Effects of m6A modifications on signaling pathways in human cancer (Review)

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Abstract. N6-methyladenosine (m6A) is one of the most prevalent post-transcriptional RNA modifications. The enzymes involved in the regulation of m6A include methyltransferase (writers), demethylase (erasers) and m6A recognition proteins (readers). Accumulating studies have demonstrated that m6A modifications have a distinct effect on various biological processes, including tumorigenesis, cell differentiation, embryonic development and neurogenic diseases, while our knowledge of the specific mechanism underlying m6A methylation in various cancer types is still limited. Various signaling pathways have an effect on tumorigenesis, invasion and apoptosis of malignant tumors. The present review summarizes the recent progress in research regarding the role of m6A in human cancer and discusses the influence of m6A on classic signaling pathways in malignant tumors.

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Abbreviations: ALKBH5, alkB homologue 5; AML, acute myeloid leukemia; CRC, colorectal cancer; EMT, epithelial-mesenchymal transition; FTO, fat mass and obesity-associated protein; GC, gastric cancer; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HIF-1α, hypoxia inducible factor α1; HIV-1, human immunodeficiency virus type 1; IGF2BP, insulin like growth factor 2 mRNA binding proteins; m6A, N6-methyladenosine; mESC, mouse embryonic stem cell; METTL3, methyltransferase-like 3; METTL14, methyltransferase-like 14; METTL16, methyltransferase-like protein 16; RBM15, RNA-binding motif protein 15; TCGA, The Cancer Genome Atlas; TGCT, testicular germ cell tumor; VIRMA, Vir like m6A methyltransferase associated; WTAP, Wilms' tumor 1-associating protein; YTH, YT521-B homology; YTHDF, YTH domain-containing family; ZC3H13, zinc finger CCCH domain-containing protein 13; ZMYM1, zinc finger MYM-type containing 1

Key words: N6-methyladenosine, cancer, methylation, signaling pathway

Contents

1. Introduction

As an essential part of the central dogma of molecular biology, mRNA and other forms of RNA serve crucial roles in biological systems by passing on genetic information. Although research on chemical modifications of RNAs began in 1965 (1), there is limited knowledge regarding the underlying regulatory mechanisms of RNA modifications in biological processes. According to the MODOMICS database (https://iimcb.genesilico.pl/modomics), 172 different RNA chemical modifications, such as 5-methylcytosine, 1-methylguanosine, N6-methyladenosine (m6A) and N1-methyladenosine, have been observed in all organisms at present. Among these modifications, m6A methylation is considered the most abundant and conserved internal transcriptional modification (2). Research on m6A methylation has been limited in the past due to a lack of accurate detection methods; however, with the development of high-throughput m6A sequencing methods (3), the understanding of the biological functions of m6A has advanced.

The process of m6A methylation is regulated by several enzymes, including writers, erasers and readers (Fig. 1) (4,5). Writers promote the formation of m6A (6-8), erasers specifically remove the methylated group from mRNAs, and readers recognize and bind m6A modifications to exert biological functions (9,10). The observation of the demethylation functions of fat mass and fat mass and obesity-associated protein (FTO) (11), and alkB homologue (ALKBH)5 (12) as an eraser, demonstrated that m6A methylation is a dynamic and reversible process. Malignant tumors are a group of abnormal cells with distinctly different functions and gene expression

compared with normal cells. Research on the mechanisms of m6A in cancer has recently advanced due to improvements in the understanding of the roles of m6A in post-transcriptional modifications (4). The present review summarizes the molecular functions and mechanisms of m6A and its three regulators in human cancer, and discusses their roles in the regulation of malignant tumor signaling pathways.

2. Brief overview of the history of m6A

Since its discovery in the 1970s, m6A has been the most prevalent modification in polyadenylated mRNAs (2). It has been estimated to be present in three m6A residues per mRNA on average (13). Since it is ubiquitous in nature, m6A can be found in yeast (14), fruit flies (15), mammals (2,16) and bacteria (17). Since m6A can undergo reverse transcription to form thymine and cannot be detected by chemical modifications, transcriptome-wide mapping of m6A remains difficult (18). In 2012, a high-throughput sequencing method based on antibodies was developed by two independent groups to map m6A distribution in the entire RNA sequence, which improved the detection efficiency of m6A (3,19).

It was originally hypothesized that the process of m6A was static; however, in 2011, FTO (11) and ALKBH5 (12) were demonstrated to be able to function as demethylases, indicating that the process of m6A is reversible. Subsequently, various proteins, including Vir like m6A methyltransferase associated (VIRMA) (8,16), insulin like growth factor 2 (IGF2BP) (10) and heterogeneous nuclear ribonucleoprotein (7), were demonstrated to function as writers and readers.

3. Readers, writers and erasers in m6A methylation

It is well-known that writers and erasers regulate m6A via methylation and demethylation, respectively (5,20,21). Furthermore, m6A groups exert biological functions by being recognized by readers, which are a type of specific binding protein (22,23).

In mammals, writers catalyze the methylation of m6A in the form of a methyltransferase complex consisting of methyltransferase-like 3 (METTL3), methyltransferase-like 14 (METTL14) (7) and Wilms' tumor 1-associating protein (WTAP) (7). METTL14 has a greater effect on m6A than METTL3 although their proportion in the complex is 1:1 (7). Previous studies have identified more writers, including methyltransferase-like protein 16 (METTL16) (24,25), zinc finger CCCH domain-containing protein 13 (ZC3H13) (26), VIRMA (8,16) and RNA-binding motif protein 15 (RBM15) (27). METTL16 is a methyltransferase which binds to the conserved U6 small nuclear RNA, non-coding RNA and precursor messenger RNA, and is involved in regulating intracellular homeostasis and mRNA splicing in response to intracellular S-adenosyl-L-methionine levels (24,25). VIRMA (also referred to as KIAA1429) can promote m6A modification and knockdown of VIRMA, resulting in a more conspicuous decrease of m6A content than the effect of METTL3 and METTL14 knockdown in A549 cells (8). RBM15 catalyzes m6A modification by binding to the U-rich region in long non-coding RNA X inactive specific transcript (27). In addition, ZC3H13 has been identified as a novel m6A writer in mice and *Drosophila* (26). The first eraser was identified in 2011 by Jia *et al* (11), who revealed that FTO could demethylate m6A. ALKBH5 was identified as the second eraser (12) as it demethylates m6A in a different way compared with FTO. The two intermediates, N6-hydroxymethyladenosine and N6-formyladenosine are first oxidized by FTO during the process of demethylation, while ALKBH5 catalyzes the direct removal of m6A (12,28). Readers can identify m6A modifications and bind to methylated RNA to transfer biological signals to downstream signaling pathways (21,22). Proteins containing the YT521-B homology (YTH) domain, such as the YTH domain-containing family (YTHDF) proteins, have been classed as readers (9). Notably, these recognition proteins of m6A exhibit distinct mechanisms. For example, Wang *et al* (29) reported the translation-promoting role of YTHDF1 and the mRNA-destabilizing role of YTHDF2. By interacting with initiation factors, including IGF2BP1 and stress granule assembly factor 1, YTHDF1 enhances the translation efficiency of target RNAs and ensures efficient protein expression from these shared transcripts. By contrast, YTHDF2 accelerates the degradation of m6A-modified transcripts to control the lifetime of the methylated transcripts (29,30). YTHDF3 serves as a hub to regulate the RNA accessibility of YTHDF1 and YTHDF2 (31).

4. m6A regulation of biological processes

m6A is widely expressed in eukaryotes and serves a crucial role in the regulation of various biological processes. In mammals, m6A modifications affect development (12), metabolism (11,32-34) and immunity (35-37). Furthermore, previous studies have indicated that m6A has effects on stem cell differentiation (38,39), human metabolic diseases (40), viral infections (41-44) and inflammation (45).

m6A is involved in the regulation of pluripotency and differentiation of stem cells. Pluripotent mouse embryonic stem cells (mESCs) undergo two different states during differentiation, naive and primed (46). m6A modifications serve key roles in the regulation of pluripotency during the transition from the naive state to the primed state (38). METTL3 depletion has a different effect on naïve and primed pluripotent stem cells. The depletion of METTL3 in naïve cells blocks differentiation and amplifies the highly expressed naïve pluripotency genes, which boosts naïve circuitry stability (38). When METTL3 and m6A are inhibited in epiblast stem cells, which are in a primed state, the expression levels of pluripotent genes are reduced, whereas the expression levels of lineage commitment markers are increased (38). By knocking out METTL3, Geula *et al* (38) revealed m6A as a timely maintainer of the balance between pluripotency and lineage priming factors, thus ensuring the orderly differentiation of mESCs. However, Batista *et al* (47) reported that the deletion of METTL3 maintains the self-renewal capacity of mESCs and mouse embryonic fibroblasts. These contradictory results may be due to the cell state. For example, different transcripts are expressed and methylated in naïve and primed embryonic stem cells (ESCs) (48). Therefore, METTL3 inactivation regulates the expression levels of genes that affect cell fate and identity, and this activity maintains pluripotency in naïve stem cells but promotes differentiation in primed stem cells (38).

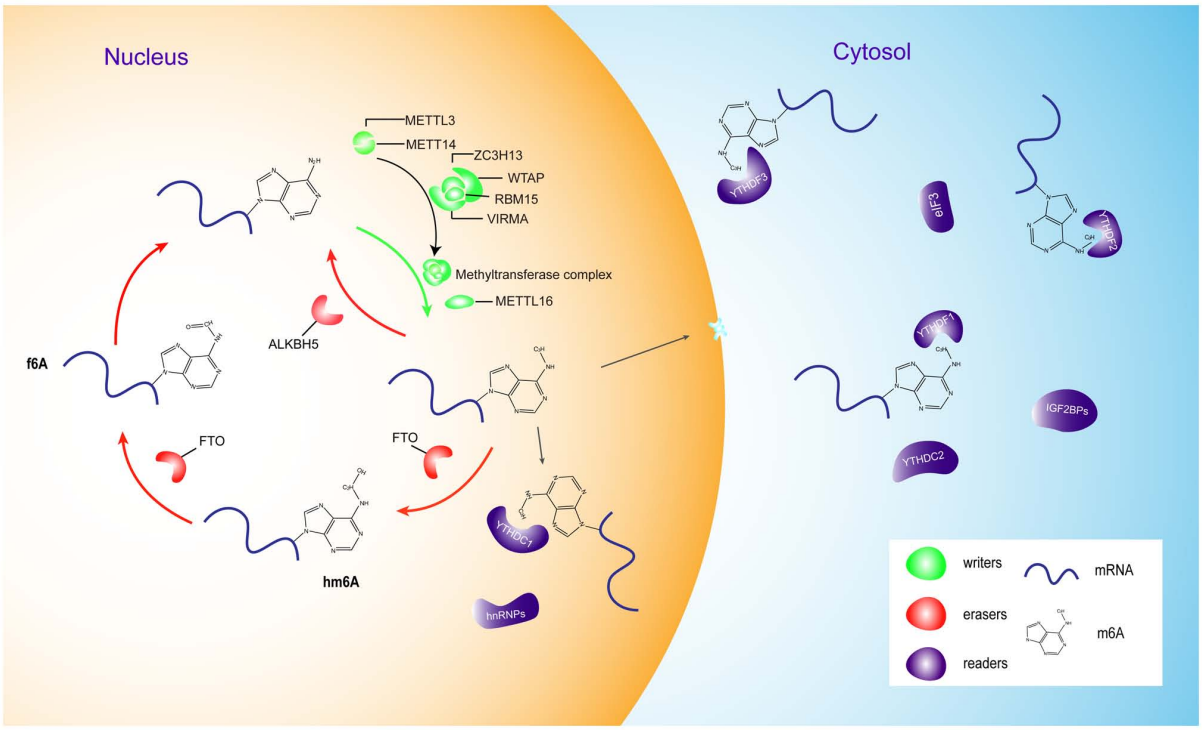

Figure 1. Process of m6A methylation. m6A methylation is a dynamic and reversible process coordinated by a series of methyltransferases (m6A writers), demethylases (m6A erasers) and m6A readers. Methylation of m6A in the nucleus occurs via two mechanisms. ALKBH5 directly catalyzes the removal of m6A. FTO can oxidize m6A to hm6A and f6A sequentially. hm6A and f6A are moderately stable and can be hydrolyzed to adenine. Readers, such as YTHDC1 and HNRNP family, bind to m6A-modified mRNA in the nucleus, while other readers, including YTHDF1/2/3, IGF2BP family, YTHDC2 and eIF3, recognize m6A modification in cytosol. N6-methyladenosine; ALKBH5, alkB homologue 5; FTO, fat mass and obesity-associated protein; hm6A, N6-hydroxymethyla denosine; f6A, N6-formyladenosine.

YTH domain containing 1 (YTHDC1) is a known m6A reader found in the nucleus (49). Similar to METLL3, the inactivation of YTHDC1 is embryonic lethal, which demonstrates that YTHDC1 is required for the development of mitotic spermatogonia in males and postnatal oocyte growth in females (50). Notably, when cytoplasmic YTHDF1and YTHDF2 are depleted, ESCs cannot emerge from diversification (50), indicating that the YTHDC1-mediated regulation of ESC differentiation occurs in the nucleus rather than in the cytoplasm.

Other erasers and readers of m6A have also been demonstrated to regulate the development and differentiation of ESCs. Knocking out YTHDF2 enhances the proliferation of mouse and human hematopoietic stem cells, highlighting its potential role in transplantation-related applications (51). Notably, m6A modification has not only been demonstrated to regulate differentiation in ESCs (38), but also in developmental cancer cells (52). Lobo *et al* (52) revealed that abundance of m6A and expression of its writer VIRMA/reader YTHDF3 are different among testicular germ cell tumor (TGCT) subtypes, with higher levels in seminomas. Higher VIRMA and YTHDF3 mRNA levels in seminomas maintain a low differentiation level compared with teratoma, which represents more differentiated TGCTs. However, Lobo *et al* (52) observed a stronger m6A immunostaining intensity in teratoma, suggesting that other writers may be responsible for establishing m6A in teratoma and/or that m6A modification may target other RNAs and even impart them a different fate.

m6A regulates biological metabolism. m6A is involved in metabolism and regulation of metabolic genes. It has been demonstrated that the demethylase FTO is involved in the metabolism of glucose and lipids in mammals (33,40). As a classic target of fat metabolism, FTO can induce mRNA expression of FOXO1, glucose-6-phosphatase catalytic subunit and diacylglycerol O-acyltransferase 2, and is closely associated with glucose metabolism in type 2 diabetes (40). FTO has also been demonstrated to regulate the expression levels of activating transcription factor 4 to control glucose production in the liver (53). Wu *et al* (54) demonstrated that FTO modulates the deposition of triglycerides and the accumulation of lipids by regulating the m6A-YTHDF2 signaling pathway. At present, the specific sites and complete mechanisms in glucose or fat production are unknown, and thus, future studies are required to address this.

m6A controls various aspects of immunity. Researchers have highlighted the roles of m6A in anti-inflammatory immunity, antitumor immunity and adaptive immunity (36,37). Yu *et al* (45) have demonstrated that YTHDF2 is involved in the inflammatory response of macrophages. Knockdown of YTHDF2 markedly increased the expression levels of IL-6, TNF-α and IL-12, which were induced by lipopolysaccharide, and the phosphorylation levels of p65, p38 and ERK1/2 in macrophages were also upregulated. Furthermore, silencing of YTHDF2 could induce upregulation of mitogen-activated protein kinase 4 and mitogen-activated protein kinase 4 by stabilizing mRNA, activating MAPK and NF-κB signaling pathways, and this aggravates the inflammatory response in macrophages. Liu *et al* (55) reported that YTHDF2 recognized

and degraded, long non-coding RNA Dpf3 in dendritic cells specifically, which markedly inhibited C-C motif chemokine receptor 7-mediated dendritic cell migration and contributed to inflammatory responses. Studies of the m6A-induced effect on antitumor immunity are emerging and still in their infancy. Han *et al* (56) demonstrated that the antigen-specific $CD8^+$ T cell antitumor response was improved in YTHDF1-deficient mice compared with mice in the wild-type group. Blocking programmed death-ligand 1 could promote tumor regression in YTHDF1-deficient mice (57). In addition, the mechanisms by which m6A regulates adaptive immunity is an emerging field of investigation (58). Li *et al* (58) first elucidated the function of m6A in $CD4^+$ T helper cells. The result suggested that deletion of METTL3 in mouse T cells disrupted T cell homeostasis and differentiation. The mRNAs of the suppressor of cytokine signaling (SOCS) family, which are involved in STAT signaling, exhibit slower mRNA decay and increased expression levels in Mettl3-deficient naïve T cells (58). This increased SOCS family activity consequently inhibits IL-7 mediated STAT5 activation and T cell homeostatic proliferation and differentiation (58).

m6A in infectious diseases. m6A modifications are involved in viral infections. Human immunodeficiency virus type 1 (HIV-1) RNA is methylated by m6A in infected cells, and readers, including YTHDF1-3, bind to methylated HIV-1 RNA to inhibit viral reverse transcription and translation (41,42). Partial knockout of m6A writers decreases HIV-1 Gag synthesis and viral release, whereas knockout of FTO has the opposite effect (42). This indicates that m6A can enhance HIV-1 protein synthesis and viral release, thereby contributing to the infection. Additionally, the proteins regulated by m6A are known to modulate the life cycle of hepatitis C virus (HCV) (43). Depletion of METTL3 and METTL14 can increase the levels of HCV infection by promoting infectious viral particle production without affecting viral RNA replication (43,59). By contrast, inhibition of the m6A demethylase FTO, but not ALKBH5, has the opposite effect (26). Furthermore, m6A has been demonstrated to serve important roles in other *Flaviviridae*, such as Zika virus (44). Lichinchi *et al* (44) revealed that the depletion or overexpression of the RNA methyltransferase could impact viral replication, demonstrating that the host RNA methyltransferase machinery acts as a key post-transcriptional regulator of Zika virus. Furthermore, YTHDF proteins binding to Zika RNA indicates another regulatory aspect of m6A readers, which serves a role in viral RNA metabolism (44). Both RNA modification layers may act as pro- or anti-viral factors in the host (44).

In addition, m6A serves a critical regulatory role in inflammation (60,61), gametogenesis (62,63) and nervous system development (64,65). Importantly, the immune regulatory role of m6A may provide a novel idea for cancer immunotherapy research.

5. Role of m6A modifications in cancer

Consistent with the regulation of m6A modifications in normal biological processes, m6A is associated with a variety of human cancer types. However, the catalysis of m6A in cancer is not unitary. Numerous studies have demonstrated that m6A serves an important role in various cancers, often via the actions of regulators that influence m6A modifications and expression of oncogenes or tumor suppressor genes. The special roles of m6A regulators in human cancer types are summarized in Table I; however, the mechanisms by which m6A regulators contribute to carcinogenesis remain to be elucidated. The present review summarizes how the three types of m6A regulatory proteins function in human cancer and discusses the role of m6A in several classic signaling pathways.

Methyltransferases/writers in cancer. Writers positively regulate m6A modifications. The aberrant expression of writer proteins in tumors affects oncogenes and tumor suppressors, thus influencing tumorigenesis (66), invasion (66) and metastasis (67). Interestingly, the mechanisms of writers in different types of cancer are not uniform. METTL3 is highly expressed in acute myeloid leukemia (AML) (68), and contributes to the translation of oncogenes. In gastrointestinal cancer, METTL3 has been demonstrated to be closely associated with the processes involved in the progression of cancer, including tumor cell proliferation, apoptosis, metastasis, angiogenesis and cancer stem cell maintenance (69). A number of studies have demonstrated that METTL3 generally acts as an oncogene in gastrointestinal cancer types, such as gastric cancer (GC) (70,71), colorectal cancer (CRC) (72), hepatocellular carcinoma (HCC) (73) and pancreatic cancer (74,75). Furthermore, the modified mRNA targets of METTL3 are diverse. For example, METTL3-mediated m6A modification can increase the expression levels of mRNA targets, including zinc finger MYM-type containing 1 (ZMYM1) (70), SEC62 homolog, preprotein translocation factor (76) and MYC (71), in a way of enhancing mRNA stability in GC, and promotes tumor cell proliferation, migration and invasion. Similarly, other writers, including METTL16 (24,25), ZC3H13 (26), VIRMA (8,16) and RBM15 (27), have been reported to have a complicated effect in other malignancies, such as hepatocellular carcinoma (77), colorectal cancer (78), prostate cancer (79) and breast cancer (80). It was hypothesized that induction mechanisms other than m6A regulation cause this phenomenon.

Demethylases/erasers in cancer. Increasing numbers of studies of erasers in cancer are being performed. These studies have identified that the m6A demethylase, FTO, serves a critical oncogenic role in AML (57,81,82). Specifically, its high expression in AMLs with mixed lineage leukemia rearrangements and fms related receptor tyrosine kinase 3-internal tandem duplication and/or nucleophosmin 1 mutations is associated with increased tumorigenesis and invasion of AML cells (81). Enhancing the expression levels of FTO can reduce the levels of m6A and mRNA transcription of ankyrin repeat and SOCS box containing 2 (ASB2) and retinoic acid receptor α (RARA) (81). ASB2 and RARA are known to regulate the differentiation of leukemia cells by inhibiting all-trans retinoic acid (81). In addition, FTO serves a crucial role in cholangiocarcinoma (83) and glioblastoma stem cells (84). In contrast to FTO, an AML study based on The Cancer Genome Atlas (TCGA) has suggested that ALKBH5, another m6A demethylase, exhibits frequent copy number loss that

Table I. Tumor-suppressing and tumor-promoting roles of m6A regulators in human cancer types.

First author, year	m6A regulator	Type of cancer	Role in cancer	Mechanism	(Refs.)
Vu *et al*, 2017	METTL3	AML	Oncogene	Promotes the translation of c-MYC, BCL2 and PTEN mRNA	(68)
Chen *et al*, 2019		BC	Oncogene	METTL3-mediated m6A modification operates a regulatory network which involves AFF4/NF-κB/MYC to promote BC progression	(118)
Shen *et al*, 2020		CRC	Oncogene	Activates glycolysis and enhances colorectal cancer progression	(72)
Li *et al*, 2019		GBM	Oncogene	Modulates mediated mRNA decay of splicing factors and alternative splicing isoform switches	(119)
Yue *et al*, 2019		GC	Oncogene	Enhances ZMYM1 mRNA stability and facilitates the EMT program and metastasis	(70)
Yang *et al*, 2020		GC	Oncogene	Mediates MYC target genes, and promotes proliferation and migration	(71)
Chen *et al*, 2020		HCC	Oncogene	Inhibits SOCS2 mRNA expression, and reduces HCC cell proliferation, migration, and colony formation	(73)
Wang *et al*, 2020		THCA	Oncogene	Regulates methylation of TCF1 mRNA and the activated Wnt signaling pathway	(120)
Weng *et al*, 2018	METTL14	AML	Oncogene	Regulates MYB and MYC mRNA via m6A modification	(121)
Ma *et al*, 2017		HCC	Suppressor	Interacts with DGCR8 and positively modulates the primary microRNA-126 process	(67)
Cui *et al*, 2017		GBM	Suppressor	Suppresses glioblastoma stem cell proliferation and self-renewal	(84)
Bansal *et al*, 2014	WTAP	AML	Oncogene	Promotes proliferation and arrests differentiation of leukemia cells	(122)
Chen *et al*, 2019		HCC	Oncogene	Facilitates progression of HCC via m6A-HuR-dependent epigenetic silencing of ETS1	(123)
Qian *et al*, 2019	VIRMA	BC	Oncogene	Promotes BC progression by modulating CDK1	(80)
Cheng *et al*, 2019		HCC	Oncogene	Promotes the migration and invasion of HCC by altering m6A modification of ID2 mRNA	(124)
Li *et al*, 2017	FTO	AML	Oncogene	Regulates expression of targets, such as ASB2 and RARA, by reducing m6A levels, and enhances leukemic oncogene-mediated cell transformation and leukemogenesis	(81)
Xu *et al*, 2017		GC	Oncogene	Unclear	(125)
Li *et al*, 2019		LC	Oncogene	Promotes the proliferation of LC cells by regulating USP7 mRNA	(126)
Li *et al*, 2019		HCC	Oncogene	Triggers the demethylation of PKM2 mRNA and accelerates translation	(127)
Zhang *et al*, 2017	ALKBH5	GBM	Oncogene	Maintains tumorigenicity by sustaining FOXM1 expression	(86)
Chao *et al*, 2020		LC	Oncogene	Affects the proliferation and invasion of LC cells by downregulating m6A modification of FOXM1 mRNA	(128)
Lin *et al*, 2019	YTHDF1	HCC	Oncogene	Mediates m6A-increased translation of Snail mRNA	(129)
Nishizawa *et al*, 2019		CRC	Oncogene	Induces the translation of m6A-modified FZD9 and Wnt6 mRNA	(88)
Mapperley *et al*, 2021	YTHDF2	AML	Suppressor	Suppresses proinflammatory signaling pathways and sustains hematopoietic stem cell function	(60)
Li *et al*, 2020		PC	Oncogene	Mediates the mRNA degradation of the tumor suppressors LHPP and NKX3-1	(130)
Dixit *et al*, 2020		GBM	Oncogene	Stabilizes oncogene MYC and VEGFA transcripts in glioblastoma stem cells	(131)

Table I. Continued.

First author, year	m6A regulator	Type of cancer	Role in cancer	Mechanism	(Refs.)
Chang *et al*, 2020	YTHDF3	BRC	Oncogene	Enhances the translation of ST6GALNAC5, GJA1 and EGFR, associated with brain metastasis	(132)
Ma *et al*, 2020	YTHDC2	LC	Suppressor	Inhibits LC tumorigenesis by suppressing SLC7A11-dependent antioxidant function	(133)
Wu *et al*, 2019	hnRNP	CRC	Oncogene	m6A-induced lncRNA RP11 can trigger the dissemination of CRC cells via post-translational upregulation of Zeb1	(134)

Tumor-suppressing and tumor-promoting roles of m6A regulators in different human cancer types are shown. This illustrates the different effects of m6A regulators and their mechanisms. m6A, N6-methyladenosine; AML, acute myeloid leukemia; BC, bladder cancer; BRC, breast cancer; CRC, colorectal cancer; GBM, glioblastoma; GC, gastric cancer; HCC, hepatic cell carcinoma; LC, lung cancer; PC, prostate cancer; THCA, thyroid carcinoma.

results in non-carcinogenic effects in AML (85). Furthermore, Zhang *et al* (86) demonstrated that ALKBH5 methylated FoxM1 to maintain proliferation and development in glioblastoma stem-like cells.

Readers in cancer. The characterization of m6A readers has provided valuable insight into to the mechanisms of m6A-mediated post-transcriptional gene regulation in cancer. It has been demonstrated that YTHDF1 is expressed at higher levels in CRC tissues, and that it contributes to malignant phenotypes and poor patient prognosis (87). A further study has indicated that YTHDF1 is induced by the oncogene c-MYC, and high YTHDF1 expression in malignant tumors can enhance the resistance to anticancer drugs, including oxaliplatin and fluorouracil (88). As another member of the YTH domain-containing family, YTHDF2 recognizes m6A modifications in the cytoplasm (31). A previous study has identified that YTHDF2 could directly bind to the 3' end of the SOCS2 transcript, and that knockdown of YTHDF2 augmented SOCS2 expression in HCC cells (73). The SOCS family of proteins are essential tumor suppressors in different cancer types, suggesting an important role of YTHDF2 in human cancer (73). YTHDC2 is known to promote the mRNA translation of hypoxia inducible factor α1 (HIF-1α) to induce the metastasis of CRC (89). Knockdown of YTHDC2 attenuates the protein expression of metastasis-related genes, such as HIF-1α, and inhibits the metastasis *in vitro* and *in vivo* (89). IGF2BP has been demonstrated to be highly expressed in a variety of malignant tumors, such as HCC, cervical cancer and AML (10,90). Huang *et al* (90) reported that IGF2BP has a positive effect on the stability and translation levels of c-MYC, indicating the potential latent relationship between IGF2BP and other readers.

6. m6A modifications in classic signaling pathways of cancer

As more research on m6A in cancer is being conducted, several studies have examined whether m6A can regulate cancer by affecting signaling pathways, and explored the specific mechanisms of m6A. As a result, studies have demonstrated that m6A can promote or inhibit malignant tumors by regulating different signaling pathways (Figs. 2 and 3).

Wnt signaling pathway. Wnt signaling is a pivotal regulatory signaling pathway that has diverse roles in cancer progression. The m6A modification targeting Wnt signaling has been a focus of cancer research. According to a study conducted by Zhang *et al* (91), the Wnt signaling pathway is activated after the levels of m6A are reduced by inhibiting METTL14 in GC. By contrast, FTO knockout exhibits the opposite effect on the Wnt signaling pathway (91). This suggests that m6A can affect the activity of the Wnt signaling pathway in GC. Similarly, E-cadherin is modulated by m6A; however, more studies are required to improve the understanding of these mechanisms (92). In endometrial cancer, FTO promotes tumor metastasis and invasion (93). FTO catalyzes demethylation modification in the 3'-untranslated region (3'-UTR) of HOXB13 mRNA, thereby inhibiting m6A modification recognition by the YTHDF2 protein (93). This leads to decreased HOXB13 mRNA decay and increased HOXB13 protein expression and activation of the Wnt signaling pathway (93). Enhanced m6A modification is also considered to be an oncogenic mechanism in hepatocellular carcinoma; METTL3 expression is upregulated and Wnt/β-catenin signaling pathway activity is induced via promotion of catenin β1 expression, which ultimately accelerates hepatocellular carcinoma development (94). The Wnt signaling pathway activates several cancer-related markers, including key regulators of the cell cycle, proliferation, invasion, angiogenesis and drug resistance (95). Therefore, examining the effect of m6A on the Wnt signaling pathway will provide guidance to explore the detailed mechanisms in cancer.

Epithelial-mesenchymal transition (EMT) signaling pathway. The EMT signaling pathway is a hot spot for cancer research due to its role in the initial process of tissue carcinogenesis. Furthermore, the markers of EMT are closely associated with tumor progression processes, such as migration, invasion, proliferation, anti-apoptosis, stemness and tumor radio/chemosensitivity of cancer cells (96,97). Yue *et al* (70) revealed the METTL3-mediated m6A modification process in GC cells and

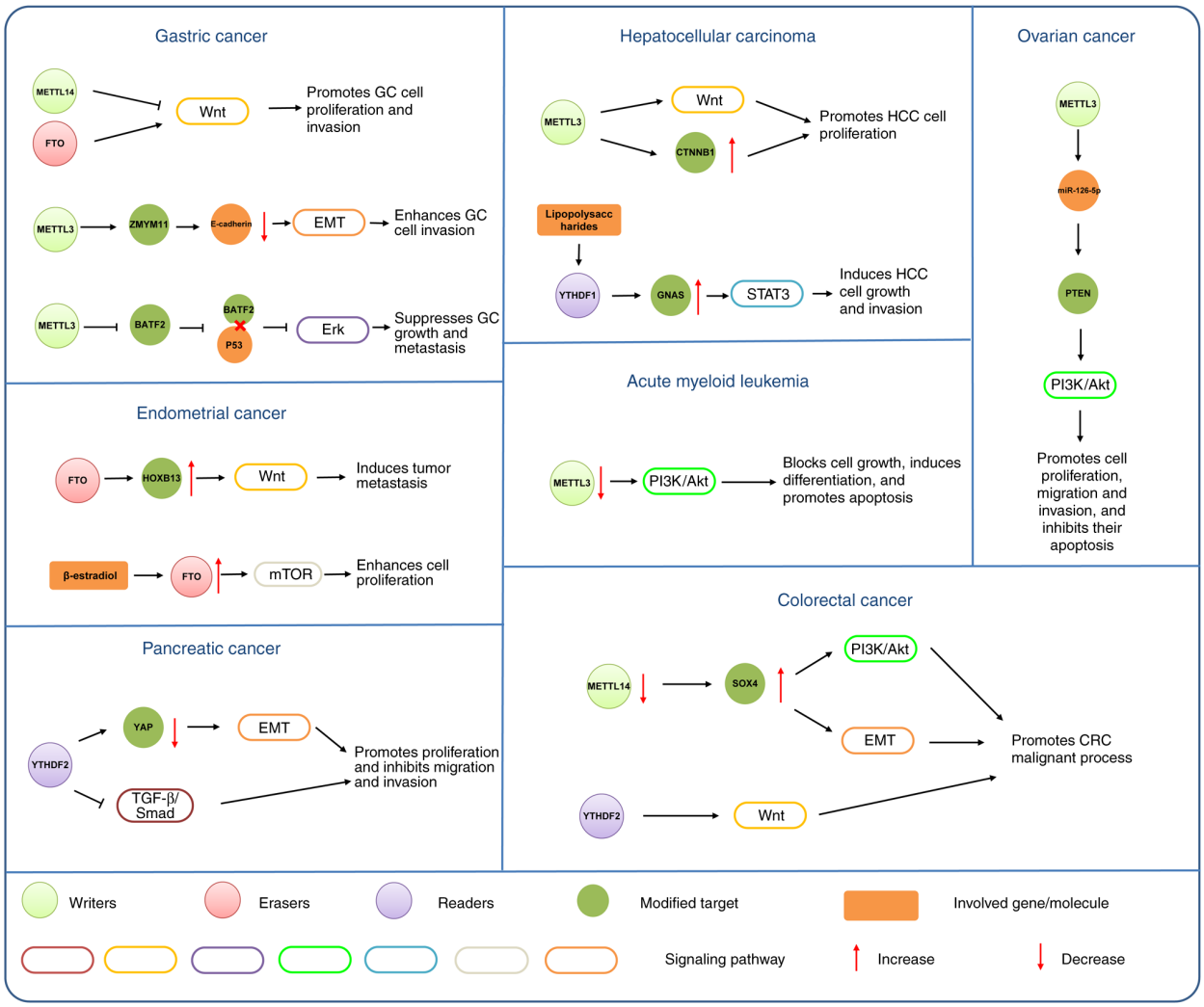

Figure 2. m6A regulators are involved in signaling pathways and promote cancer progression. Diagram showing the mechanism via which m6A regulators affect classic signaling pathways to promote tumor progression in gastric cancer, hepatocellular carcinoma, endometrial cancer, pancreatic cancer, acute myeloid leukemia, ovarian cancer and cocancer. m6A, N6-methyladenosine.

identified ZMYM1 as a target of METTL3. The elevated expression levels of ZMYM1 repress the activation of E-cadherin promoter by recruiting C-Terminal Binding Protein/Human lysine specific demethylase l/CoREST complex, thus facilitating the EMT process. YTHDF2 is highly expressed in various cancer types and is involved in dual regulation (60,98). In pancreatic cancer, YTHDF2 knockdown increases the expression levels of YAP, which is a key protein of the TGF-β/Smad signaling pathway (98). A previous study has demonstrated that there are two m6A sites in YY1 associated protein 1 (YAP), which suggests that YTHDF2 directly binds to YAP mRNA to decrease the stability of mRNA and regulate EMT via YAP signaling inhibition (98). Progress has also been achieved in the development of novel drug targets based on m6A modifications. Chen *et al* (99) reported that simvastatin induced METTL3 downregulation in lung cancer tissues, which further influenced EMT via m6A modification on EZH2 mRNA and inhibited the malignant progression of lung cancer.

PI3K/Akt signaling pathway. The PI3K/Akt signaling pathway is important for cancer progression. Although aberrant activity of the PI3K/Akt signaling pathway could be associated with tumorigenesis, it also has a great impact on the proliferation, adhesion, invasion and angiogenesis of malignant tumors (100). Increasing evidence suggests that m6A modification is involved in carcinogenesis by targeting the PI3K/Akt signaling pathway (101-105). In renal cell carcinoma, METTL3 inhibits the PI3K/Akt/mTOR signaling pathway and serves a role as a tumor suppressor gene (101). Zhao *et al* (102) conducted an analysis for sequencing data of gastrointestinal cancer from TCGA and Gene Expression Omnibus, and demonstrated that m6A modification directly modulates PI3K/Akt and mTOR signaling pathway activity by regulating critical kinases in human gastrointestinal cancer. This conclusion was supported and validated by a study by Chen *et al* (103). According to Chen *et al* (103), knockdown of METTL14 markedly abolished SOX4 mRNA m6A modification and elevated SOX4 mRNA expression, whereas METTL14-mediated SOX4 mRNA degradation stimulated PI3K/Akt signaling and inhibited CRC malignant process. A study revealed that m6A modification can affect the activity of the PI3K/Akt signaling pathway by regulating miRNA (104). Bi *et al* (104) demonstrated that METTL3 promoted miR-126-5p maturation by modifying pri-miR-126-5p in ovarian cancer. METTL3 knockdown inhibits the effect of miR-126-5p to

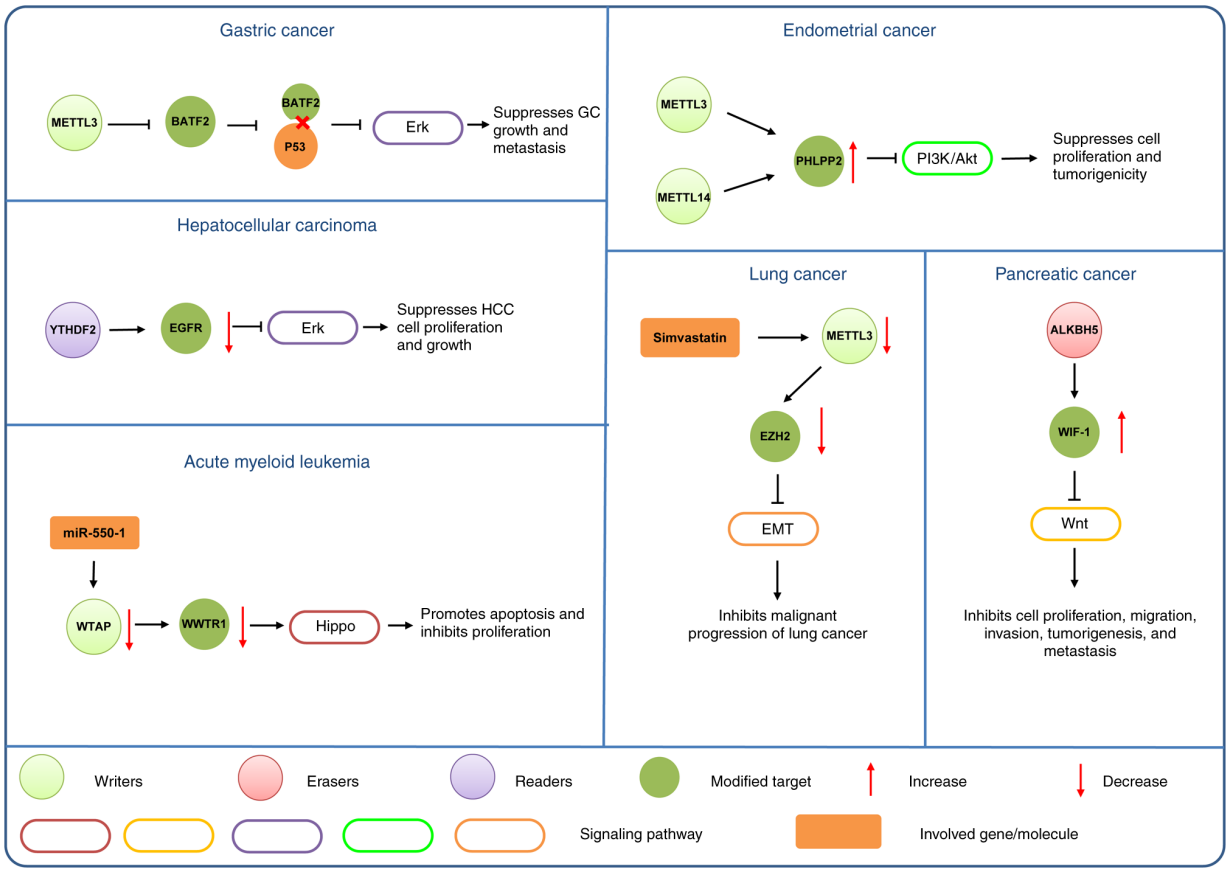

Figure 3. m6A functions as an inhibitor in human cancer by regulating signaling pathways. m6A inhibits cancer progression by regulating signaling pathways in gastric cancer, hepatocellular carcinoma, acute myeloid leukemia, endometrial cancer, lung cancer and pancreatic cancer. m6A, N6-methyladenosine.

upregulate PTEN, which prevents PI3K/Akt/mTOR signaling pathway activation. Furthermore, Liu *et al* (105) demonstrated that reductions of m6A methylation mediated by METTL14 mutation or reduced expression levels of METTL3 lead to the activation of the Akt signaling pathway by decreasing PHLPP2 expression and increasing mTORC2 expression, which promotes cell proliferation in endometrial cancer.

ERK signaling pathway. The ERK signaling pathway has been demonstrated to be important for cancer progression. The substrates of ERK signaling are broad, which make ERKs key regulators of proliferation, migration, apoptosis and chemo-immune-resistance, as well as appealing therapeutic targets in cancer (106). Zhong *et al* (107) revealed that YTHDF2 directly bound to the m6A modification site of the EGFR 3'-UTR to promote the degradation of EGFR mRNA in HCC cells, and this mechanism suppressed MEK and ERK activation, cell proliferation and tumor growth. However, previous studies, have revealed the interaction between m6A modification and the ERK signaling pathway (108,109). Xie *et al* (109) demonstrated that basic leucine zipper ATF-like transcription factor 2 (BATF2) could bind to p53 and enhanced its protein stability, thereby inhibiting the phosphorylation of ERK in GC, and m6A modification mediated by METTL3 could repress BATF2 mRNA expression, which provides potential prognostic and therapeutic targets for GC treatment. Conversely, the ERK signaling pathway has been demonstrated to have a positive effect on m6A deposition (108). Sun *et al* (108) demonstrated that ERK could phosphorylate METTL3 at S43/S50/S525 and WTAP at S306/S341, thus stabilizing the m6A methyltransferase complex in ESC and malignant tumor cells.

Other signaling pathways in cancer. In addition to the aforementioned representative signaling pathways, researchers have reported that m6A modification also serves a crucial role in other classic signaling pathways. Ghazi *et al* (110) investigated the effects of fusaric acid on p53 expression and its epigenetic regulation via promoter methylation and m6A modification in HCC cells. The results revealed that fusaric acid epigenetically decreased p53 expression by altering its m6A modification (110). Similarly, Ding *et al* (111) reported that lipopolysaccharides stimulation promotes GNAS complex locus (GNAS) expression by increasing the m6A methylation levels of GNAS mRNA, thus inducing HCC cell proliferation and invasion by interacting with the STAT3 signaling pathway in HCC. In addition, Zhang *et al* (112) demonstrated that β-estradiol can accelerate FTO nuclear localization and increase the proliferation of endometrial cancer cells by modulating the mTOR signaling pathway; however, the mechanism by which estrogen receptor-α mediates FTO nuclear accumulation is unclear (113).

7. Therapeutic implications of m6A in cancer

At present, m6A modification is mechanistically linked to the progression and prognosis of several types of cancer. Given the

complicated process of m6A catalysis in cancer, m6a and its regulatory proteins may be novel therapeutic targets for cancer diagnosis and prognosis. For example, METTL3 has been considered as an oncogenic factor in numerous human types of cancer. According to recent studies, METTL3 may be an independent prognostic factor for patients with GC (114), CRC (115) and HCC (73). Similarly, evidence also supports the proliferative roles of FTO in cancer (81). FB23-2, a promising FTO inhibitor, has been demonstrated to negatively regulate proliferation and progression of human AML cell lines by inhibiting FTO (57). Furthermore, R-2HG, another small-molecule inhibitor of FTO, exhibits anticancer activity in AML (18). Additionally, clinical data have demonstrated that the expression levels of ALKBH1 are negatively associated with tumor size and TNM stage, and that the expression levels of FTO are associated with improved overall survival in patients with GC (116). Despite extensive efforts being devoted to study m6A in cancer, a number of issues associated with the function and mechanism of m6A remain unknown. Considering that novel m6A readers and writers are constantly emerging, m6A-mediated biological functions require further exploration. Additionally, multifarious modification targets and sites suggest that the specific mechanisms of m6A is not unitary even in the same type of malignant tumor. This should be clarified. The rapid development of detection methods and several novel inhibitors of m6A-related factors will provide practical assistance for researchers.

8. Conclusion

Increasing studies suggest that m6A is deeply involved in the regulation of gene expression. m6A can determine the fate during development and differentiation, and aberrant m6A modifications can affect classic signaling pathways, including the PI3K/AKT (91,101,105,112), Wnt (91,92) and mTOR (101,113) signaling pathways, in cancer. The dual role of m6A regulation in cancer is unclear, and may be due to differences in cell types and states. At present, m6A is gaining attention in cancer research, and may provide promising targets for cancer therapies. Future research may focus more on the specific mechanism of m6A methyltransferases and demethylases, or the specificity and sensitivity of readers. The regulatory role of m6A modification in cancer is described as a 'double-edged sword' implying that clinical applications require further investigation (117). Furthermore, the development of methods for the detection and analysis of m6A is required to improve the understanding of the underlying mechanisms.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

FL searched the literature and drafted the manuscript for the study. FL designed the figures. XS and FL revised the manuscript and assessed all the raw data. XS and FL are responsible for confirming the authenticity of the data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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