

# Importance of N<sup>6</sup>-methyladenosine RNA modification in lung cancer (Review)

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**Abstract.** The N<sup>6</sup>-methyladenosine (m<sup>6</sup>A) modification is the most common mRNA modification in eukaryotes and exerts biological functions by affecting RNA metabolism. The m<sup>6</sup>A modification is installed by m<sup>6</sup>A methyltransferases, removed by demethylases and recognized by m<sup>6</sup>A-binding proteins. The interaction between these three elements maintains the dynamic equilibrium of m<sup>6</sup>A in cells. Accumulating evidence indicates that m<sup>6</sup>A RNA methylation has a significant impact on RNA metabolism and is involved in the pathogenesis of cancer. Lung cancer is the leading cause of cancer-related deaths worldwide. The treatment options for lung cancer have developed considerably over the past few years; however, the survival rate of patients with lung cancer still remains very low. Although diagnostic methods and targeted therapies have been rapidly developed in recent years, the underlying mechanism and importance of m<sup>6</sup>A RNA methylation in the pathogenesis of lung cancer remains ambiguous. The current review summarized the biological functions of m<sup>6</sup>A modification and considers the potential roles of m<sup>6</sup>A regulators in the occurrence and development of lung cancer.

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## 1. Introduction

RNA modifications are used to fine-tune the structural features of infrastructural RNAs. In recent years, RNA modifications have been found to be reversible and involved in important biological processes, through continued efforts to map and quantify various RNA modifications in a transcriptome-wide manner (1). The N<sup>6</sup>-methyladenosine (m<sup>6</sup>A) methylation modification is the most prevalent internal modification of eukaryotic mRNA. The latest discoveries of the locations, functions, and mechanisms of m<sup>6</sup>A provide new insights into the regulation mechanism of RNA expression (2). Evidence supports the involvement of m<sup>6</sup>A modifications in precursor mRNA (pre-mRNA) splicing, mRNA stability, RNA structure, translation, and processing of primary transcripts of microRNAs (miRNAs) (3). The m<sup>6</sup>A sites are enriched near the stop codons and 3'-untranslated regions (UTRs), and an association exists between the m<sup>6</sup>A residues and the mRNA-binding site in the 3'-UTR (4,5).

The m<sup>6</sup>A modification appears to be reversible under the combined action of the enzymes involved (6,7). The m<sup>6</sup>A modification is mediated, removed, and recognized by methyltransferases, demethylases, and m<sup>6</sup>A-binding proteins, respectively (8). Methyltransferase-like 3 (METTL3) (9), methyltransferase-like 14 (METTL14) (10), methyltransferase-like 16 (METTL16) (11), Wilms tumor 1-associating protein (WTAP) (12), RNA-binding motif protein 15/15B (RBM15/15B) (13), and vir-like m<sup>6</sup>A methyltransferase-associated protein (VIRMA/KIAA1429) (14) are considered to be the components of 'writers' that catalyze the formation of m<sup>6</sup>A; 'erasers' such as the obesity-associated protein (FTO) and alkB homolog 5 (ALKBH5) (15,16) remove the methyl code from target mRNAs; 'readers' such as the YTH domain protein families (YTHDF) and heterogeneous nuclear ribonucleoprotein (HNRNP) families (17,18) are capable of recognizing m<sup>6</sup>A methylation and generating a functional signal (19). YTH domain proteins read m<sup>6</sup>A through a conserved aromatic cage (20) and two other proteins, FMRP translational regulator 1 (FMR1) and leucine-rich pentatricopeptide repeat-containing (LRPPRC), can also recognize this modification (21,22). Therefore, the m<sup>6</sup>A modification is a highly dynamic and reversible process (Fig. 1) (23).

Lung cancer is the most common malignant tumor with high morbidity and mortality rates worldwide. Non-small

cell lung cancer (NSCLC) accounts for 85-90% of all cases of lung cancer, including lung squamous cell carcinoma (LUSC), lung adenocarcinoma (LUAD), and large cell anaplastic carcinoma (LCAC) (24). According to the latest data on cancer incidence, the 5-year survival rate of patients with NSCLC is as low as approximately 15%, which can be attributed to atypical symptoms in the early phase of the disease and lack of effective treatment (25,26). The epigenetic m<sup>6</sup>A modifications are involved in the progression, auxiliary diagnosis, and prognosis of lung cancer (27). Moreover, m<sup>6</sup>A modification is an important factor affecting the growth, survival, and invasion of cancer cells (28,29). Here, we review and summarize the molecular mechanisms and functions of m<sup>6</sup>A RNA modification in lung cancer. Further, we discuss the role of m<sup>6</sup>A modification in lung cancer to provide a new theoretical basis for m<sup>6</sup>A research.

## 2. m<sup>6</sup>A writers in lung cancer

The m<sup>6</sup>A methyltransferase writer complex, which catalyzes the m<sup>6</sup>A mRNA methylation in lung cancer, primarily consists of METTL3, METTL14, METTL16, and WTAP. METTL3 (also known as MTA70) is the methyltransferase primarily responsible for the m<sup>6</sup>A modification. METTL3 and METTL14 form a stable heterodimer core complex of METTL3-METTL14, which affects the cellular m<sup>6</sup>A deposition on mammalian nuclear RNAs. WTAP does not exhibit methylation activity; however, it interacts with the METTL3-METTL14 complex to significantly impact the cellular m<sup>6</sup>A deposition (10).

**METTL3.** METTL3 levels are upregulated in lung cancer tissues, which are higher in advanced stage lung cancer patients (30). METTL3 increases the translation of target mRNAs by recruiting the eukaryotic translation initiation factor (eIF)3 to the translation initiation complex in H1299 cells (31). It directly interacts with certain components of the multi-subunit eIF3 complex. Meanwhile, the METTL3-eIF3H interaction is essential for promoting translation, formation of densely packed polyribosomes, and oncogenic transformation of A549 cells (Fig. 2A). Disruption of the METTL3-eIF3H interaction eliminates the ability of METTL3 to promote translation, influence polysome conformation, and enhance oncogenic transformation (32). Furthermore, Lin *et al* found that METTL3 enhanced RNA translation without the aid of methyltransferase and reader protein activity. METTL3 increases RNA translation by directly recruiting translation initiation factors. METTL3 knockdown inhibits the recruitment of eIF3 to both the cap-binding protein 80 (CBP80)- and eIF4E-cap binding proteins (33). Inhibition of m<sup>6</sup>A with METTL3 short hairpin RNA (shMETTL3) significantly decreases the expression of eIFs in lung cancer cells (34).

METTL3 regulates the translation of genes related to tumor progression and apoptosis. METTL3 knockdown in A549 cells decreases the expression of bromodomain containing 4 (BRD4) and other targets, and the cells expressing METTL3 are more sensitive to pharmacological BRD4 inhibition. METTL3 promotes translation only when it is tethered to the reporter mRNA at sites close to the stop codon and assists

the mRNA looping mechanism for ribosome recycling and translational control (32). The mRNAs of several oncogenes, such as the epidermal growth factor receptor (EGFR), tafazzin (TAZ), MAPKAPK2 (MK2), and DNA methyltransferase 3 alpha (DNMT3A), have one or more m<sup>6</sup>A peaks near the stop codon. The analysis of m<sup>6</sup>A levels in EGFR revealed that METTL3 binds to the EGFR mRNA in A549 cells (33). METTL3 knockdown significantly increases E-cadherin expression and decreases the expression of Fibronectin and Vimentin in A549 and LC-2/ad cells. Moreover, it inhibits the expression changes of these epithelial-mesenchymal transition (EMT)-related marker genes stimulated by transforming growth factor  $\beta$  (TGF- $\beta$ ) treatment. These results suggest the involvement of endogenous METTL3 in the transcriptional regulation of TGF- $\beta$ -induced EMT program (35). Furthermore, the expression of metastasis-associated lung adenocarcinoma transcript 1 (MALAT1), a transcript related to lung cancer metastasis and prognosis, is increased due to a higher level of m<sup>6</sup>A modification mediated by METTL3. Meanwhile, the METTL3/YTHDF3 complex increases the stability of MALAT1. METTL3 catalyzes the m<sup>6</sup>A methylation modification of the nuclear effector yes-associated protein (YAP) of the Hippo signaling pathway, promotes its translation, and mediates the proliferation and metastasis of NSCLC (31).

The characteristics of METTL3 in activation and post-translational modification (such as SUMOylation) of METTL3 may directly affect the proliferation and xenograft tumor growth of lung cancer cells. SUMOylation of METTL3 mediates the m<sup>6</sup>A mRNA modification and subsequent differences in gene expression profiles (36).

miRNAs can be differentially expressed and act as oncogenic or tumor suppressor miRNAs, which are based on the roles of miRNA-regulated genes (37). The m<sup>6</sup>A modification may control arsenite-induced proliferation and apoptosis of cells by affecting miRNAs (38). Studies have provided new insights into the mechanism of METTL3 regulation by miRNAs, thus signifying the potential application of METTL3 as a therapeutic target in NSCLC. For example, miR-33a inhibits NSCLC cell proliferation by targeting the 3'-UTR of METTL3 mRNA (39). Moreover, miR-600 attenuates the METTL3 expression and regulates cell proliferation, metastasis, and apoptosis by regulating the PKB Protein Kinase (AKT) and  $\beta$ -catenin signaling pathways (40). METTL3 has been reported to increase the splicing of precursor miR-143-3p, accelerate the processing and maturation of miR-143-3p. Moreover, miR-143-3p/VASH1 axis acts as adverse prognosis factors for *in vivo* progression and overall survival rate of lung cancer (41). In summary, miRNA is an important bridge for m<sup>6</sup>A to influence the proliferation, metastasis, invasion, and apoptosis of lung cancer cells.

**Other m<sup>6</sup>A methyltransferases.** WTAP is the target of miRNAs and accelerates the progression of NSCLC (42). METTL16 is a recently confirmed m<sup>6</sup>A RNA methyltransferase that interacts with the 3'-terminal RNA triple helix of MALAT1 in lung cancer (43). The three-m<sup>6</sup>A-regulator signature (KIAA1429, METTL3 and IGF2BP1) is recognized as an independent prognostic model to categorize lung cancers into high- and low-risk groups for patient stratification, prognostic assessment, and personalized treatment in lung cancer.

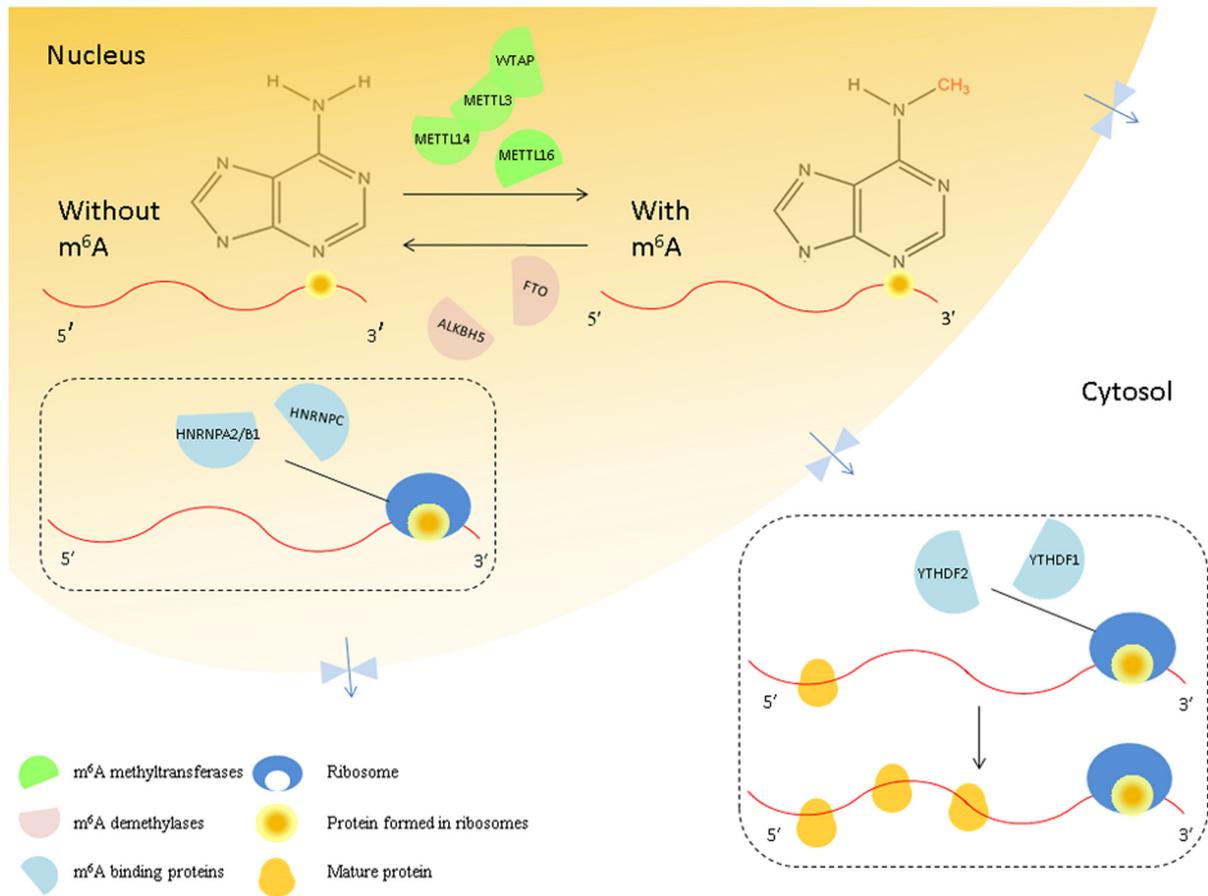


Figure 1. Interaction between the mRNA methylation 'writers,' 'erasers' and 'readers.' In the nucleus, m<sup>6</sup>A methyltransferases (green semicircles) are named 'writers'. Their binding proteins (blue semicircles) are named 'readers.' The demethylases (pink semicircles) are named 'erasers.' The m<sup>6</sup>A 'writers' include METTL3/14/16 and WTAP, and the 'erasers' that can eliminate the m<sup>6</sup>A from mRNAs include FTO and ALKBH5. Furthermore, m<sup>6</sup>A is recognized by the 'readers,' such as YTHDF1/2/3, HNRNPA2/B1, and HNRNPC. YTHDF1/2/3 (yellow circles) promotes the translation of m<sup>6</sup>A-modified mRNAs in the cytosol. m<sup>6</sup>A, N<sup>6</sup>-methyladenosine; METTL, methyltransferase-like; WTAP, Wilms tumor 1-associating protein; FTO, Fat mass and obesity-associated protein; ALKBH5, alkB homolog 5; YTHDF, YTH domain protein families; HNRNP A2/B1, heterogeneous nuclear ribonucleoprotein A2/B1; HNRNPC, heterogeneous nuclear ribonucleoprotein C.

KIAA1429 and insulin-like growth factor 2 mRNA-binding protein 1 (IGF2BP1) are significantly associated with multiple biological processes, including proliferation, apoptosis, metastasis, energy metabolism, drug resistance, and recurrence; additionally, they target potential genes related to lung cancer (44). Proteinase activated-receptor 2 (PAR2) participates in cancer metastasis promoted by serine proteinases. Knock down of NOP2/sun domain family, member 2 (NSun2), a new RNA methyltransferase, blocks the reduction in miR-125b induced by PAR2. NSun2 is shown to interfere in the mature processing of miR-125b from pri- and pre-miR-125b2 in A549 cells. Furthermore, PAR2 activation increased the level of m<sup>6</sup>A-containing pre-miR-125b in NSun2-dependent manner (45). Overall, these data reveal the existence of a complex network of interactions between the m<sup>6</sup>A methylases and oncogenes that can regulate the proliferation, metastasis, invasion, and apoptosis of lung cancer cells.

### 3. m<sup>6</sup>A erasers in lung cancer

m<sup>6</sup>A is deposited by the methyltransferase complex and cleared by the demethylases FTO and ALKBH5. These demethylases participate in the biological processes of lung

cancer (46,47). m<sup>6</sup>A in the nuclear RNA is a substrate of FTO, and FTO causes an enzymatic alteration that may be related to mRNA transcription (48). ALKBH5, a primary m<sup>6</sup>A demethylase, plays important roles in lung cancer by regulating proliferation, migration, invasion, metastasis, and tumor growth (49).

**FTO.** Increased METTL3 and decreased FTO levels demonstrate that the dysregulated writer and/or eraser may affect the m<sup>6</sup>A content in both the cells and tissues of LUAD patients (34). RNA sequencing analysis has revealed that some genes are influenced by m<sup>6</sup>A demethylation, most of which are associated with lung cancer, such as laminin  $\gamma$ 2, nerve growth factor inducible, integrin alpha 11, thrombospondin 1, and proprotein convertase subtilisin/kexin type 9. FTO enhances LUAC cell progression by activating cell migration (15).

In LUSC, FTO acts as a prognostic factor responsible for aberrant m<sup>6</sup>A modifications (50). The proliferation and invasion of cells in LUSC was found to be effectively decreased by FTO knockdown. Furthermore, overexpression of FTO rather than its mutant form promotes the malignant phenotype of cells. Mechanism analysis demonstrated that FTO decreases the m<sup>6</sup>A modification of the myeloid zinc finger 1 (MZF1)

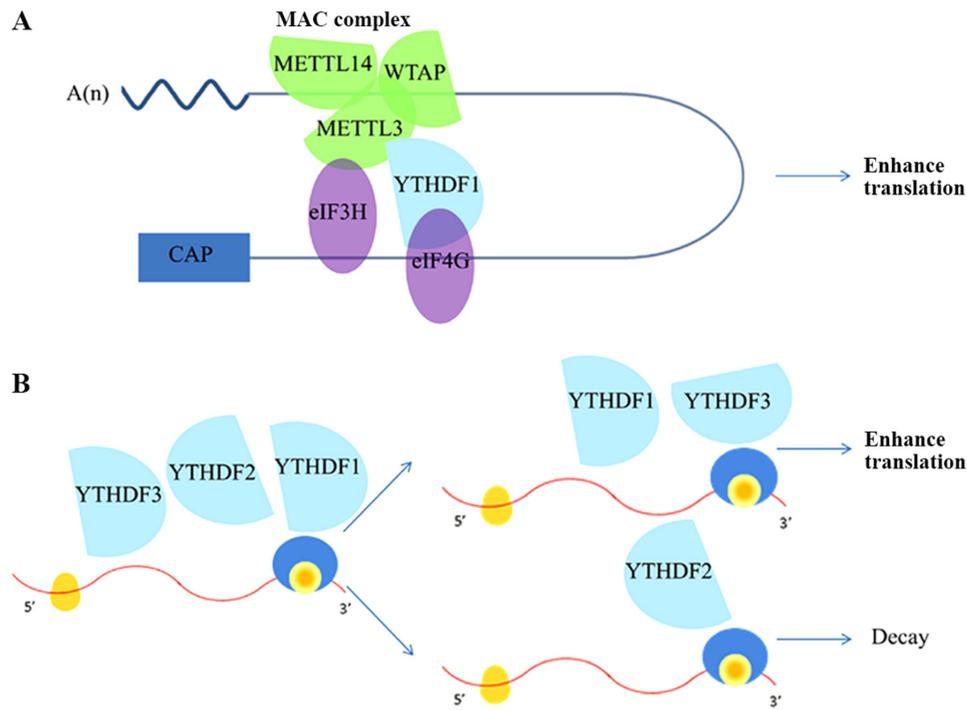


Figure 2. Functional roles of m<sup>6</sup>A modification on mRNA expression. (A) RNA circularization. METTL3, an RNA methyltransferase located at the 3'-UTR, combines with METTL14 and WTAP to form the MAC complex, which increases the translation of specific mRNAs independently through its catalytic activity and recruitment of eIF3H. YTHDF1 binds to eIF4G at 5'-UTR, which promotes the process of RNA circularization. (B) YTHDF1 and YTHDF3 synergistically promote protein synthesis, while YTHDF2 affects the attenuation of methylated mRNA. YTHDF1/2/3 serves a key role in the metabolism of N<sup>6</sup>-methyladenosine-modified mRNAs in the cytoplasm. METTL, methyltransferase-like; UTR, untranslated region; WTAP, Wilms tumor 1-associated protein; MAC, m<sup>6</sup>A-METTL complex; eIF3H, eukaryotic translation initiation factor 3H; YTHDF, YTH domain protein families.

transcript and strengthens its stability, resulting in increased MZF1 expression, as well as promotion of the occurrence and development of lung cancer (47).

In addition, FTO represses the m<sup>6</sup>A levels and strengthens the mRNA stability of ubiquitin-specific protease 7 (USP7), which relies on the demethylase activity of FTO. FTO down-regulation inhibits proliferation and growth of NSCLC cells by facilitating the expression of USP7 (46). Therefore, over-expression of FTO promotes the proliferation, migration, and invasion abilities of lung cancer cells.

**ALKBH5.** ALKBH5 is upregulated in NSCLC and is closely associated with a poor prognosis. Functionally, ALKBH5 facilitates proliferation and inhibits apoptosis of the NSCLC cells *in vitro*, whereas ALKBH5 knockdown reduces tumor growth *in vivo* (16). The overexpression of ALKBH5 results in the increase translation efficiency of factor forkhead box M1 (FOX M1) mRNA by decreasing the level of m<sup>6</sup>A in FOX M1, which promotes the growth of LUAD cells (51). Mechanistically, ALKBH5 knockout inhibits the growth and invasion of A549 and NCI-H566 cells under intermittent hypoxia by down-regulating the m<sup>6</sup>A modification of FOX M1 and increasing the FOX M1 levels (51). Mechanistically, methylated RNA immunoprecipitation sequencing revealed that ALKBH5 targets the 3'-UTR of tissue inhibitor of metalloproteinase 3 (TIMP3). ALKBH5 inhibits the TIMP3 transcript stability, thereby reducing its translation (16). Due to the upregulation of ALKBH5 in NSCLC, the oncogene ubiquitin conjugating enzyme E2C (UBE2C) is stabilized epitranscriptionally with the remaining lower m<sup>6</sup>A levels within its mature RNAs.

Activation of UBE2C is associated with adverse prognosis and enhances proliferation, clonogenicity, and invasive growth of NSCLC cells (52). Furthermore, ALKBH5 restrains tumor growth and metastasis by decreasing the expression and activity of YAP in a YTHDF- and miR-107/large tumor suppressor kinase 2 (LATS2)-mediated manner. YAP expression is negatively correlated with ALKBH5 expression and plays an opposite role in the regulation of cellular proliferation, invasion, migration, and EMT of NSCLC cells (53). Collectively, m<sup>6</sup>A demethylases affect the proliferation, invasion, and apoptosis of lung cancer cells by downregulating the m<sup>6</sup>A modification of mRNA.

#### 4. m<sup>6</sup>A readers in lung cancer

m<sup>6</sup>A also affects biological processes by recruiting reader proteins that specifically recognize m<sup>6</sup>A RNA methylation and affect the downstream functions (54,55). YTH N<sup>6</sup>-methyladenosine RNA-binding protein (YTHDF)1, YTHDF2, YTHDF3, YTH domain containing 1 (YTHDC)1, and YTHDC2 read mRNA with the m<sup>6</sup>A modification specifically in the cytoplasm (56). In the cytoplasm, the m<sup>6</sup>A-binding protein YTHDF1 promotes the translation of m<sup>6</sup>A-modified mRNAs, and YTHDF2 facilitates the decay of m<sup>6</sup>A-modified transcripts. YTHDF3 accelerates protein synthesis in synergy with YTHDF1 and impacts the methylated mRNA decay mediated by YTHDF2. Cells deficient in all three YTHDFs show the most dramatic accumulation of m<sup>6</sup>A-modified transcripts (8). In addition to the YTH domain m<sup>6</sup>A readers, other readers such as the heterogeneous nuclear

ribonucleoprotein A2/B1 (HNRNP A2/B1) and heterogeneous nuclear ribonucleoprotein C (HNRNPC) do not directly interact with m<sup>6</sup>A but bind to the transcripts containing m<sup>6</sup>A (57).

**YTH domain family.** The expression of YTHDF1 and YTHDF3 is higher, but that of YTHDF2 is lower, in human lung cancer tissues compared to adjacent normal lung tissues. These alterations are related to the functions of mRNA translation and decay of the pre-mRNA target genes (Fig. 2B). Similarly, YTHDF1 knockdown partly blocks the pre-mRNA processing factor 6 (PRPF6) expression and cell growth (34). YTHDF1 positively facilitates protein synthesis by interacting with the translation machinery (58). YTHDF1 knockdown restrains NSCLC cell proliferation and xenograft tumor formation by regulating the translational efficiency of cyclin-dependent kinase (CDK)2, CDK4, and cyclin D1, whereas YTHDF1 depletion inhibits lung cancer progression. High expression of YTHDF1 is related to better clinical outcomes, with its depletion rendering cancer cells resistant to cisplatin treatment. Mechanistic studies identified the Keap1-Nrf2 axis as the downstream mediator of YTHDF1 (59). YTHDF2 is upregulated in lung cancer tissues, promotes lung cancer cell growth and enhances the pentose phosphate pathway (PPP) flux, which is important for tumor growth. Mechanistically, YTHDF2 directly binds to the m<sup>6</sup>A modification site of 6-phosphogluconate dehydrogenase(6-PGD) 3'-UTR to promote 6-PGD mRNA translation in lung cancer cells (60). YTHDF3 with METTL3, YTHDF1, and eIF3b directly promotes YAP translation via interaction with the translation initiation machinery in NSCLC. Meanwhile, MALAT1 stability is increased by the METTL3/YTHDF3 complex. Both YAP and MALAT1 promote carcinogenic activity and are associated with the occurrence of lung cancer (31). YTHDF1 and YTHDF2 competitively interacted with YTHDF3 in an m<sup>6</sup>A-independent manner to regulate YAP expression. YTHDF2 facilitated YAP mRNA decay via the AGO2 system, whereas YTHDF1 promoted YAP mRNA translation by interacting with eIF3a; both these activities are regulated by m<sup>6</sup>A modification (53).

In addition, the m<sup>6</sup>A-binding protein YTHDC2 is associated with tumor progression in lung cancer (27). YTHDC2 is frequently suppressed in LUAD. Downregulation of YTHDC2 is associated with poor clinical outcome of LUAD. Moreover, YTHDC2 exhibits antitumor activity in human LUAD cells. Mechanistically, YTHDC2, through its m<sup>6</sup>A-recognizing YTH domain, inhibits cystine uptake and blocked the downstream antioxidant program. Furthermore, solute carrier 7A11 (SLC7A11), the catalytic subunit of cystine transporter system Xc-, is identified to be the direct target of YTHDC2. YTHDC2 destabilizes SLC7A11 mRNA in an m<sup>6</sup>A-dependent manner because YTHDC2 preferentially bound to m<sup>6</sup>A-modified SLC7A11 mRNA and thereafter promotes its decay (61). In summary, these YTH domain family proteins may influence the fundamental biological processes in an integrated and cooperative manner in lung cancer.

**HNRNP family.** HNRNP A2/B1 is overexpressed in lung cancer and in other cancers, such as liver cancer, breast cancer, and pancreatic cancer (62,63). This overexpression is not associated with the histological classification of lung

cancer but with the clonal expansion of the tumor in NSCLC patients (64,65). He *et al* demonstrated that hnRNP A2/B1 formed complexes with the transcripts of many of the verified downstream genes, suggesting that HNRNP A2/B1 contributes to the regulation of these genes (66). The expression of HNRNP A2/B1 protein is correlated with the expression of anaxoleto (AXL). The expression of HNRNP A2/B1 and AXL affects the prognosis of patients with NSCLC (67). CACNA1G-AS1 (CAS1) increases the level of HNRNP A2/B1, which enhances cancer cell invasion and migration in NSCLC (68). In addition, expression of HNRNP A2/B1 may affect the function of EMT by regulating the E-cadherin expression in non-epithelial lung cancer cell lines (62). These results reinforce the conclusion that HNRNP A2/B1 is associated with cellular processes that affect the cell cycle and proliferation.

HNRNPC is another RNA-binding protein 'reader' of m<sup>6</sup>A methylation, and is related to the progression of various cancers. HNRNPC is upregulated in progressed lung cancer (27). HNRNPC expression is significantly related to poor overall survival in patients with LUAD, indicating that HNRNPC may be a cancer-promoting factor and a potential prognostic biomarker in LUAD (69). Overexpression of HNRNPC significantly enhances lung cancer cell proliferation, migration, and invasion both *in vitro* and *in vivo*. In NSCLC cell lines, HNRNPC interacts with KH-type splicing regulatory protein (KHSRP), which is considered to be a metastasis-associated candidate molecule. KHSRP and HNRNPC are significantly associated with advanced tumor progression and metastasis (both lymph node and distant) and may induce invasion and metastasis in human lung cancer (70).

**The other m<sup>6</sup>A binding proteins.** IGF2BPs, a class of RNA-binding proteins, including IGF2BP1, IGF2BP2, and IGF2BP3, are considered to be the 'reader' of m<sup>6</sup>A methylation and remarkably affects cancer occurrence and development. Studies have proved that IGF2BP3 has prognostic potential in multiple public databases compared with other members of the IGF2BPs family. IGF2BP3 is abnormally highly expressed in LUAD tissue, and can lead to worse overall survival. IGF2BP3 expression could serve for independently predicting the prognosis of LUAD patients. In summary, IGF2BP3 may be an oncogene and potential prognostic biomarker of LUAD (71).

m<sup>6</sup>A regulators regulate the expression of the downstream target genes by mediating the mRNA stability, translation efficiency, and mRNA decay to affect the proliferation, migration, and invasion of lung cancer cells (Table I). Knowledge about the mechanism of m<sup>6</sup>A methylation is limited, and the supplementary discoveries of regulatory patterns mediated by m<sup>6</sup>A in lung cancer are worth verifying in future studies.

## 5. m<sup>6</sup>A as a potential therapeutic target in lung cancer

The treatment options for lung cancer have developed considerably over the past years; however, most patients are diagnosed at an advanced stage of the disease due to the insidious symptoms of early-stage lung cancer. Thus, the survival rate of lung cancer patients remains low (72). Most RNA methylation regulators had distinct expressions in tumor tissues and adjacent tissues. The patients in the high-risk group were more likely to have a higher stage, more lymph node metastases, and

Table I. Potential mechanisms and target genes of m<sup>6</sup>A regulators in lung cancer.

A, Writer m <sup>6</sup> A components			
Proteins	Related targets	Roles in lung cancer	(Refs.)
METTL3	EGFR, TAZ, eIF, CBP80, BRD4, DNMT3A, JUNB, EZH2, ATG7, LC3B, SQSTM1	Promotes growth, translation, survival and invasion of lung cancer cells	(28,30,25,49)
METTL14	Unknown	Forms complex with METTL3	(10)
METTL1 <sup>6</sup>	MALAT	Combines with metastasis-related lung adenocarcinoma transcripts to promote lung cancer activity	(85)
WTAP	Unknown	Forms complex with METTL3	(86)
B, Eraser m <sup>6</sup> A components			
Proteins	Related targets	Roles in lung cancer	(Refs.)
FTO	USP7, MZF1	Promotes growth of lung cancer cells	(46,47)
ALKBH5	FOXMI, YAP UBE2C, TIMP3	Promotes growth and invasion of lung adenocarcinoma cells and stabilize mRNA transcripts	(16,53)
C, Reader m <sup>6</sup> A components			
Proteins	Related targets	Roles in lung cancer	(Refs.)
YTHDF1	eIF, G3BP1	Promotes translational efficiency	(58)
YTHDF2	6-PGD	Reduces the stability of the target transcript	(60)
YTHDF3	YAP	Regulates the stability of mRNA and cooperates with YTHDF1 to promote protein synthesis	(53)
HNRNP A2/B1	AXL, COX-2, PGE2	Regulates expression of transcription-related factors and determines cell fate transition	(64,87,88)
HNRNPC	KHSRP, uPAR	Promotes proliferation, migration, and invasion of lung cancer cells	(70,89)

6-PGD, 6-phosphogluconate dehydrogenase; ATG, autophagy protein; AXL, anexeletto; BRD4, bromodomain-containing protein 4; CBP80, cap-binding protein 80; COX-2, cytochrome c oxidase subunit II; DNMT3A, DNA methyltransferase 3 alpha; EGFR, epidermal growth factor receptor; eIF, eukaryotic translation initiation factors; EZH2, enhancer of zest homolog 2; FOXMI, factor forkhead box M1; G3BP1, GTPase-activating protein-(SH3domain)-binding protein 1; JUNB, recombinant Jun B proto oncogene; KHSRP, KH-type splicing regulatory protein; LC3B, light chain 3B; MZF1, myeloid zinc finger 1; MALAT1, metastasis-associated lung adenocarcinoma transcript 1; PGE2, prostaglandin E2; SQSTM1, Sequestosome 1; TAZ, tafazzin; TIMP3, tissue inhibitors of metalloproteinase 3; UBE2C, ubiquitin conjugated enzyme E2C; uPAR, urokinase plasminogen activator receptor; USP7, ubiquitin-specific protease 7, YAP, yes-associated protein.

distant metastases, showing a poor clinical outcome. Different molecular phenotypes constructed by RNA methylation regulators can be independent ri7sk factors for the prognosis of LUAD (73). The expression of m<sup>6</sup>A methylation regulators between high- and low-risk LUSC patients is significantly different, and the high-risk LUSC patients have significantly low levels of ALKBH5, METTL3, HNRNPC, and KIAA1429. Thus, m<sup>6</sup>A methylation regulators may result in a poor prognosis in patients with low-risk LUSC (74).

Recently, cancer immunotherapy has become involved in treating all forms of cancer and has changed the landscape of cancer care. LUAD is the most common histological subtype in lung cancer. LUAD subtypes are identified on the basis of the immunogenomic profiling of 29 immune signatures.

There are three LUAD subtypes: Immunity High, Immunity Medium, and Immunity Low. The Immunity High subtype exhibits more sensitivity to immunotherapy and chemotherapy. Immunity High is significantly associated with decreased gene expression, such as METTL3, RBM15, YTHDC1, YTHDF1, and YTHDF2, which are involved in m<sup>6</sup>A mRNA methylation. And the level of m<sup>6</sup>A RNA methylation, associates with cancer initiation and progression, is reduced in the Immunity High subtype (75). Interleukin-37 (IL-37) plays a crucial protective role in lung cancer. Treatment of IL-37 in lung cancer cells induced widespread and dynamic RNA m<sup>6</sup>A methylation. It could lead to changes in m<sup>6</sup>A methylation level and relates molecule expression level in A546 cells, such as METTL3, METTL14, WTAP, ALKBH5 and FTO, and may

downregulate the proliferation by inhibiting Wnt5a/5b pathway in A549 cells. It concludes that IL-37 suppresses tumor growth through regulation of RNA m<sup>6</sup>A methylation in lung cancer cells (76). These findings suggest that m<sup>6</sup>A RNA methylation is important determinants of initiation, progression and prognosis in lung cancer and may provide potential prognostic biomarker or therapeutic target for immunotherapeutic and chemotherapeutic development.

m<sup>6</sup>A performs multi-functional roles in EMT modulation, suggesting the critical roles of m<sup>6</sup>A in cancer progression regulation. EMT plays a critical role in lung cancer progression; thus, it is important to identify the factors that inhibit EMT in lung cancer treatment (77). METTL3 down-regulation in lung cancer tissues influences EMT via m<sup>6</sup>A modification of the enhancer of zeste homolog 2 (EZH2), contributes to the macrophage recruitment, and reduces the malignant progression of lung cancer (30). Knockdown of METTL3 inhibits the TGF- $\beta$ -induced morphological conversion of the cell and increases the cell migration potential as well as changes in the expression of EMT-related marker genes (35). TGF- $\beta$ 1-induced EMT is inhibited in METTL3 knockdown cells. The expression of TGF- $\beta$ 1 is up-regulated, while self-stimulated expression of TGF- $\beta$ 1 is suppressed in METTL3 cells. m<sup>6</sup>A promotes TGF- $\beta$ 1 mRNA decay, but impairs TGF- $\beta$ 1 translation progress. Besides this, the autocrine of TGF- $\beta$ 1 is disrupted in METTL3 cells through interrupting TGF- $\beta$ 1 dimer formation. Snail, which is down-regulated in METTL3 cells, is a key factor responding to TGF- $\beta$ 1-induced EMT (78). In addition, YTHDF1 is positively correlated with the growth, invasion, and EMT of NSCLC cells, while YTHDF2 plays an opposite role in these cell processes (53). Recent studies have demonstrated the inhibitory effect of simvastatin on tumor cell proliferation. Simvastatin causes METTL3 downregulation in lung cancer tissues, resulting in EMT via m<sup>6</sup>A modification of mRNA, thus restraining the malignant progression of lung cancer (30). These results indicate that m<sup>6</sup>A regulators can be potential therapeutic targets for EMT in lung cancer cells.

In recent years, epigenetics, especially m<sup>6</sup>A RNA modification, has been further understood and explored with the rapid advances in detection methods and high-throughput sequencing techniques (79). It has been widely illustrated that the dysregulation of m<sup>6</sup>A RNA modification is related to various types of cancers, as well as the drug resistance to anti-tumor therapy (80). In three LUAD cell lines, treatment with ammonium tetra thiomolybdate (ATTM) at high concentrations inhibited the cell growth, while at low ATTM concentrations the cell growth was promoted. Treatment with ATTM significantly increased the level of METTL3 but reduced the FTO levels. Additionally, ATTM upregulates METTL14 expression, which is not consistent with The Cancer Genome Atlas (TCGA) analysis. This difference may be due to the uncertainty in gene expression between the mRNA and protein levels. This unique expression contributes to ATTM-induced increase in m<sup>6</sup>A in A549 cells (34). METTL3 promotes the translation of important oncogenes like EGFR in lung cancer. EGFR inhibitors, such as gefitinib and erlotinib, have gained approval for the treatment of patients with NSCLC (81). FTO inhibitor rhein enhances the antitumor activity of pemetrexed through influencing autophagy and

apoptosis by modulating the p PI3K-AKT-mTOR pathway and B-cell lymphoma-2 (Bcl-2) family of proteins in A549 cells. It demonstrates that the potential application of rhein as a candidate drug in combination with pemetrexed is promising for treatment of the human lung cancer (82). m<sup>6</sup>A methyltransferase METTL3-mediated autophagy plays an important role in reversing gefitinib resistance by  $\beta$ -elemene in NSCLC cells. Mechanistically,  $\beta$ -elemene can reverse gefitinib resistance by inhibiting the late stage of autophagy in a manner of chloroquine, which inhibits the maturation of autophagosomes into autolysosomes through attenuating the lysosomal acidification. In this reversing process, METTL3 can positively regulate this autophagy process by targeting autophagy protein (ATG5), ATG7, light chain 3B (LC3B) and Sequestosome 1 (SQSTM1) (83). Functional enrichment analysis of the m<sup>6</sup>A-modified genes revealed that m<sup>6</sup>A methylation might modify the cell cycle to influence the response to afatinib. Furthermore, these m<sup>6</sup>A-modified genes are over-represented in the putative drug resistance-associated genes and FDA-approved drug targets and have a higher average degree and clustering coefficient than other genes in the protein-protein interaction network (84). Overall, the action of m<sup>6</sup>A regulators may contribute to drug resistance in tumor therapy and prognosis.

## 6. Conclusion

m<sup>6</sup>A methylation has a huge impact on RNA production/metabolism and is involved in the pathogenesis of many diseases, including cancer. In the occurrence and development of lung cancer, m<sup>6</sup>A-modified mRNA regulates RNA transcription, splicing, processing, translation, and decay. Accumulating evidence reveals that m<sup>6</sup>A regulators and their mechanisms of action play vital roles in lung cancer. The m<sup>6</sup>A modification directly or indirectly affects cell proliferation, metastasis, invasion, and apoptosis. Systematic study of the functions and potential molecular mechanisms of m<sup>6</sup>A regulators will further improve our understanding of the complex networks associated with lung cancer. Inhibitors targeting m<sup>6</sup>A regulators may have great therapeutic potential in the treatment of lung cancer. In addition to the known modulating effects of m<sup>6</sup>A methylation, the underlying mechanism of m<sup>6</sup>A modification in lung cancer needs to be further investigated.

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### Availability of data and materials

Not applicable.

### Authors' contributions

YW and XS designed the review. MZ, MX, YC, and ZL were involved in the collection and collation of references. YW wrote, reviewed, and edited the manuscript. WZ critically revised the manuscript for intellectual content. All authors read and approved the final version of the manuscript.

### Ethics approval and consent to participate

Not applicable.

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Not applicable.

### Competing interests

The authors declare that they have no competing interests.

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