Minireview

Emerging role of N6-methyladenosine RNA methylation in lung diseases

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Impact Statement

The N6-methyladenosine (m^6A) methylation is the most abundant internal modification on mRNA. It is confirmed to be closely related to the occurrence, development, targeted therapy, diagnosis, and prognosis of multiple lung diseases. In this review, we conclude that, the m^6A methylation affects the occurrence and development of asthma, chronic obstructive pulmonary disease, pulmonary interstitial disease, and lung cancer through inflammation and immune function. Meanwhile, their dysregulations are associated with drug resistance and poor prognosis in lung cancer patients. The m^6A regulators can be viable therapeutic targets for drug resistance and new drug targets for targeted therapy. The increased level of m^6A modification in circulating tumor cells (CTCs) is indicated to be a non-invasive diagnostic method for lung cancer. More importantly, models composed of multiple m^6A regulators are also used to evaluate the diagnosis, risk or prognosis of lung diseases. Therefore, we believe that m^6A methylation plays an extremely important role in lung diseases.

Abstract

In recent years, with the increase of air pollution, smoking, aging, and respiratory infection, the incidence rate and mortality of lung diseases are increasing annually, which has become a major hazard to human health. N6-methyladenosine (m^6A) RNA methylation is the most abundant modifications in eukaryotes, and such modified RNA can be specifically recognized and combined by m^6A recognition proteins and then mediate RNA splicing, maturation, enucleation, degradation, and translation. More and more studies have revealed that the m^6A modification is involved in the pathogenesis and development of some diseases; however, the mechanisms of m^6A in lung diseases are poorly understood. In this review, we summarize the latest progress in the biological function of m^6A modifications in lung diseases and discuss the potential therapeutic and prognostic strategies. The dysregulation of global m^6A levels and m^6A regulators may affect the occurrence and development of asthma, chronic obstructive pulmonary disease, lung cancer, and other lung diseases through inflammation and immune function. In lung cancer, this modification has an important impact on malignant cell proliferation, migration, invasion, and drug resistance. In addition, abnormally changed m^6A-modified proteins in lung cancer tissue samples and circulating tumor cells (CTCs) may be used as diagnostic and prognostic markers of lung cancer. Models composed of multiple m^6A regulators can be used to evaluate the risk prediction or prognosis of asthma and pulmonary fibrosis. In general, the in-depth study of m^6A modifications is a frontier direction in disease research. It provides novel insights for understanding of the molecular mechanisms underlying disease occurrence, development, and drug resistance, as well as for the development of effective novel therapeutics.

Keywords: N6-methyladenosine, asthma, chronic obstructive pulmonary disease, idiopathic pulmonary fibrosis, lung cancer

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Introduction

Environmental exposure and inflammation can cause most categories of lung diseases.[1] Lung diseases include asthma, chronic obstructive pulmonary disease (COPD), idiopathic pulmonary fibrosis (IPF), pulmonary hypertension (PH), and lung cancer.[2] COPD is a common chronic disease, which is the fourth leading cause of death in the world, with the characteristics of airflow obstruction. IPF is a kind of chronic progressive fibrosis interstitial pneumonia, which is characterized by dyspnea and gradual deterioration of lung function.[3] High mortality of lung cancer is associated with common diagnosis at an advanced stage that hampers curative therapy and leads to a poor 5-year survival rate.[4]

N6-methyladenosine (m^6A) refers to the methylation modification of adenine nucleoside N6, which is enriched near the stop codon and 3′-untranslated terminal region (UTR).[5,6] There are three types of regulators for RNA m^6A methylation modification. The methyltransferases ("writers") are responsible for catalysis while the demethylases ("erasers") are responsible for removing methylation. Binding proteins ("readers") recruit downstream functional complexes to perform their functions (Figure 1). The m^6A modification functionally regulates the eukaryotic transcriptome and

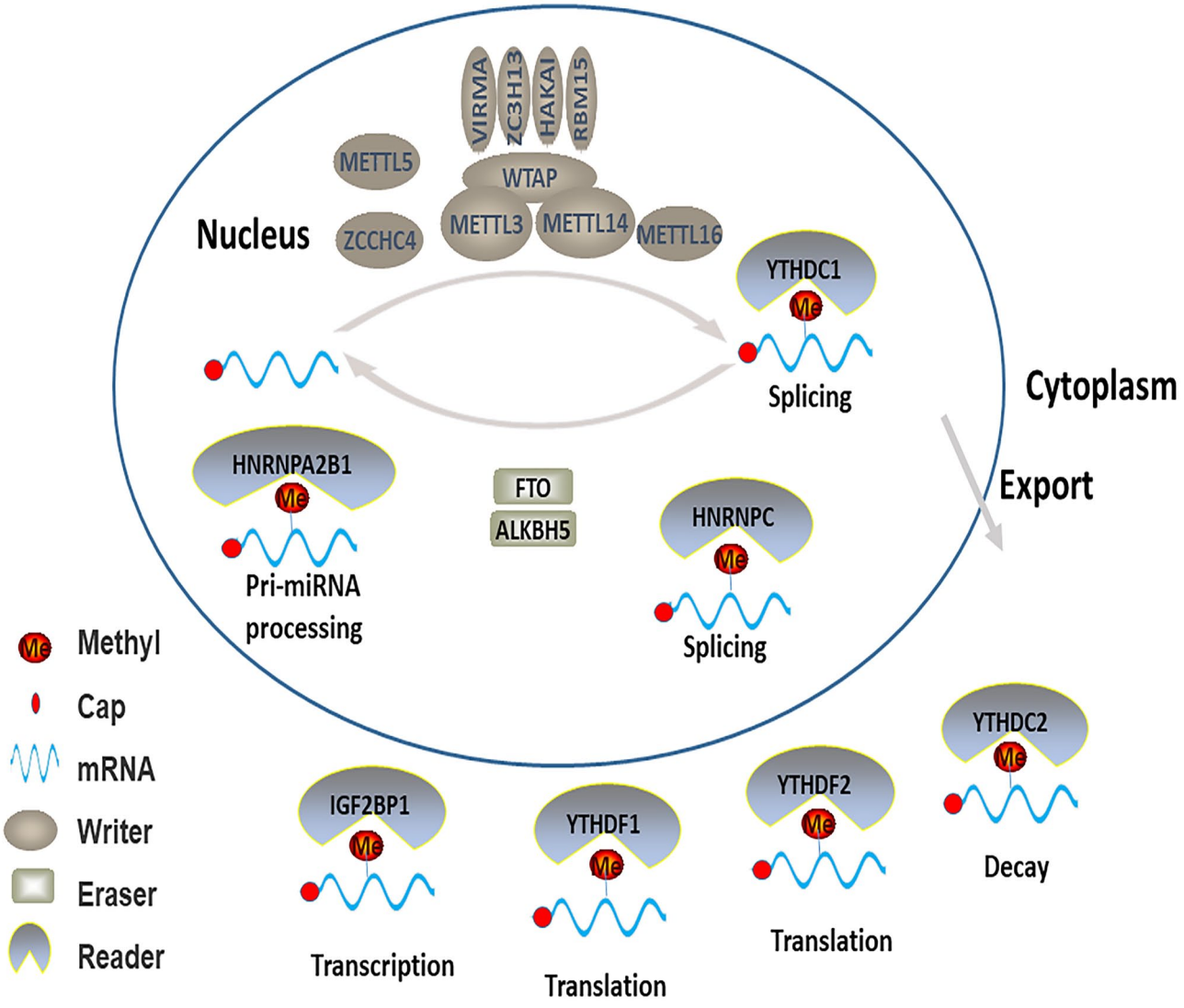

Figure 1. The dynamic and reversible processes and consequences of m^{6}A methylation. (A color version of this figure is available in the online journal.)

affects the splicing, nuclear export, translation, and stability of mRNA.[5,7,8] Nevertheless, the underlying mechanisms of how m^{6}A RNA methylation plays the biological function are poorly understood. Recently, emerging evidences have reported that m^{6}A modifications play critical roles in a series of cancers, metabolic diseases, infertility, and neural development.[9–15] These modifications appear to play a carcinogenic role in some cancers while have anti-tumor effects in others. Therefore, studying the biological functions of genes regulated by m^{6}A in different diseases and identifying the key m^{6}A target genes are of great significance to understand the pathogenesis of multiple diseases. In this comprehensive review, we describe the roles played by m^{6}A modifications of mRNA in the development of lung diseases.

RNA modification-related proteins

RNA m^{6}A methyltransferases consist of the METTL3 (methyltransferase-like 3) complex, METTL16, METTL5, ZCCHC4 (zinc finger CCHC-type containing 4).[16,17] METTL3 complex functions with METTL3 acting as the catalytic core and METTL14 as the RNA-binding platform.[18] While several new components, including WTAP (Wilms' tumor 1-associated protein), RBM15 (RNA-binding motif protein 15), KIAA1429 (also known as VIRMA), ZC3H13 (zinc finger CCCH domain-containing protein 13), and HAKAI (also known as CBLL1) function as regulatory subunits to regulate the activity of METTL3 in cells.[19–22] Studies have indicated that METTL3 promotes the translation of a large subset of oncogenic mRNAs by interacting with eukaryotic translation initiation factors.[23] New studies suggested that METTL16, METTL5, and ZCCHC4 are also identified as m^{6}A methyltransferases, which can function independently and catalyze m^{6}A modification on some structural RNA.[24–26] As the homologous protein of METTL3, METTL16 regulates the m^{6}A modification of U6 snRNA.[24] METTL5 catalyzes m^{6}A on 18S rRNA, while ZCCHC4 mainly methylates human 28S rRNA.[25,26] METTL5 can enhance the metabolic stability in cells by forming a heterodimer complex with a special structure with methyltransferase activator TRMT112.[17,26] At the same time, m^{6}A modification of ZCCHC4 mainly affects translation and cell proliferation.

FTO (fat mass and obesity-associated protein) and ALKBH5 (alkB homolog 5) are common demethylases that reverse m^{6}A modification which considered m^{6}A erasers.[27,28] FTO is the first discovered RNA demethylase and mediates the demethylation of internal m^{6}A.[27] In a study of lung squamous cell carcinoma, the high expression of FTO was significantly correlated with the poor prognosis of patients.[29] FTO also functioned as an oncogene by regulating the expression of MZF1 which can affect proliferation and invasion, and inhibit apoptosis.[29] As the second m^{6}A demethylase identified, ALKBH5 has been proved to affect biological processes, such as proliferation, invasion, and metastasis.[28,30–32] Interestingly enough, it is found that ALKBH5 plays a carcinogenic or antitumor role in cancers.[32,33] Therefore, the potential mechanism of ALKBH5 in cancer is not only unclear, but also controversial.[34]

The common RNA m^{6}A modification readers include YTH family proteins (YTHDF1–3 and YTHDC1-2), HNRNP proteins (HNRNPA2B1, HNRNPC, and HNRNPG) and IGF2BPs (insulin-like growth factor 2 mRNA-binding proteins).[16,35] These readers can mediate different pathways of function. For instance, YTHDF2/3 has been shown to accelerate mRNA decay,[36,37] while YTHDF1 can promote the translation of methylated RNA.[38] YTHDC1 promotes exon inclusion of mRNA.[21] The RNA-binding proteins HNRNPA2B1, HNRNPC, and HNRNPG mediate alternative splicing effects.[39–42] Moreover, HNRNPA2B1 can recruit DGCR8 to RNA to promote primary microRNA (miRNA) processing.[40]

Role of m^{6}A RNA methylation in lung diseases

Role of m^{6}A RNA methylation in lung diseases related to environmental poisons

Researchers have conducted in-depth study on the molecular mechanism of lung diseases caused by smoking, whereas the regulatory effect of m^{6}A after exposure to environmental pollutants is unclear. One study showed that the global m^{6}A RNA methylation level was decreased in A549 cells after treating with particulate matter and sodium arsenite.[43] After the analysis of the population study, it was observed that the expression of several m^{6}A regulators in the high $PM_{2.5}$ exposure group was significantly higher than that in the low exposure control group.[43] Cheng's group[44] found that the exposure of human bronchial epithelial (HBE) cells to cigarette smoke extract elevated METTL3 expression, which increased the m^{6}A modification of the transcriptional repressor ZBTB4. The downregulation of ZBTB4 was associated with elevated EZH2, which can reduce the level of E-cadherin and induce epithelial–mesenchymal transition (EMT). HBE cells chronically treated with arsenite sodium were identified to have a malignant phenotype and increased levels of cellular proliferation.[45] Further analysis showed that after treating cells with arsenite, the RNA m^{6}A modification which synergistically regulated by METTL3, METTL14, WTAP, and FTO was significantly increased.[45] These studies suggest that m^{6}A modification affects the malignant proliferation and EMT process of HBE cells exposure to environmental toxicants, such as arsenite or particulate matter.

Role of m^{6}A RNA methylation in asthma

Asthma is an immune-related disease. Its airway hyperresponsiveness induces bronchoconstriction and chronic inflammation.[2] In one study, researchers identified 11 significant m^{6}A regulators associated with asthma from the GSE40888 dataset.[46] Subsequently, five candidate m^{6}A regulators were screened according to the nomogram model to predict the risk of childhood asthma. Therefore, we believe that the m^{6}A regulators have assignable influence upon the occurrence and development of childhood asthma. In primary human airway epithelium, the loss of FTO led to destabilization of the mRNA of the master ciliary transcription factor FOXJ1 and a severe loss of ciliated cells.[47] After Fto gene knockout, mice showed a strong asthma like phenotype after allergen stimulation due to the loss of ciliated cells. Therefore, FTO demethylation can stabilize FOXJ1 mRNA which facilitate the formation of motor cilia, and then inhibit the occurrence and development of asthma.

Role of m^{6}A RNA methylation in COPD

COPD is a progressive inflammatory disease of the airways, alveoli, and microvasculature. The key functional characteristic of COPD is the irreversible limitation of airflow.[48] Previous reports showed that METTL3, FTO, IGF2BP3, and YTHDC2 genes were significantly elevated in COPD tissues at mRNA level compared with control samples.[49] According to the analysis of signaling pathways and biological processes, these genes are closely related to promote the development of COPD. This reveals that these m^{6}A regulators may play roles in COPD occurrence. Emphysema refers to parenchymal destruction that causes loss of alveolar units and the characteristic gas trapping and hyperinflation that is found in patients with COPD.[50] Cigarette smoke induced METTL3 overexpression and produced excessive mature miR-93 in bronchial epithelial cells. The miR-93 activated the c-Jun N-terminal kinase (JNK) pathway and elevated the level of matrix metalloproteinases (MMP9 and MMP12) that induced emphysema.[51] Based on the above literatures, METTL3 plays a vital role in pulmonary inflammation.

Role of m^{6}A RNA methylation in IPF

IPF is a kind of progressive fibrosis interstitial pneumonia. It is the end-stage of diffuse pulmonary parenchymal disease and a risk factor for lung cancer.[52,53] Han's group found that both m^{6}A modifications of pri-miR-126 and its binding with DGCR8 were decreased after carbon black treatment, which resulted in the reduction of mature miR-126 and the activation of PI3K/AKT/mTOR pathway, finally driving fibrogenesis in the lungs.[54] A new study showed that the expression of ALKBH5 was both increased in lung tissue of mice inhaled with silica and fibroblasts stimulated by transforming growth factor β1.[55] Their study of the molecular mechanism of these phenomena showed that after demethylation by ALKBH5, the maturation process of pri-miR-320a-3p was blocked and miR-320a-3p decreased. More in-depth study demonstrated that miR-320a-3p regulates fibrosis through binding to the 3'-UTR of FOXM1 mRNA.[55] Therefore, we believe that m^{6}A regulatory protein can promote pulmonary fibrosis by reducing the formation of mature miRNA.

Table 1. Multiple functions exerted by m^{6}A RNA methylation in lung cancer.

	Protein	Role in cancer	Biological function	Mechanism	Refs
1	METTL3	Oncogene	Promote growth, survival, and invasion	Promote translation of EGFR and TAZ	Lin *et al*.[61]
			Promote drug resistance and metastasis	Promote translation of YAP by regulating the lncRNA MALAT1-miR-1914-3p-YAP axis	Jin *et al*.[62]
			Promote the proliferation	Strengthen the stability of the lncRNA ABHD11-AS1	Song *et al*.[63]
			Promote cell migration	Increase mRNA stability of JUNB	Wanna-Udom *et al*.[64]
			Promote invasion and angiogenesis	Increase the splicing of precursor miR-143-3p and promote miR-143-3p processing	Wang *et al*.[65]
2	WTAP	Oncogene	Promote tumorigenesis	lncRNA PCGEM1 sponge miR-433-3p	Weng *et al*.[80]
3	HAKAI	Oncogene	Promote cell proliferation and invasion	Promote G1/S cell cycle transition, decrease E-cadherin expression and increase expression of MMP2 and MMP9	Hui *et al*.[67]
		Oncogene	Promote cell migration and invasion/ Cisplatin resistance	Decrease E-cadherin and increase N-cadherin/ increase AKT activity	Liu *et al*.[68]
		Oncogene	Promote cell migration/ gefitinib resistance	Decrease E-cadherin	Weng *et al*.[69]
4	KIAA1429	Oncogene	Promote tumor growth and metastasis	Promote MUC3A expression	Zhao and Xie[70]
5	FTO	Oncogene	Promote tumor growth	Increase mRNA stabilize of USP7	Li *et al*.[71]
		Oncogene	Promote cell proliferation, migration, and invasion	R96Q (an FTO missense mutant) inhibit proliferation and invasion	Ding *et al*.[72]
6	ALKBH5	Oncogene	Promote proliferation and reduce apoptosis	Decrease mRNA stability of TIMP3	Zhu *et al*.[73]
			Promote proliferation and invasion	Promote translation of FOXM1	Chao *et al*.[30]
		Suppressor	inhibit tumor growth and metastasis	Decrease YAP activity by regulating miR-107/ LATS2 axis	Jin *et al*.[31]
7	YTHDF1	Oncogene	Promote tumor growth and metastasis	Promote translation of YAP	Jin *et al*.[31]
			Promote proliferation/ Reduce cisplatin resistance	Promote translation of CDK2, CDK4, and cyclin D1/silent Keap1-Nrf2-AKR1C1	Shi *et al*.[74]
8	YTHDF2	Oncogene	Promote tumor growth and metastasis	Promote YAP mRNA decay via the AGO2 system	Jin *et al*.[31]
		Oncogene	Promote tumor growth	Promote translation of 6PGD	Sheng *et al*.[75]
9	YTHDC2	Suppressor	Decrease tumorigenesis	Decrease mRNA stability of SLC7A11	Ma *et al*.[10]
10	HNRNPA2B1	Oncogene	Promote cell growth	Promote the processing of primary miR-106b-5p by LINC01234	Chen *et al*.[76]
11	IGF2BP1	Oncogene	Promote tumor cell growth and invasion	Enhance SRF-dependent transcriptional activity	Muller *et al*.[77]

There are some similarities between the current global pandemic coronavirus disease and IPF. As reported, genes related to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) are broadly regulated by m^{6}A RNA modification. Li's group researched the networks of m^{6}A-SARS-CoV-2-related genes in bronchoalveolar lavage cells of IPF patients from the GEO database.[56] Through a variety of regression analysis combined with survival information, eight m^{6}A-related-CoV genes were obtained, and a prognostic risk prediction model was established.

Role of m^{6}A RNA methylation in PH

PH frequently complicates the course of treatment for patients with various chronic lung diseases.[57] However, the incidence and development of PH in lung cancer patients are largely neglected.[58] Post-transcriptional modifications are implicated in the vascular remodeling of PH. Hu's group demonstrated elevated m^{6}A levels of total RNA in mouse and rat models of PH as well as in patients with idiopathic pulmonary arterial hypertension.[59] Targeting YTHDF1 via gene silencing in human pulmonary artery smooth muscle cells (HPASMCs) and in a novel YTHDF1 knockout mouse attenuates cell proliferation and vascular remodeling in PH, at least in part, by decreased translation of MAGED1. METTL3 mRNA and protein were upregulated in pulmonary artery smooth muscle cells (PASMCs) and hypoxic rat models with PH.[60] The downregulation of METTL3 was accompanied by the weakening of PASMC proliferation and migration. YTHDF2, which can recognize m^{6}A-modified PTEN and promote the degradation, was significantly increased in PASMCs under hypoxia. The decreased expression of PTEN can activate the PI3K/Akt signaling pathway and contribute to the overproliferation of PASMCs. These studies show that METTL3, YTHDF1, and YTHDF2 play significant regulatory roles in the proliferation, migration and vascular remodeling of PASMCs.

Multiple functions of m^{6}A RNA methylation in lung cancer

At present, the role and mechanism of m^{6}A modification in tumorigenesis and development are hot topics in tumor biology. In the development of lung cancer, the abnormal changes of m^{6}A gene level are closely related to proliferation, migration, invasion, metastasis, and drug resistance. Therefore, the study of biological function of m^{6}A modification and identify the key factors to clarify the pathogenesis of lung cancer can provide the basis for the diagnosis and treatment of lung cancer. More and more evidence shows that m^{6}A plays a dual role in cancers. It affects the occurrence and development of cancer by enhancing or inhibiting the expression of oncogenes and tumor suppressor genes. We summarize the potential mechanisms of m^{6}A regulators in lung cancer in Table 1. Furthermore, the Figure 2 presents the functions and targets of m^{6}A writers, erasers, and readers in lung diseases.

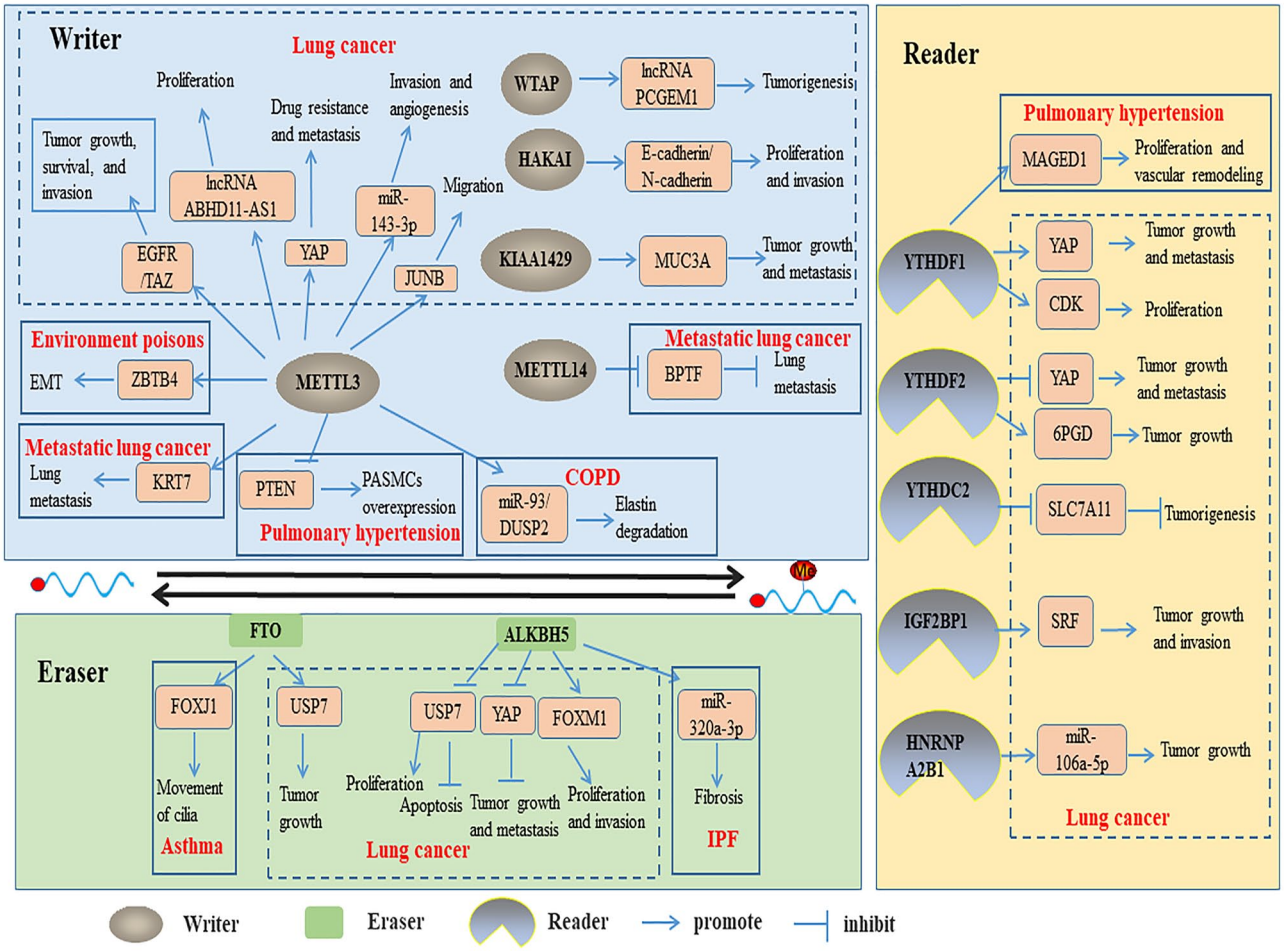

Figure 2. Multiple functions of m⁶A RNA methylation in lung diseases. (A color version of this figure is available in the online journal.)

Role of m⁶A RNA methylation in lung cancer cell proliferation

Lung cancer is formed by the malignant proliferation of lung epithelial cells, which is related to excessive cell division, a disordered cell cycle and abnormal apoptosis regulation. Existing evidence suggests that m⁶A RNA methylation is associated with cancer cell proliferation in a variety of cancers. METTL3 expression was increased in both lung adenocarcinoma patients from TCGA (The Cancer Genome Atlas) cohort and lung adenocarcinoma cell lines, and correlated with tumor stage.[23,61] METTL3 interacts with translation initiation factors and specifically promotes the translation of initiation factor-dependent reporter mRNAs.[14,61,62] As a result, METTL3 increased the expression of epidermal growth factor receptor (EGFR), transcriptional coactivator with PDZ-binding motif (TAZ), and Yes-associated protein (YAP) and promoted cell growth, survival and invasion.[61,62] SUMOylation of METTL3 repressed its methyltransferase activity and promoted tumor growth in non–small cell lung cancer (NSCLC).[66] Meanwhile, miRNA-related studies also revealed that miR-33a and miR-600 can inhibit the expression of METTL3 by targeting the mRNA, thereby inhibiting the proliferation NSCLC.[78,79] The overexpression of METTL3 enhanced the stability of lncRNA ABHD11-AS1 transcripts and promoted the proliferation of NSCLC.[63] These studies pointed out that METTL3 plays a cancer promoting role in lung cancer. Besides, highly expressed lncRNA PCGEM1 upregulated WTAP and accelerated the progression of NSCLC by sponging miR-433-3p.[80] On the contrary, the inhibition of HAKAI can promote the G1/S cell cycle transition thereby promoting cell proliferation in NSCLC.[67]

Some studies indicated that the mRNA and protein levels of FTO were excessive in human NSCLC cell lines and tissues.[71] FTO knockdown with short hairpin RNAs (shRNAs) inhibited the proliferation rate and colony formation ability of lung cancer cells *in vitro* and *in vivo*.[71] Mechanistically, the decrease of m⁶A level caused by FTO contributes to the increase of ubiquitin-specific protease 7 (USP7) mRNA stability, and further promotes the growth of NSCLC cells.[71] In the mice model of intermittent hypoxia, ALKBH5 was increased in lung adenocarcinoma cells, tissues and subcutaneous tumors.[30,73] Functionally, ALKBH5 enhanced the proliferation of NSCLC cells and reduced the apoptosis by repressing the stability of TIMP3 mRNA.[73] In lung adenocarcinoma cells, the level of m⁶A in FOXM1 mRNA was increased with knockdown of ALKBH5, which decreased the translation efficiency and led to the downregulation of FOXM1 protein.[30] These changes significantly inhibited the growth and invasion of lung adenocarcinoma cells. However, a recent study showed that ALKBH5 was significantly underexpressed in NSCLC. ALKBH5 decreased the activity

of YAP by regulating the miR-107/LATS2 axis and inhibited proliferation and metastasis,[31] which was not consistent with other studies. Similarly, the m^{6}A methylation modification was removed by FTO and ALKBH5, which increased the stability of USP7 mRNA but decreased the stability of TIMP3 mRNA. Moreover, the results obtained by removing the m^{6}A modification of different genes by ALKBH5 were completely different. We speculated that the m^{6}A modification of different genes was changed and selectively recognized by different m^{6}A reader proteins, resulting in differences in post-transcriptional regulation. The specific molecular mechanism underlying the selective recognition ability of m^{6}A recognition proteins still needs to be further investigated.

YTHDF family and YAP have complex dual functions. YTHDF2 facilitated YAP mRNA decay via the AGO2 system, whereas YTHDF1/3 promoted YAP mRNA translation by interacting with eIF3a/eIF3b.[31,62] In addition, one group revealed that YTHDF1 deficiency can affect the translation efficiency of cyclins, thereby inhibiting proliferation, colony formation and xenograft tumor formation.[74] YTHDF2 promoted 6-phosphogluconate dehydrogenase (6PGD) mRNA translation in lung cancer cells by binding to the m^{6}A modification site of 6PGD,[75] to accelerate glucose metabolism through the pentose phosphate pathway and provide raw materials for the malignant proliferation of NSCLC. The increased LINC01234 which mediated by c-Myc interacted with HNRNPA2B1 and promoted the processing of primary miR-106b-5p. While the inhibition of cryptochrome 2 (CRY2) and upregulation of c-Myc caused by miR-106b-5p can enhance NSCLC cell growth. This formed a positive-feedback loop involving the m^{6}A reader, c-Myc, and miR-106b-5p, which can promote the growth of NSCLC cell.[76]

Role of m^{6}A RNA methylation in lung cancer cell migration and metastasis

Researches on the effect of m^{6}A RNA methylation on cancer cell migration and metastasis has become increasingly mature. The expression of METTL3 was excessive in EMT which induced by transforming growth factor beta (TGF-β) in lung cancer cells.[64] The knockdown of METTL3 reduced the mRNA stability of JUNB, thereby antagonizing EMT phenotype. Rescue experiments confirmed that JUNB overexpression partially restored EMT inhibited by METTL3 knockdown.[64] METTL3 was down regulated in lung cancer with Simvastatin treatment, which changed the m^{6}A modification of EZH2 mRNA and inhibited the EMT progression.[81] MiR-143-3p is known to enhance the invasiveness and angiogenesis of lung cancer by targeting angiostatin-1. Wang et al. found that METTL3 can promote the expression of miR-143-3p by increasing the splicing of miRNA precursor, and finally promoting the metastasis of lung cancer cells.[65] METTL3 can also enhance metastasis and drug resistance of NSCLC by increasing the stability of YAP mRNA which regulated with lncRNA MALAT1 and miR-1914-3p.[62] In addition to METTL3, KIAA1429 was significantly overexpressed in lung adenocarcinoma (LUAD) with larger tumor diameter, more prone to metastasis, and lower overall survival. KIAA1429 downregulation significantly inhibited the migration, proliferation, and invasion of LUAD cells by inhibiting MUC3A expression and arresting cell cycle in the G1 phase.[70] Moreover, HAKAI regulates the migration and invasion of NSCLC cells by changing the EMT markers E-cadherin and N-cadherin.[67,68]

Overexpression of FTO can enhance the proliferation, migration, and invasion of lung cancer cells. Nevertheless, the FTO missense mutant R96Q, lacking demethylase activity, blunted the effects. Interestingly, RNA sequencing of FTO or R96Q overexpressed cells indicated that most genes regulated by m^{6}A mRNA demethylation were associated with lung cancer.[72]

Role of m^{6}A RNA methylation in drug resistance

Drug resistance refers to the resistance of cancer cells to chemotherapy, radiotherapy, and targeted drugs, which is one of the major causes for cancer recurrence.[82] Recent studies indicated that m^{6}A regulators are dysregulated in a variety of cancers and play not to be neglected roles in drug resistance and cancer recurrence. m^{6}A RNA modification can be considered as a feasible therapeutic target for overcoming drug resistance.

The elevated expression of YAP1 caused by METTL3 enhancing the stability of YAP1 m^{6}A methylation mediated cisplatin resistance in NSCLC.[62] One study suggested that both cancer specimens with acquired gefitinib resistance and gefitinib-resistant NSCLC cells showed increased expression of HAKAI, accompanied by decreased expression of E-cadherin.[69] At the same time, the suppression of HAKAI significantly enhanced chemosensitivity to cisplatin.[68] Notably, the depletion of YTHDF1 renders cisplatin resistant in cancerous cells through the KEAP1/NRF2/AKR1C1 axis. Meanwhile, the high expression of YTHDF1 in patients with NSCLC is often accompanied by better clinical results.[74]

In a study of the relationship between m^{6}A modifications and afatinib resistance in NSCLC cells, researchers found that the scores of m^{6}A enrichment in afatinib-resistant lines (H1299) was higher than in afatinib-sensitive lines (A549).[83] Further gene function enrichment analysis revealed that the differentially expressed genes in the two groups were related to the cell cycle. Researchers speculated that m^{6}A-modified genes may cause afatinib resistance in NSCLC by interfering with normal cell cycle.

Role of m^{6}A RNA methylation in lung cancer diagnosis, treatment and prognosis

The pivotal role of m^{6}A RNA methylation in the occurrence and development of cancer provides more opportunities for early diagnosis and treatment. Huang et al. found that m^{6}A modification was upregulated in circulating tumor cells (CTCs) of lung cancer patients, and further analyzed the m^{6}A levels of RNA in individual cells for validation.[84] The results and methods provided a potential basis for evaluating whether lung cancer patients have metastasis and good prognosis. A systematic analysis of m^{6}A regulatory factors in LUAD and normal samples revealed that 12 m^{6}A methylation-related genes displayed aberrant expression.[85] Furthermore, the team finally established a diagnostic score model with 11 genes and a risk score model with 10 genes. It is worth exciting that the diagnostic score model discriminated each stage of lung adenocarcinoma in the training and validation cohorts, even stage IA.

As an important oncogene of many tumors, FTO is considered to be a promising therapeutic target. However, the identified FTO inhibitors have low sensitivity and specificity, and their clinical effects are limited. FTO inhibitor R-2HG enhanced the sensitivity of acute myeloid leukemia (AML) cells to therapeutic agents, while another inhibitor meclofenamic acid inhibited the growth and survival of glioblastoma (GBM) cells.[86–88] According to the characteristics of genetic variation, Li's group found that mutations in FTO and YTHDF3 were related to poor overall survival.[89] The m6Sig scoring tool they set up had a positive correlation with PD-L1 expression, which could reflect the tumor microenvironment and prognosis of LUAD patients. The therapeutic advantage of the high m6Sig group was also confirmed in the anti PD-L1 immunotherapy cohort.[89] Clinically, most cases of acinar LUAD subtype exhibited simultaneous downregulation of YTHDC2 and elevation of SLC7A11, accompanied by adverse clinical results.[10] The METTL3-eIF3h interaction enhances translation and carcinogenicity. Some studies have confirmed that the depletion of METTL3 can inhibit tumorigenicity and improve the sensitivity of lung cancer cells to BRD4 inhibition.[23] Thus, METTL3-eIF3h can be considered as a possible therapeutic target for lung cancer. The classification of chemotherapy benefit prediction based on m^{6}A regulator constructed by least absolute shrinkage and selection operator (LASSO) Cox model has been proved to be more powerful than other parameters.[90] Further cytological experiments revealed that three m^{6}A regulators-ZCCHC4, G3BP1, and RBMX from the model were considered to be novel targets to overcome chemotherapy resistance of small cell lung cancers (SCLCs).

The information obtained from TCGA databases showed that compared with normal patients, some m^{6}A-related genes were upregulated in LUAD and differentially expressed in different races, ages, and tumor, node, metastasis (TNM) stages.[91] Patients with high expression of METTL3, YTHDF1, and YTHDF2 had better survival rate and were promising biomarkers for the prognosis of LUAD.[91] Wu's group developed a prognostic model of m^{6}A regulatory gene composed by IGF2BP1, IGF2BP2, HNRNPA2B1, METTL3, and HNRNPC, which can accurately assess the risk level of LUAD patients.[92] IGF2BP1 promoted growth and invasion of lung cancer cells by promoting serum response factor (SRF)-dependent transcriptional activity. Meanwhile, SRF/IGF2BP1-dependent genes indicated a poor overall survival probability in lung cancer.[77] Importantly, nomogram based on prognostic characteristics can accurately predict the survival probability of LUAD patients and provide beneficial guidance for clinical treatment.

Role of m^{6}A RNA methylation in metastatic lung cancer

Metastatic growth is the last but most fatal stage in the malignant progression of tumors.[93] Metastatic lung cancer refers to any part of the malignant tumor that has spread through a variety of ways of metastasis to form a lung tumor, which can be caused by hematogeneous dissemination, lymphatic metastasis, or direct invasion of adjacent organs.[94] The incidence of lung metastasis is different in different parts of the tumor, among which thyroid cancer, breast cancer, renal cancer, choriocarcinoma, and osteosarcoma have the highest incidence. Researchers found that METTL3 mRNA was increased, while FTO was decreased in established breast cancer lung metastasis cells.[95] Specifically, increased METTL3 methylated KRT7-AS to increase the stability of a KRT7-AS/KRT7 mRNA duplex. Furthermore, YTHDF1/eEF-1 was involved in translational elongation of FTO regulated KRT7 mRNA. In general, m^{6}A methylation of KRT7 mRNA promoted breast cancer lung metastasis. Utilizing TCGA and researchers' own datasets, they found that METTL14 was low expressed in metastatic renal cell carcinoma samples with poor prognosis.[96] From the mechanism analysis, m^{6}A modification mediated by METTL14 negatively regulated the mRNA stability of BPTF and drove lung metastasis.

Discussion

M6A modification is the most common post-transcriptional modification in mRNA. Increasing evidence shows that m^{6}A plays an assignable role in tumorigenesis, metastasis, and angiogenesis. Within recent years, extensive experimental data have shown that global m^{6}A levels and regulators are abnormally expressed in multiple lung diseases. They affect the occurrence and development of asthma, COPD, IPF, and lung cancer through inflammation and immune function. Meanwhile, their dysregulation will also affect the drug resistance and poor prognosis of cancer patients. Studies in human lung cancer cell lines have indicated that m^{6}A regulators, such as METTL3, YTHDF1, and HAKAI can be viable therapeutic targets for drug resistance.[62,68,69,74] The increased level of m^{6}A modification in CTCs was indicated to be a non-invasive diagnostic method for lung cancer.[84] Models composed of multiple m^{6}A regulators have also been used to evaluate the diagnosis, risk or prognosis of lung diseases, such as asthma, SARS-CoV-2, and lung cancer.[46,56,85,91] In recent years, targeted therapy focused on m^{6}A modification has become a research hotspot for new drug targets, and it mainly includes FTO inhibitors, METTL3-14/WTAP activators, and combination therapy.[86,97,98]

With the discovery of m^{6}A-related proteins and the study of potential mechanisms, unknown problems have been gradually exposed. First, many m^{6}A methyltransferases have been identified; however, research on lung diseases focuses on METTL3, with few studies focused on other methyltransferases. Thus, whether other methyltransferases are involved in the occurrence and development of lung diseases has not been clarified. Second, the affect of m^{6}A modification in lung cancer is also controversial. Some m^{6}A-related proteins have dual effects and simultaneously promote and inhibit cancer. For example, ALKBH5 can promote the proliferation and invasion of NSCLC and reduce apoptosis.[30,73] whereas another study indicated that it can inhibit the growth and metastasis of NSCLC.[31] YTHDF1 deficiency can inhibit the proliferation of NSCLC, although its high expression is associated with good prognosis.[74] How these m^{6}A-modified proteins play different roles and how different m^{6}A recognition proteins selectively recognize and bind to target RNA will be the focus of future research. Third, the development of inhibitors or activators of m^{6}A-related proteins is still in its infancy and is mainly used in preclinical experimental

research; thus, its therapeutic effect on cancer patients has not been reported.

With the in-depth research of m^{6}A-modified protein, the detection methods have also been developed. Traditional methods for the analysis of m^{6}A, such as MeRIP-seq and miCLIP-seq, cannot accurately locate and quantify m^{6}A, and these methods rely on the specific antibody of m^{6}A. The specificity and sensitivity of antibodies are limited due to changes in affinity and batch effects as well as the cross reaction between the antibody and other similar modifications (such as m^{6}A$_m$). Therefore, it has become the most popular research hotspot in the field to detect the level of RNA modification with new technology. Based on the sensitivity of the newly discovered RNA endonuclease MazF to m^{6}A, a new high-throughput m^{6}A identification method can quantify the methylation level with single base resolution, accurately detect the whole transcriptome range of m^{6}A, and does not rely on antibodies, which overcomes the limitations of traditional m^{6}A identification methods. Nanopore sequencing calculates the modifications carried by nucleotides by detecting the electrical signals of nucleotides passing through the nanopore. Although these two high-throughput sequencing technologies have good application prospects, but the current calculation methods and technologies still have high false-positive rates, which need to be further improved. However, the realization of these technologies will be the key to clarifying the dynamic regulatory mechanism and molecular function of m^{6}A modification.

In addition to m^{6}A, new chemical modifications of RNA, such as N1 methyladenosine modification (m^{1}A), methylcytosine modification (m^{5}C) and m^{6}A$_m$, have also been hot spots in recent research. At present, research on m^{5}C and m^{1}A is still in its infancy. Whether they play a regulatory role in multiple biological processes, like as m^{6}A modification, needs to be further studied in the future. The development of more accurate detection methods, such as nanopore sequencing and single base resolution technology, is very important for the establishment of high reliability RNA modification profiles. The function of m^{6}A$_m$ is still controversial. First, m^{6}A$_m$ was identified to be related to RNA stability. Later studies found that m^{6}A$_m$ only affected a small part of the half-life of mRNA, and m^{6}A$_m$ could also affect the translation efficiency of mRNA. Therefore, further studies are needed on the role of m^{6}Am in mRNA fate determination.

Overall, the study of m^{6}A modification in disease is a new frontier. It not only provides new insights into the molecular mechanisms of disease occurrence, immune response, and drug resistance, but also contributes to the development of new therapies. Targeting dysregulated m^{6}A regulators by inhibitors alone or combined with other therapeutic drugs may have potential efficacy.

AUTHORS' CONTRIBUTIONS

XLM had the idea for the article, reviewed the full text articles, and wrote the initial draft of the manuscript. ZLY conducted the literature search, reviewed full text articles, and contributed to the extraction of informations. YCX extracted information from articles and plotted the figure. XLM wrote the final version in collaboration with LLQ. All authors read and approved the final manuscript.

DECLARATION OF CONFLICTING INTERESTS

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