermore, FTO is demonstrated to demethylate m^6^Am of snRNA which may influence mRNA splicing [36]. This finding is confirmed by an antibody-independent technique MAZTER-seq, that neither knockdown nor overexpression of FTO impact the m^6^A levels in human embryonic stem cells and HEK293T cells. However, overexpressed ALKBH5 induces a subtle decrease in methylation levels [37]. However, many studies have demonstrated that FTO acts on specific m^6^A sites and impacts targeted genes in cancer. This discrepancy may be due to the limitations of the detection and statistical methods used in previous studies, which could not distinguish between m^6^A and m^6^Am. In contrast, demethylases may be recruited to specific transcripts or induced cytosolic translocation to activate the demethylation program in some cancers [38, 39]. However, FTO does not affect global m^6^A, possibly because it affects certain gene expressions that promote m^6^A. Overall, m^6^A demethylation is rare, and the reversibility is unlikely to be a major mechanism in most cells.

**Readers**

The m^6^A recognized by different readers can execute diverse downstream biological functions (Fig. 1). m^6^A is mainly recognized by the YT521-B homology domain-containing proteins (including YTHDC1, YTHDC2, YTHDF1, YTHDF2, and YTHDF3), which has an aromatic cage to specifically accommodate methyl mark [40,
YTHDC2 plays a role in spermatogenesis rather than promoting mRNA degradation in an m6A-dependent manner. Zaccara et al. proposed a distinctly unified model that all m6A sites bind all three YTHDF proteins with sequence motifs characteristic of superfamily 2 DExHBox helicases, an ankyrin repeat pair insertion, and two C-terminal HA2 and OB domain extensions, which confers YTHDC2 helicase activity. Studies have shown that YTHDC2 plays a critical role in the meiotic entry process by interacting with m6A-modified transcripts and reducing their stability or enhancing translation [44–47]. However, Jain et al. argued that the helicase activity of YTHDC2 is involved in spermatogenesis rather than recognizing m6A. To lay down the principles underlying its molecular function, they found that mutation of the m6A binding pocket of YTHDC2 has no significant effect on gametogenesis and mouse fertility. Cross-linking and immunoprecipitation (CLIP) data suggested that YTHDC2 binds to the site containing U-rich and UG-rich motifs and affects their steady-state levels rather than binding to m6A-modified sites to regulate translation. Mutation of the ATPase motif in the helicase domain of YTHDC2 did not affect meiotic entry. However, it blocked the progression of meiotic prophase I, causing catastrophic failure of spermatogenesis and leading to sterility [48]. It may be consistent that YTHDC2 recruits 5′-3′ RNA exonuclease XRN1 as a partner to reduce the target RNA stability [47]. This provides insight into how gene expression regulation has diversified over the course of evolution.

The YTHDF family contains three paralogs, YTHDF1, YTHDF2, and YTHDF3, each of which has been reported to have different functions with limited redundancy. YTHDF1 promotes mRNA translation, YTHDF2 enhances mRNA degradation, and YTHDF3 cooperates with YTHDF1 and YTHDF2 to accelerate translation and degradation [49–52]. However, the latest research proved that the YTHDF proteins enhance mRNA degradation in a redundant manner rather than translation efficiency [53]. Zaccara et al. proposed a distinctly unified model that all m6A sites bind all three YTHDF proteins to promote mRNA degradation in an m6A-dependent manner in HeLa cell and leukemia cell line models rather than enhance translation. This redundancy may be due to the similar characteristics of YTHDF proteins, including RNA-binding properties, m6A binding preferences and affinities in transcriptome, high confidence with RNA degradation machinery, and intracellular localization. The mRNA degradation efficiency was highest when all three proteins were present, and compensation cannot occur when all these proteins are depleted; the mRNA is the most stable. Notably, the previous studies that YTHDF paralogs promote translation, which was affected by bioinformatic and technical issues, may be incorrect [53]. Together, these diverse lines of evidence suggest that YTHDF proteins do not promote translation; however, we cannot exclude their role in promoting translation in other cell lines or conditions. Because YTHDF proteins show significantly different expression levels in different tissues, their ability may also be affected by different phosphorylation modes, specific stimuli, and cellular contexts [49, 54–58]. Lasman et al. found that this functional compensation is context-dependent, which affects mRNA stability via an m6A-dependent manner rather than translation [59]. They explored the effects of YTHDF proteins in vivo in mouse gametogenesis, postnatal viability, and in vitro in mouse embryonic stem cells (mESCs). Only YTHDF2 is crucial for mouse gametogenesis, which may be due to the different expression spatial or temporal space, cell types, and intracellular localization. YTHDF proteins show compensation for one another in a dosage-dependent manner during embryonic development and gestation. In mESCs, only triple knockout of YTHDF paralogs shows the effect on mRNA decay and differentiation.

In addition to these canonical readers, other proteins also act as indirect m6A readers with a distinct binding mechanism, including insulin-like growth factor 2 mRNA binding proteins (IGF2BPs) (including IGF2BP1, IGF2BP2, and IGF2BP3) and RNA binding proteins (RBPs) (including HNRNPA2B1, HNRNPC, and HNRNPG) [60–62]. Instead of directly recognizing and binding to m6A, these proteins bind to target RNAs via an indirect mechanism termed "m6A-switch", that m6A can alter its local RNA structure and enhance the accessibility of its base-paired residues or nearby regions to modulate protein binding. The m6A-switch is proven to regulate HNRNPC binding activities, affecting target mRNAs’ abundance and alternative splicing, demonstrating the regulatory role of m6A-switch on gene expression and RNA maturation [60]. The HNRNPA2B1 protein was initially proposed as a direct m6A mark reader interacting with the consensus motif via its RNA-recognition motifs (RRMs) and regulating alternative splicing [63]. However, Wu et al. found that HNRNPA2B1 may mediate the effects of m6A via the m6A-switch mechanism [62]. Their structural study showed no aromatic cage-like surface in crystal structures. HNRNPA2B1 does not recognize or show enhanced binding to the m6A-modified RNA substrates in vitro. In vivo study showed that only a small fraction of the m6A nuclear sites is located near HNRNPA2B1. Therefore, the m6A-switches may account for the enhanced HNRNPA2B1 binding to m6A [61]. Moreover,
HNRNPG is also proved an indirect reader to use the m^6^A-switch mechanism, which binds to m^6^A-modified RNAs by a low-complexity region [62]. The three IGF2BP paralogs are highly similar, with two N-terminal RRM domains and four C-terminal K-Homology (KH) domains, characterized by a conserved αβ-topology that can be structurally and functionally included in two di-domains (KH1-2 and KH3-4) [64]. Functionally, IGF2BP1-3 fortifies the stability and increases m^6^A-modified mRNAs translation efficiency [8]. Nevertheless, the latest research takes a different view of their roles as m^6^A readers. Sun et al. used the in vivo click selective 2-hydroxyl acylation and profiling experiments (icSHAPE), a technique they developed to map RNA structure in three compartments – chromatin, nucleoplasm, and cytoplasm, to help determine the precise relationship between RNA structure and cellular processes, including transcription, translation, RNA decay, RBP interaction, and RNA modification. They used the cytoplastic RNA structurome data to filter published CLIP-seq data and calculated m^6^A modification effect on protein binding. The results showed that while the canonical readers bind most strongly to the m^6^A sites, HNRNPC and IGF2BP protein binding peaks at a distance. RNA pull-down experiment showed that IGF2BP3 exhibits enhanced binding to the m^6^A-modified RNAs and uracil mutations compared to the unmethylated RNAs. This suggests that IGF2BP proteins may also be able to read the structural changes induced by m^6^A-switch [65].

**Impaired antigen processing presentation and tumor immune escape (TIE)**

Tumor antigens (tAgs) are expressed on the surface of cells by HLA-I and undergo two distinct pathways to induce an effective antitumor response. First, tAgs are taken up by dendritic cells (DCs) and cross-presented to prime naive CD8^+^ T cells [66]. Second, tAgs are directly presented by tumor cells to be recognized and killed by primed CD8^+^ T cells. Under constant T-cell selection, tumor cells can avoid immune recognition via different evasion mechanisms [67]. In both pathways, tumors impair antigen presentation by disrupting DC function or reducing HLA-I expression to escape immune recognition, eventually leading to TIE.

**Dendritic cell defects and TIE**

DCs take up dying tumor cells that release dangerous signals, undergo maturation, migrate to the draining lymph nodes, and process and load tAgs onto HLA-I to CD8^+^ T cells (Fig. 2). METTL3-mediated m^6^A modification was demonstrated to promote DC maturation and activation in an innate immune response model. Mechanistically, CD40, CD80, and TLR4 signaling adaptor Tirap transcripts are methylated by METTL3, promoting their translation in DC to enhance T cell activation and cytokine production [68]. In contrast, m^6^A modification generally displays the opposite role in antitumor immunity models. In melanoma and hepatocellular carcinoma (HCC), activating the β-catenin signaling pathway can prevent the infiltration of DCs and CD8^+^ T cells within the tumor microenvironment (TME) by inhibiting the secretion of the CC chemokine ligands CCL4 or CCL5, which directly impairs the therapeutic benefit of ICI [69, 70]. Numerous studies have demonstrated that m^6^A modifications can activate the β-catenin signaling pathway in different cancers (Table 1). For example, m^6^A modification promotes the stability of circRNA-SORE mRNA, which enhances sorafenib resistance through the circRNA-SORE/miR-103a-2-5p/miR-660-3p/Wnt2b/β-catenin pathway in HCC [72]. YTHDF2 decreases IncAY levels in an m^6^A-dependent manner, which promotes the proliferation and migration of HCC cells via the IncAY/BMI1/Wnt/β-catenin signaling pathway [85]. These studies have demonstrated that m^6^A modification promotes progression and drug resistance by activating the β-catenin signaling pathway in various cancer types. We envision that m^6^A may promote TIE by activating the β-catenin signaling pathway, which needs further studies. Prostaglandin E_2 (PGE_2) secretion in a cyclooxygenase 2 (COX2)-dependent manner can impair the accumulation and viability of DCs in tumors and suppress DC maturation [87–89]. In lung cancer (LC), HNRNPA2B1 upregulates COX2 expression, PGE_2 production, and promotes tumor growth [90]. Moreover, m^6^A modification induces TIE by increasing antigen degradation in DCs [91]. m^6^A modifies transcripts encoding lysosomal proteases to promote lysosomal cathepsins translation in DCs, which is associated with enhanced ingested antigens degradation [92, 93]. In summary, m^6^A modification plays a vital role in regulating the cross-presentation function of DCs and priming of CD8^+^ T cells, including maturation, migration, activity, and antigen degradation of DCs.

**Defective class I human leukocyte antigen presentation in tumors**

After priming with DCs, CD8^+^ T cells migrate from the draining lymph nodes to the tumor and recognize the tAgs present on HLA-I by the tumor cells for elimination. In addition to the failure in the cross-presentation function of DCs, malfunctions also occur in the tAg presentation pathway of tumor cells. The antigen processing and presenting machinery (APM) in tumor cells mainly consists of the ubiquitin-protease system and HLA-I complex. The presentation processes are divided into four steps: (i) processing proteins into peptides, (ii) peptide transportation, (iii) installation of peptides on the HLA-I
Fig. 2  Dendritic cells (DCs) in antitumor immunity. DCs are recruited into the tumor bed by chemokines, such as CC chemokine ligands 4 (CCL4), CCL5, and XC-chemokine ligand 1 (XCL1). FMS-like tyrosine kinase 3 ligand (FLT3L) promotes the differentiation and survival of DCs. Immature DCs take up dying tumor cells that release damage-associated molecular patterns, migrate to the draining lymph nodes, and process and load cancer antigens onto human leukocyte antigen (HLA)-I and HLA-II for presentation to CD8+ and CD4+ T cells, respectively. Naive CD4+ T cells are primed first, which allows DCs to prime CD8+ T cells via CD40-CD40L signaling. Moreover, intratumoral DCs generate chemokines CXC-chemokine ligand 9 (CXCL9) and CXCL10 to recruit effector CD8+ T cells from draining lymph nodes. Tumors can change DC functions to achieve tumor immune escape. Vascular endothelial growth factor (VEGF) prevents DC differentiation and maturation. Activation of β-catenin signaling and expression of prostaglandin E2 (PGE2) prevent the recruitment of DCs to the tumor bed by blocking chemokine secretion, including CCL4, CCL5, and XCL1. PGE2 prevents the recruitment and maturation of DCs. Tumor cells, CD4+ regulatory T cells (Treg), myeloid-derived suppressor cells (MDSCs), and M2 macrophages produce cytokines, including tumor growth factor-β (TGFβ), interleukin (IL)-6, IL-10, PGE2, and VEGF, to prevent DC maturation. CCL4, CC-chemokine ligands 4; CCL9, CXC-chemokine ligand 9; DAMP, damage-associated molecular pattern; DC, dendritic cell; FLT3L, FMS-like tyrosine kinase 3 ligand; HLA-I, class I human leukocyte antigen; IL-6, interleukin-6; MDSC, myeloid-derived suppressor cell; PGE2, prostaglandin E2; TGFβ, transforming growth factor-β; Treg cell, regulatory T cell; VEGF, vascular endothelial growth factor; XCL1, XC-chemokine ligand 1.
complex, and (iv) peptide-HLA-I complex translocation and presentation (Fig. 3). m^6_A modification has been reported to be correlated with APM in tumors, such as HCC, pancreatic cancer (PC), esophageal cancer (EC), and breast cancer (BC) [94–97]. In BC, higher m^6_A modification levels are associated with elevated expression of HLA-A and more tumor-infiltrating CD8^+ T cells, helper T cells, and natural killer cells, but decreased expression of programmed cell death protein ligand 1 (PD-L1), PD-L2, T-cell immunoglobulin and mucin-domain containing 3 (TIM3), and CCR4. Lower m^6_A modification is related to the hallmarks of PI3K/AKT signaling in cancer, KRAS signaling, angiogenesis, and shorter overall survival (OS) [97]. In HCC, downregulated METTL3 expression is related to increased MHC, co-stimulatory, and adhesion molecules [94]. However, current studies are based on the analysis of public databases that require further experimental verification.

Immunosuppressive cells and TIE

CD4^+ regulatory T cells (Tregs) and TIE

Based on different antigen signals and cytokine stimulations, CD4^+ T cells can differentiate into numerous subtypes, such as Tregs and helper T cells 1, 2, and 17 (Th1, Th2, and Th17). Tregs generally play pro-tumor roles, are immunosuppressive, and are associated with TIE [98]. Tregs inhibit the function of antitumor T cells by producing inhibitory cytokines, including interleukin (IL)-10, IL-35, and TGF-β [99]. Tregs can also inhibit the activation of CD8^+ T cells by expressing cytotoxic T lymphocyte antigen 4 (CTLA4), an inhibitory molecule [98]. Tregs inhibit T-cell activation by blocking the maturation and function of DCs [99]. Studies have shown that METTL3 regulates T-cell homeostasis and differentiation in mouse T cells. METTL3-mediated m^6_A modification promotes mRNA degradation of SOCS family genes and enhances T-cell differentiation and proliferation via the IL7R/JAK/STAT5 pathway [100]. In a further study, they demonstrated in vivo that METTL3-mediated m^6_A modification promotes the IL2-STAT5 pathway and immunosuppressive functions of Tregs [101].

Myeloid-derived suppressor cells (MDSCs) and TIE

MDSCs are potent immunosuppressive cells in cancer, promote tumor initiation and metastasis, and have attracted attention as targeted therapeutic interventions.
[102]. MDSCs induce TIEs via various pathways. First, MDSCs significantly inhibit MHC II expression, cross-presentation, DC activation, and T-cell stimulation by upregulating indoleamine 2,3-dioxygenase (IDO) and myeloperoxidase. Second, increased IDO levels enhance the differentiation and suppressive functions of Tregs. Moreover, the IDO-induced depletion of tryptophan attenuates cytotoxic T lymphocyte (CTL) proliferation. Furthermore, upregulated arginase 1 causes CTL disability and apoptosis [103]. In CC, the expression of METTL3 and CD33\(^+\) MDSCs in tumor tissues is higher than that in adjacent normal tissues, and their expression is positively correlated. Their levels in tumor samples are significantly related to poor disease-free survival (DFS) and OS. More importantly, the expression of METTL3 is an independent factor for DFS and OS in patients, whereas the number of CD33\(^+\) MDSCs is an independent predictor of DFS. Furthermore, knockdown of METTL3
potently reduces CD33+ CD11b+ HLA-DR− MDSCs and tumor-derived MDSCs in CD33+ or HeLa cells [104].

**Tumor-associated macrophages (TAMs) and TIE**

Macrophages are myeloid cells with various phenotypes, of which M1 and M2 subtypes are extreme. M1 can kill tumor cells by emerging as antigen-presenting cells (APCs) or generating nitric oxide, type 1 cytokines, and chemokines. M2 macrophages are activated by IL-4, IL-13, TGF-β, and/or glucocorticoids. M2 macrophages produce cytokines and type II chemokines to accelerate tumor growth. In turn, stromal and tumor-associated factors in the TME can polarize macrophages to the M2 type, particularly the TAM type, to drive TIE. In BC, M2 macrophages produce high levels of IL-10, which effectively attenuate CD8+ T cell-dependent responses to paclitaxel by downregulating IL-12 in intratumoral DCs [105]. FTO has been demonstrated to promote both M1 and M2 polarizations. Gu et al. found that FTO knockdown reduced the mRNA stability of STAT1 in M1 and PPAR-γ in M2 in a YTHDF2-dependent manner [106]. Furthermore, IGF2BP2 has been reported to shift M1 macrophages to M2 activation by stabilizing TSC1 and PPAR-γ in an m6A-dependent manner [107]. ALKBH5 was demonstrated to promote macrophage recruitment, and M2 polarization and phagocytosis in glioblastoma multiforme cells and induce immunosuppression in allograft tumors. ALKBH5-mediated m6A demethylation stabilizes NEAT1, leading to TAM recruitment and TIE via ALKBH5/NEAT1/CXCL8/IL-8 pathway [108].

In summary, immunosuppressive Tregs, MDSCs, and M2 macrophages can help tumors obtain TIE in different ways, which prevents the immune system from recognizing and destroying the tumor. These immunosuppressive cells are regulated by m6A modification to obtain TIE, which may be a promising target for antagonizing TIE and reinforcing the effect of antitumor immunotherapy.

**Immune checkpoint molecules and TIE**

Immune checkpoints have emerged as a large number of inhibitory pathways in the immune system that are critical for maintaining self-tolerance and regulating the duration and magnitude of the peripheral tissue physiological immune response to reduce collateral tissue damage. Tumors acquire TIE through these immune checkpoint pathways [109]. Co-inhibitory and co-stimulatory receptor signaling pathways play crucial roles in T cell activation, differentiation, effector function, and survival. Therefore, promising therapeutic approaches to reinvigorate a T-cell response can be achieved using inhibitors of co-inhibitory factors or agonists of co-stimulatory factors. The currently known co-inhibitory receptors include CTLA4, programmed cell death protein 1 (PD-1), PD-L1, lymphocyte activation gene 3, TIM3, and T cell immunoreceptor with immunoglobulin and ITIM domain (TIGIT). In addition, the co-stimulatory factors include B- and T-cell lymphocyte attenuator, glucocorticoid-induced tumor necrosis factor (TNF) receptor family-related protein, OX40, 41BB, and inducible T cell co-stimulatory. Next, we review the research progress on m6A in immune checkpoint regulation.

The discovery and clinical implementation of ICIs targeting CTLA4, PD-1, and PD-L1 have revolutionized cancer treatment and have been recognized by the 2018 Nobel Prize for Medicine and Physiology. PD-L1, also known as B7 homolog 1 or cluster of differentiation 274, is the first functionally characterized ligand of co-inhibitory PD-1. PD-L1 is a transmembrane protein expressed in various tissues, but it is primarily found in T cells, B cells, DCs, monocytes, and various tumor cells. However, PD-1 is mainly expressed on the surfaces of activated T cells, B cells, and DCs. T cells are the basis of the immune response, and their activation requires the interaction of two signals: (i) via the CD3 complex upon the binding of the T-cell receptor (TCR) expressed on the surface of T cells with HLA-I and its cognate peptide antigen on APCs or a target cell and (ii) another co-stimulatory receptor, CD28, which binds to the B7 family of co-stimulatory molecules mainly expressed on APCs. T cells differentiate, proliferate, produce cytokines, and subsequently form memory T cells [110]. PD-1 interacts with its ligand PD-L1, leading to the dephosphorylation of downstream TCR signaling molecules and inhibition of TCR-mediated IL2 production and T-cell proliferation, which causes TIE [111]. Currently, anti-PD-1/PD-L1 antibodies are approved for use in patients with a wide range of tumor types [112]. Antibodies against PD-1 include nivolumab, pembrolizumab, and cemiplimab. The PD-L1 antibodies include durvalumab, atezolizumab, and avelumab. m6A modification has been found to modulate PL-L1/PD-1 expression and promote immunosuppression, thus contributing to TIE [38, 113–115]. Knockdown of m6A regulators or targeted inhibitors can potentially enhance the immunotherapeutic effects of these antibodies. ALKBH5 was verified to promote PD-L1 expression, consequently reshaping TME and affecting immunotherapy efficacy in intrahepatic cholangiocarcinoma. Knockdown of ALKBH5 increases m6A modification in the 3'UTR of PD-L1 mRNA, thereby promoting its degradation in a YTHDF2-dependent manner. Moreover, ALKBH5 inhibits the expansion and cytotoxicity of T cells by promoting PD-L1 expression. Single-cell mass cytometry analysis showed that ALKBH5 promotes PD-L1 expression in monocytes/macrophages and reduces the infiltration of MDSCs. Analysis of samples from patients receiving anti-PD1 immunotherapy showed that tumors with strong
nuclear expression patterns of ALKBH5 are more sensitive to PD-L1 immunotherapy [115]. FTO promotes melanoma tumorigenesis and inhibits anti-PD-1 blockade immunotherapy. FTO knockdown increases m6A modification of PD-1 (PDCD1), CXCR4, and SOX10, contributing to enhanced RNA decay via the m6A reader YTHDF2 in melanoma cells. It has been consistently proven that loss of FTO sensitizes melanoma cells to interferon-γ and sensitizes melanoma to anti-PD-1 treatment in mice, which depends on adaptive immunity [38]. In addition to PD-1/PD-L1, other immune checkpoint molecules are also associated with m6A modifications and related regulators, such as CTLA-4, TIGIT, and TIM-3 [116, 117].

**Speculative functions of m6A modification in TIE**

In addition to the mechanisms discussed above, many other signaling pathways can modulate TIE, including metabolic alterations, acquisition of stemness, and epithelial-mesenchymal transition (EMT). m6A modification has been proven to play crucial roles in these pathways. Therefore, we speculate that m6A may also impact TIE via these pathways, which are discussed in the following section.

**Aerobic glycolysis and TIE**

Despite aerobic conditions, tumor cells prefer to produce energy through glycolysis rather than aerobic oxidation, along with more lactic acid generation, known as the Warburg effect [118]. Excess lactic acid acidifies the TME, endows tumor cells with stronger viability and aggressiveness, and has been identified as an important therapeutic target [119]. More importantly, the acidic TME impairs the immune response by weakening cytotoxic T cell function, blocking DC maturation, and enhancing helper cell activities [120]. In melanoma, ALKBH5 knockdown effectively promotes sensitivity to anti-PD-1 treatment. Mechanistically, during anti-PD-1 treatment, ALKBH5 promotes lactate generation and consequent accumulation of Tregs and MDSCs in TME by stabilizing Mct4/Slc16a3 mRNA, which is a pivotal enzyme mediating the transmembrane transport of lactate [121]. Many studies have found that m6A modifications play critical roles in regulating glycolysis.

The glycolysis process in tumor cells and the regulation of glycolysis-related enzymes by m6A are summarized in Fig. 4 and Table 2. Glucose, the feedstock for glycolysis, is transported into the cells by glucose transporters (GLUTs), which are membrane proteins on the cell surface. Abnormal expression of GLUTs promotes glucose intake and glycolysis. In colorectal cancer (CRC), METTL3 promotes glycolysis and cancer progression in an m6A-dependent manner. METTL3-mediated m6A modification promotes SLC2A1 (GLUT1) and hexokinase 2 expression through IGF2BP2/3-dependent mRNA stability regulation, and activates glycolysis [122]. METTL3 was also shown to promote CRC progression by activating the m6A/GLUT1/mTORC1 pathway. Mechanistically, METTL3 promoted GLUT1 translation in an m6A-dependent manner, increasing glucose uptake and lactate production and further activating the mTORC1 signaling pathway [123].

Glycolysis is triggered after glucose is taken into cells. Its progression is maintained by several enzymes, such as hexokinase (HK), aldose enzyme, glucose phosphate isomerase (GPI), phosphofructokinase (PFK), aldolase (ALDO), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), phosphoglycerate kinase (PGK), phosphoglycerate mutase (PGM), enolase (Eno), pyruvate kinase (PK), pyruvate dehydrogenase (PDH), and lactate dehydrogenase (LDH), which have been reported to be regulated by m6A modification (Fig. 4, Table 2). In cervical cancer (CC), METTL3 promotes proliferation and aerobic glycolysis of CC cells. METTL3 binds to the 3′-UTR of HK2 mRNA to catalyze m6A modification, which is recognized by YTHDF1 to enhance HK2 stability [126]. In general, m6A is shown to enhance glycolysis by regulating the expression of key enzymes. METTL3 promotes glucose uptake, lactate generation, and ATP level, which can be reversed by METTL3 knockdown. Downregulation of METTL3 suppresses tumor growth and glycolysis progression in tumor cells and xenograft mouse models. Since m6A has the function of reprogramming energy metabolism, we hypothesized that this process is accompanied by reprogramming TME. Consequently, targeting m6A may effectively overcome TIE and effectively treat a variety of cancers in combination with immunotherapy.

In tumor cells, hypoxia-inducible factor-1α (HIF-1α) has been reported to enhance glycolysis by upregulating glycolysis-related enzymes, inhibiting PDH and mitochondrial oxidative phosphorylation, and subsequently leading to TME acidification and TIE [173–175]. METTL3 promotes glycolysis and malignant biological behaviors of HCC cells by methylating HIF-1α [133]. Moreover, the m6A reader YTHDC2 can promote metastasis by enhancing HIF-1α translation in CRC [134]. However, further studies are required to determine whether this occurs by regulating m6A modification. Although m6A modification has been shown to affect glycolysis from multiple pathways, its effect on TIE deserves further exploration.

**Cancer stem cell-like characteristics and TIE**

Cancer stem cells (CSCs) are subpopulations with strong self-renewal ability, pluripotency, and tumorigenicity that are closely related to tumor initiation, metastasis, drug resistance, and recurrence. They can also obtain
Aberrant m\(^6\)A modifications are related to the initiation and maintenance of CSC-like phenotypes in tumor cells (Table 2). FTO promotes the self-renewal of leukemia stem cells (LSCs) and TIE, which can be reversed by small-molecule FTO inhibitors (FTOis) [39]. FTOis (CS1 and CS2) impairs LSC self-renewal properties by occupying the catalytic pocket of FTO to inhibit its demethylase activity, which leads to increased m\(^6\)A abundance and decreased expression of \(MYC\) and \(CEBPA\). In contrast, FTO promotes \(LILRB4\) expression, an immune checkpoint gene, by demethylating \(LILRB4\) to inhibit the m\(^6\)A-mediated degradation. FTOis effectively overcomes TIE and sensitizes acute myeloid leukemia (AML) cells to T cell cytotoxicity. More importantly, LSCs share numerous characteristics with hematopoietic stem cells (HSCs). Therefore, eliminating LSCs without damaging HSCs as much as possible is the key to treating leukemia. Knockdown of \(YTHDF2\) promotes HSC self-renewal to enhance HSC ex vivo expansion without any noticeable lineage bias or leukemic potential by stabilizing \(Tal1\) mRNA [177]. This may help overcome the limitations of umbilical cord blood in treating leukemia, as there are insufficient HSCs in a single human umbilical cord blood unit. METTL3 promotes glioblastoma stem cell (GSC) self-renewal and tumorigenesis by enhancing m\(^6\)A modifications to stabilize transcripts of some CSC-related genes, such as \(AMAD19\), \(SOX2\), \(SRSF3/6/11\), \(NOTCH1\), and \(HES1\) [136–139]. Furthermore, \(YTHDF2\) promotes glioblastoma stemness by stabilizing \(MYC\) and \(VEGFA\) in an m\(^6\)A-dependent manner [153]. In a word, m\(^6\)A promotes the initiation and maintenance of CSC-like phenotypes in tumor cells. Inhibitors targeting m\(^6\)A can effectively reverse stem-like phenotype and block TIE. Further studies are needed to dissect whether m\(^6\)A reverses TIE by affecting CSCs. What is more, none of the existing immunotherapy approaches selectively targets CSCs. Therefore, understanding this mechanism will provide a solution for optimizing or identifying new immunotherapy strategies and their combinations.

![Diagram of glucose metabolism in tumor cells](image-url)

**Fig. 4** Overview of the regulation of tumor immune escape-associated glycolytic enzymes by N\(^6\)-methyladenosine modification in tumor cells. The afferent blood delivers glucose to tissues, where it reaches the cells by diffusion. Glucose is taken up by specific glucose transporters (GLUTs), which are first converted to glucose-6-phosphate by hexokinase (HK) and then to pyruvate by various enzymes, including glucose phosphate isomerase (GPI), phosphofructokinase (PFK), aldolase (ALDO), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), phosphoglycerate kinase (PGK), phosphoglycerate mutase (PGM), enolase (Eno), pyruvate kinase (PK), pyruvate dehydrogenase (PDH), and lactate dehydrogenase (LDH). ALDO, aldolase; Eno, enolase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GLUT, glucose transporter; GPI, glucose phosphate isomerase; HK, hexokinase; LDH, lactate dehydrogenase; PDH, pyruvate dehydrogenase; PFK, phosphofructokinase; PGK, phosphoglycerate kinase; PGM, phosphoglycerate mutase; PK, pyruvate kinase.
## Table 2: The functions and mechanisms of m6A modification on pathways affecting TIE

| Regulator | Cancer                  | Mechanism                                                                 | Functional classification                                                                 | Refs          |
|-----------|-------------------------|---------------------------------------------------------------------------|------------------------------------------------------------------------------------------|---------------|
| METTL3    | CRC                     | Stabilizes HK2 and GLUT1.                                                 | Enhances glycolysis to promote CRC progression.                                           | [122]         |
| METTL3    | CRC                     | Promotes GLUT1 translation.                                               | Promotes CRC tumorigenesis by activating m6A/GLUT1/mTORC1 axis.                         | [123]         |
| IGF2BP2   | PC                      | Stabilizes GLUT1.                                                         | Promotes glycolysis and proliferation of PC.                                              | [124]         |
| HNRNPA2B1 | PC                      | Promotes GLUT1 expression.                                                | Promotes glycolysis and proliferation of PC cell.                                         | [125]         |
| METTL3    | CC                      | Promotes HK2 stability.                                                   | Enhances CC aerobic glycolysis and tumorigenesis.                                         | [126]         |
| WTAP      | lymphoma                | Promotes HK2 stability.                                                   | Enhances lymphoma cell proliferation.                                                     | [127]         |
| WTAP      | GC                      | Stabilizes HK2.                                                           | Promotes GC cell proliferation and glycolytic capacity.                                  | [128]         |
| IGF2BP2   | glioblastoma            | Promotes HK2 mRNA stability.                                              | Enhances glioblastoma aerobic glycolysis.                                                | [129]         |
| FTO       | leukemia                | Promotes stability of PFKP and LDHB mRNA.                                | Enhances aerobic glycolysis of leukemia cells.                                            | [130]         |
| METTL3    | CC                      | Promotes the translation elongation and mRNA stability of FOX1.          | Promotes CC cell glycolysis and proliferation.                                           | [131]         |
| METTL3    | HCC                     | METTL3/IGF2BP1-mediated m6A stabilizes LNCAROD.                           | Promotes HCC cell glycolysis, proliferation, migration, invasion and chemoresistance via METTL3/IGF2BP1/LNCAROD/PKM2 pathway. | [132]         |
| METTL3    | HCC                     | Promotes HIF-1α expression.                                               | Promotes the metabolic reprogramming and malignant biological behaviors of HCC cells.  | [133]         |
| YTHDC2    | CRC                     | Promotes HIF-1α translation.                                              | Promotes CRC metastasis.                                                                 | [134]         |
| METTL3    | CRC                     | Stabilizes SOX2.                                                          | Promotes cell self-renewal, stem cell frequency and migration in vitro and suppresses CRC tumorigenesis and metastasis in vivo. | [135]         |
| METTL3    | glioblastoma            | Promotes SOX2 stability.                                                  | Promotes the GSC maintenance and glioma cell differentiation.                            | [136]         |
| METTL3/14, FTO | glioblastoma             | Promotes AMAD19 expression.                                               | FTO promotes and METTL3 inhibits GSCs growth and self-renewal.                           | [137]         |
| METTL3    | glioblastoma            | Stabilizes SRSF3/6/11.                                                    | Promotes the growth and self-renewal of GSCs.                                            | [138]         |
| METTL3    | glioblastoma            | Stabilizes NOTCH1 and HES1.                                               | Promotes GSC maintenance and glioma progression.                                         | [139]         |
| METTL3    | bladder cancer          | Promotes AFF4 expression.                                                 | Promotes self-renewal of bladder cancer stem cells.                                      | [140, 141]    |
| METTL3    | cutaneous squamous cell carcinoma | Promotes ΔNp63 expression.                               | Promotes cutaneous squamous cell carcinoma cell stem-like properties.                 | [142]         |
| METTL3    | CRC                     | Stabilizes CBX8 mRNA.                                                    | Promotes stemness and suppresses chemo sensitivity of CRC.                              | [143]         |
| METTL3    | LC                      | Promotes RMRP stability.                                                  | Promotes the CSCs properties and EMT, which promote the resistance to radiation therapy and cisplatin. | [144]         |
| METTL3    | kidney cancer           | Promotes ABCD1 translation.                                               | Promotes cell migration, spheroid formation and tumor growth.                           | [145]         |
| METTL3    | oral squamous cell carcinoma | Downregulates p38 expression.                       | Promotes stem-like capacities in oral squamous cell carcinoma cells.                   | [146]         |
| METTL14   | leukemia                | Enhance MYB and MYC mRNA stability and translation.                      | Promotes self-renewal of LSCs.                                                          | [147]         |
| METTL14   | EC                      | Upregulates mir-99a-5p by promoting pri-mir-99a processing.              | Promotes CSCs persistence and the radio-resistance.                                      | [148]         |
Epithelial-mesenchymal transition and TIE

EMT is a cellular reprogramming process that detaches epithelial cells from each other and the underlying basement membrane, eventually transforming them into mesenchymal cells. EMT increases the developmental and metastatic potential of cancer cells and drug resistance [178]. An increasing number of studies have shown that EMT can regulate antitumor immunity. For example, in melanoma cells, SNAIL-induced EMT stimulates the secretion of TGF-β and thrombospondin 1, which promotes the formation of Treg cells and impairs the antigen-presenting capacity of DCs [179]. This attenuates the immunogenicity of melanoma cells and their sensitivity to immunotherapy, which can be restored by inhibiting SNAIL [179]. Furthermore, EMT promotes immunosuppression and TIE in BC cells. Tumors derived from more...
mesenchymal carcinoma cell lines express lower MHC-I and higher PD-L1 and contain within their stroma Treg cells, M2 macrophages, and exhausted CD8+ T cells than tumors derived from more epithelial carcinoma cell lines [180]. The above evidence indicates that EMT can promote immunosuppression of tumor cells and obtain TIE.

EMT promotes the expression of the EMT-inducing transcription factors (TFs) ZEB, SNAIL, and TWIST, which activate mesenchymal state-related genes (N-cadherin, vimentin, fibronectin, β1 and β3 integrins, and matrix metalloproteinases) and resilient epithelial state-related genes (E-cadherin, epithelial cell adhesion molecule, occludins, claudins, α6β4 integrins, and cytokeratins). Studies have demonstrated that upregulated m6A levels promote EMT in cancer cells, such as HeLa cells (CC), HepG2 cells (liver cancer), Huh7 cells (liver cancer), and A549 cells (LC). Increased m6A modification promotes SNAIL mRNA translation, which can be enhanced or inhibited by ALKBH5 or METTL3 knockdown. Depletion of METTL3 blocks invasion, migration, and EMT of cancer cells and tumor metastasis [181]. Furthermore, METTL3-mediated m6A also potentiates EMT by regulating integrin-β1 and ZMYM1/E-cadherin pathway [159, 169]. In addition to these EMT-inducing TFs, m6A also induces EMT by regulating the expression of other genes, such as MALAT1, HMGAI, circ1662, miR-20a-5p, FOXM1, DLXAP9, YAP, and ZBTB14 [160–163, 165, 166, 171, 172]. In addition to its roles in regulating RNA stability and translation processes, m6A has been found to promote EMT by promoting the splicing of precursor miRNAs. For example, METTL3-mediated m6A modification promotes the splicing of precursor miR-143-3p to produce mature miRNAs, thus contributing to enhanced EMT via the METTL3/miR-143-3p/VASH1 pathway in LC cells [164]. In liver cancer, METTL3 determines the fate of the HSP5 transcript to process it into circHSP5 rather than mRNA. Increased circHSP5 acts as a miR-370 sponge to promote HMGAI expression and potentiate EMT [167]. Abnormal m6A promotes EMT by regulating EMT-inducing TFs and other genes; however, its role in TIE remains unclear. Whether regulation of EMT through m6A modification can attenuate TIE and promote immunotherapy efficacy remains to be further explored.

**Targeting m6A modification in cancer therapy**

The m6A levels of specific RNA transcripts have been shown to influence tumor development [182]. Therefore, inhibitors targeting m6A regulators may be effective new approaches for tumor therapy (Table 3). Rhein derived from the rhizome of *Rheum palmatum*, which was identified as the first competitive inhibitor target Alkb subfamily by structure-based in silico high-throughput screening and further structural optimization [183]. It competitively bounds to FTO or AlkB catalytic domain to form a complex and prevent the recognition of m6A substrates inside cells, which can increase the cellular m6A on mRNA. Rhein shows several bioactivities; however, its cellular targets remain largely unknown [196, 197]. Rhein also inhibits ALKBH2 activity, which is responsible for demethylating N6-methyladenosine modification in vitro. Furthermore, rhein inhibits other Fe2+- and 2OG-dependent hydroxylases by high-throughput screening in the NIH Molecular Libraries Probe Development Center Network (MLPCN) program, including the Jumonji domain containing 2A and 2E (JMJD2) histone demethylases and prolyl-4-hydroxylase. Rhein binding to FTO still has the possibility of multiple orientations. The crystal structure of small molecules in complexes with FTO has not been determined. In addition, increased m6A distributions due to rhein in cells may result from the direct inhibition of cellular demethylation via FTO or other members of nucleic acid demethylases. Characterizing the cell specificity of rhein will also be important to demonstrate its use as a cellular probe for nucleic acid demethylase in future studies. Rhein has been shown to have anticancer activity against various cancers. For example, rhein inhibited tumor growth in 4T1 BC xenografts [184]. Another inhibitor, meclofenamic acid (MA), was identified by a high-throughput fluorescence polarization assay and selectively inhibited FTO demethylation of m6A over ALKBH5 [186]. This slightly higher selectivity for FTO in the Alkb subfamily mainly depends on its structure. MA is neither a mimic of 2OG nor a chelator of iron, and the structural complex of MA bound to FTO is a β-hairpin motif which is a part of the nucleotide recognition lid (NRL) for providing hydrophobic interactions with MA. In contrast to FTO, ALKBH5 lacks this part of the NRL loop, resulting in leakage upon MA binding. In addition, MA could not inhibit ALKBH2 and ALKBH3 which have this region of NRL. The existence of the hydrophilic and bulky residues in the part of the NRL might significantly disturb the inhibitor MA binding to ALKBH2 and ALKBH3. Based on this structural complex, it should be possible to design more optimized analogs for FTO specificity and potency. In addition, MA increases m6A abundance in HeLa cells in an FTO activity-dependent manner. In glioblastoma, MA2 (an ethyl ester form of MA) significantly inhibits GSC growth and self-renewal, effectively suppresses GSC-induced tumorigenesis, and prolongs the lifespan of GSC-grafted mice [137]. In later studies, MA2 was shown to enhance the effect of the chemotherapeutic drug temozolomide on the suppression of glioma...
| Drugs | Regulator | Cancer | Function | Structure | Refs. |
|-------|-----------|--------|----------|-----------|-------|
| Rhein | FTO       | BC     | Suppresses tumor growth of BC in vivo. | ![Structure](image1.png) | [183, 184] |
|       |           |        | Augments antiproliferative effects of atezolizumab based on BC regression. | | [185] |
| MA2   | FTO       | glioblastoma | Inhibits GSCs growth and self-renewal in vitro, and tumor growth in vivo. | ![Structure](image2.png) | [137, 186] |
|       |           |        | Enhances the effect of the chemotherapy drug temozolomide on suppressing proliferation of glioma cells. | | [187] |
| MO-I-500 | FTO     | BC     | Inhibits the survival and/or colony formation of a triple-negative inflammatory BC cell line. | ![Structure](image3.png) | [188–190] |
| FB23-2 | FTO       | AML    | Suppresses proliferation and promotes the differentiation/apoptosis of AML cells and in vitro, and inhibits tumor growth in vivo. | | [191] |
|       |           |        | | ![Structure](image4.png) | | |
| R-2HG | FTO       | AML    | Inhibits cell growth, promotes cell cycle arrest and apoptosis of leukemia cells. | | [192] |
| CS1   | FTO       | AML, BC, PC, and glioblastoma | Suppresses CSC maintenance and immune evasion of AML. | | [39] |
| CS2   | IGF2BP1   | LC, OC | Inhibits proliferation and anchorage-independent growth of IGF2BP1-positive cancer cells. Blocks tumor cells' growth and spread in xenograft tumors. Synergizes with palbociclib at low concentrations of both compounds. | | [193, 194] |
| BTYNB | METTL3    | AML    | Inhibits AML cells growth and promotes differentiation and apoptosis in vitro, and disrupts engraftment and prolonged survival in vivo. | | [195] |

**Abbreviations:** AML acute myeloid leukemia, BC breast cancer, CSC cancer stem cell, GSC glioblastoma stem cell, LC lung cancer, MA meclofenamic acid, OC ovarian cancer, PC pancreatic cancer, R-2HG R-2-hydroxyglutarate
cell proliferation [187]. Subsequently, many FTOis can effectively inhibit the proliferation, promote the apoptosis of AML cells in vitro and suppress tumor growth in vivo, including FB23-2, R-2HG, CS1, and CS2 [39, 191, 192].

In addition to FTO, other m^6^A regulators are key targets for treating m^6^A-associated tumors. BTYNB is screened as a potent and selective inhibitor among 16,000 small molecules via the fluorescence anisotropy-based assay, which inhibits IGF2BP1 binding to a specific high-affinity binding site in the coding region stability determinant of c-Myc mRNA. BTYNB effectively reduces the expression of c-Myc mRNA and protein. It inhibits proliferation and anchorage-independent growth of IGF2BP1-positive cancer cells [193]. Furthermore, IGF2BP1 acts as the dependent E2F-transcription super-enhancer. The E2F pathway is regulated by IGF2BP1 in an m^6^A-dependent manner. BTYNB blocks E2F1 expression at both mRNA and protein levels, as well as inhibits E2F/IGF2BP1-driven gene expression by reducing the binding of IGF2BP1 to E2F1 mRNA. BTYNB effectively blocks tumor cells' growth and spread in xenograft tumors. Moreover, BTYNB synergizes with palbociclib at low concentrations of both compounds, suggesting that BTYNB is beneficial for combination therapy to impinge tumor cell proliferation [194]. However, BTYNB showed the ability to reduce IGF2BP1-dependent stabilization of mRNAs, and its putative off-target effects are unknown. Therefore, we hypothesized that BTYNB might interfere with the interaction between IGF2BP1 and m^6^A sites. STM2457 is a highly potent and selective inhibitor of METTL3 without disrupting the METTL3–METTL14 complex, identified by high-throughput screening. STM2457 effectively inhibits AML cell growth and promotes differentiation and apoptosis while reducing m^6^A levels of known AML-related mRNAs to block their translation and expression. In in vivo studies, STM2457 disrupted engraftment and prolonged survival in AML mouse models [195]. In addition to these competitive inhibitors, there are other ways to inhibit the functions of m^6^A regulators. First, the catalytic capacity of METTL3 depends on the heterodimer structure formed with METTL14; hence, it would be reasonable to design inhibitors based on protein-protein interaction strategies. Second, structural analysis of METTL3 suggested that the binding sites of the substrate SAM are merged into a large pocket; therefore, the development of bi-substrate inhibitors occupying both binding sites may be another effective strategy. Third, the proteolysis-targeting chimeras strategy is a promising technology to degrade target proteins via proteasomes. Targeted m^6^A modification for clinical application is still in the initial stage and has not yet entered clinical trials. However, with increasing knowledge of the function and mechanism of m^6^A modification in cancer, it is expected that drugs targeting m^6^A modification will be developed and applied to clinical treatment in the near future.

**Targeting m^6^A modification in TIE**

Regulation of the antitumor immune response by m^6^A RNA methylation is still in its infancy. However, recent studies have shown the possibility of combining immunotherapy with newly developed m^6^A regulator inhibitors for cancer therapy. Rhein significantly enhances the antiproliferative effects of atezolizumab (an anti-PD-L1 antibody) in 4T1BC xenografts. Moreover, the proportion of CD8^+^ T cells in the spleen and tumor is significantly increased in the combination therapy group and is significantly different from that in the monotherapy groups. Serum levels of TNF-α and IL-6 are significantly elevated in the rhein and combination therapy groups. In addition, the levels of various apoptotic factors in the tumor tissues are significantly higher in the combination treatment group [185]. Two FTOis are identified by structure-based virtual screening, CS1 and CS2, which show strong antitumor activity in leukemia. Inhibition of FTO by CS1 or CS2 significantly suppresses LSC self-renewal and promotes the immune response by suppressing the expression of the immune checkpoint gene LILRB4. They reverse TIE by abolishing the FTO-induced stability of LILRB4 mRNA and enhancing the sensitivity of AML cells to T-cell cytotoxicity [39]. ALKBH5 promotes lactate generation and Tregs and MDSC accumulation by stabilizing Mct4/Slc16a3 mRNA in melanoma cells. A specific ALKBH5 inhibitor, ALK-04, is identified by in silico screening of compounds using the X-ray crystal structure of ALKBH5 and by performing structure-activity relationship studies on a library of synthesized compounds. ALK-04 potently enhances anti-PD-1 therapy response in in vivo experiments [121]. These studies show not only the inhibition of m^6^A demethylases as a potential anticancer target but also their potential to reverse TIE. Although inhibitors of m^6^A modification regulators have been demonstrated to have anticancer roles by modulating tumor immunity, none have been tested in a clinical setting.

**Conclusions and perspectives**

In this review, we outline the different TIE mechanisms and summarize the increasing excitement surrounding the development of the regulatory roles of m^6^A modification involved in these mechanisms. Due to many new related discoveries in recent years, updating the academic progress of m^6^A modification is still necessary. Here, we summarize the research progress of m^6^A modification and the core function of m^6^A in TIE. Although
m^6^A modification is directly related to TIE, the exact molecular mechanism underlying its regulation remains unclear. The complex TIE mechanism in tumor cells is the cause of the low response rate to immunotherapy. Therefore, m^6^A modification may be considered a potential candidate target for targeting the TIE mechanism and is expected to be vital to overcoming immunotherapy-related challenges.

In the pathogenesis of tumors, m^6^A modification regulates RNA splicing, decay, nuclear export, stability, and translation, promotes the expression of onco-genes, or inhibits the expression of tumor suppressor genes, thereby inducing TIE. The putatively dynamic and reversible characteristics of m^6^A modifications make them attractive in the field of anticancer therapy. In light of new findings on the physiological roles of m^6^A regulators. At present, it is more important to clarify the ‘real’ regulator and their specific physiological function in certain cancers. This will help to explore the clear mechanism of m^6^A in TIE and target it more accurately. m^6^A RNA demethylase inhibitors have shown the potential to enhance immunotherapy [39, 121, 185]. The m^6^A methyltransferases METTL3 and METTL14 also show antitumor functions by reprogramming macrophages [198, 199]. SAM, the methyl donor for RNA, has shown anticancer activity in various cancer types by targeting histone methylation and DNA hypomethylation. It is unknown whether SAM inhibits tumor growth and metastasis by upregulating m^6^A RNA methylation levels or promoting immunotherapy. This means that m^6^A agonists, not just inhibitors, may also inhibit the growth of certain tumors. In addition, m^6^A modification exhibits cellular heterogeneity; that is, the same writer, eraser, and reader proteins may have different biological functions in different cells. This may cause it to act in the opposite manner in tumor or immune-related cells. Furthermore, other hypotheses could explain the paradox. First, m^6^A regulators may function independently of their m^6^A catalytic activity. Second, as recognized by different readers, genes modified by m^6^A modification can undergo different fates. Third, the location of m^6^A modifications in different regions of the same mRNA transcript may lead to different results. Therefore, additional studies are required to clarify the roles of m^6^A modifications in TIE, including the contributions of specific regulators, targets, modes of action, and TME.

Further studies are required to determine whether m^6^A modification is likely to provide new insights into identifying patients with tumors susceptible to specific drugs, identifying prognostic indicators, and developing targeted drugs. We believe that this approach is highly personalized. Utilizing a detailed understanding of m^6^A levels and each patient’s immune status will be the basis for the next step in immunotherapy. In addition, m^6^A status, the TME-infiltration characteristics, and the immune system can change over time, especially during treatment. Therefore, continuous monitoring of m^6^A regulators and immune markers is essential for developing and adjusting treatment regimens.

Both DNA and histone methylation inhibitors have been shown to enhance the efficacy of immunotherapy. Therefore, the mechanism by which m^6^A modification interacts with DNA and histone epigenetics to regulate gene expression and whether there is a potential association between m^6^A modification and other types of methylation remains unclear. In addition, based on the theories and mechanisms obtained from these studies, we explored whether m^6^A inhibitors combined with other epigenetic drugs can synergistically promote immunotherapy. Moreover, dual inhibitors targeting both m^6^A regulators and immune checkpoints may achieve better efficacy and fewer side effects.

With the continuous development of this field, it will be necessary to continue to study the mechanism of m^6^A modifications leading to TIE as a theoretical basis and simultaneously accelerate clinical trials to translate the theories obtained. In this way, the outcomes of patients with cancer can be further improved.

**Abbreviations**

3′ UTR: 3′ untranslated region; 5′ UTR: 5′ untranslated region; ALKBH5: Alkb homolog 5; ALDO: Aldolase; AML: Acute myeloid leukemia; APC: Antigen-presenting cell; APM: Antigen processing and presenting machinery; BC: Breast cancer; CC: Cervical cancer; CLIP: Cross-linking and immunoprecipitation; COX2: Cyclooxygenase 2; CRC: Colorectal cancer; CTL: Cytotoxic T lymphocyte; CTLA4: Cytotoxic T lymphocyte antigen 4; CSC: Cancer stem cell; DC: Dendritic cell; DFS: Disease-free survival; EC: Esophageal cancer; EMT: Epithelial-mesenchymal transition; Eo: Eosinophil; EOT: Epithelial origin tumor; F: Flow cytometry; FMT: Faecal microbiota transplantation; GC: Gastric cancer; GEM: Gemcitabine; GPCR: G protein-coupled receptor; GLUT: Glucose transporter; GR: Glucose regulated protein; HCC: Hepatocellular carcinoma; HIF-1α: Hypoxia-inducible factor 1α; HK: Hexokinase; HLA: Class I human leukocyte antigens; HSC: Hematopoietic stem cell; ICC: Immune checkpoint combination; icSHAPE: In vivo click selective 2-hydroxyl acylation and profiling experiment; IDO: Indoleamine 2,3-dioxygenase; IGF2BP: Insulin-like growth factor 2 mRNA binding protein; I-10: Interleukin-10; IMJ2: JunMoni domain containing 2; KH: K homology; LC: Lung cancer; LDH: Lactate dehydrogenase; LSC: Leukemia stem cell; m^6^A: N^6^-methyladenosine; m^6^ACE-seq: m^6^A-Crosslinking-Exonuclease-sequencing; m^6^Am: N^6^,2′-O-dimethyladenosine; MA: Meclofenamic acid; MDSC: Myeloid-derived suppressor cell; MelRIP-seq: Methylated RNA immunoprecipitation sequencing; mESC: Mouse embryonic stem cell; METTL3: Methyltransferase-like protein 3; MHC: Major histocompatibility complex; miCLIP: m^6^A individual-nucleotide-resolution cross-linking and immunoprecipitation; MLPCN: Molecular Libraries Probe Development Center Network; MTC: Methyltransferase complex; NOL: Nucleotide recognition lid; OS: Overall survival; PC: Pancreatic cancer; PCIF1: Phosphorylated CTD interacting factor 1; PDH: Pyruvate dehydrogenase; PD-L1: Programmed death ligand 1; PD-1: Programmed cell death protein 1; PGE2: Prostaglandin E2; PFK: Phosphofructokinase; PGK: Phosphoglycerate kinase; PGM: Phosphoglycerate mutase; PK: Pyruvate kinase; RBP: RNA binding protein; RRN: RNA recognition motif; SAM: S-adenosyl methionine; tAg: tumor antigen; TAM: Tumor-associated macrophages; TCR: T-cell receptor; TF: Transcription factor; Th1: Type 1 helper T cell; TIE: Tumor immune escape; TIGIT: T cell immunoreceptor with immunoglobulin and ITIM domain; TIM3: T-cell immunoglobulin and mucin-domain containing 3; TME: Tumor microenvironment; tAg: tumor antigen; tAg: tumor antigen; UTR: Untranslated region; UTR: Untranslated region; VEGF: Vascular endothelial growth factor; VEGFR2: Vascular endothelial growth factor receptor 2; VHL: Von Hippel-Lindau; Wnt: Wingless-related integration site.
Tumor microenvironment; TNF: Tumor necrosis factor; Treg: CD4+ regulatory T cell; rRNA: Transfer RNA; WTAP: Wilms tumor 1-associated protein; ZCCHC4: Zinc finger CCHC-type containing 4.

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Authors’ contributions
D.P., S.X., and W.L. conceived the idea, design the study; W.L. retrieved and analyzed the data, and drafted the manuscript; W.L., Y.H. and X.Z. revised and polished the manuscript. All authors read and approved the final manuscript.

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The authors declare that they have no competing interests.

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