Research Progress on N6-Methyladenosine Editing in Cancer Therapy

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Abstract: One of the most prevalent internal alterations on mRNAs in eukaryotes is N6-methyladenosine (m6A). By altering several facets of RNA metabolism, such as translation, splicing, nuclear export, stability, degradation, and microRNA processing, m6A alteration governs and modulates gene expression. Several proteins known as "writers," "erasers," and "readers" control m6A alterations. METTL3, METTL14, WTAP, RBM15/15B, VIRMA, and ZC3H13 are among the writers; FTO, ALKBH5, and ALKBH3 are among the erasers; and YTHDF1/2/3, YTHDC1/2, IGF2BP1/2/3, HNRNP, and eIF3 are among the readers. Cancer is a significant socioeconomic and public health concern of the twenty-first century, accounting for around 22.8% of non-communicable disease-related deaths worldwide. Nonetheless, the studies demonstrate that new cancer treatments may be developed by adjusting the amounts of m6A alterations in particular genes. In order to help researchers better comprehend the development, this review aims to provide an overview of the research on m6A editing in cancer therapy.

1 INTRODUCTION

Over the past century, the severity of non-communicable diseases (NCDs) has gradually increased due to population growth and aging. Among these diseases, cancer is responsible for nearly 22.8% of global deaths from non-communicable diseases, making it a major social and public health issue of the twenty-first century (Li et al. 2024). Recent findings, however, suggest that altering the amounts of N6-methyladenosine (m6A) alterations in particular genes may result in the creation of novel cancer treatments in therapeutic trials aimed at illnesses like cancer. Translation regulation, mRNA degradation, nuclear export, pre-mRNA splicing, 3' end processing, and non-coding RNA (ncRNA) processing are all facets of eukaryotic RNA metabolism that m6A is involved in (Wang et al. 2017, Li et al. 2024). For example, m6A modifications targeting tumor-related genes have the ability to stop tumor cells from replicating and spreading.

m6A regulates key features of cancer cells, and its reversible RNA methylation affects transcription, splicing, mRNA stability, and translation rates (Wang et al. 2017). As a result, both transcriptional and post-transcriptional regulatory mechanisms related to m6A coexist across a wide range of malignancies, impacting key genes. The majority of species, including bacteria and humans, exhibit the evolutionarily conserved RNA alteration known as methylation of RNA m6A, which is the addition of a methyl group to the N6 position of adenosine. The post-transcriptional modification process known as m6A RNA modification is dynamic and reversible, and this property makes it a key role in rapid cell communication. Selective polyadenylation, translation efficiency, nuclear output, mRNA stability and splicing, and RNA metabolism are all significantly influenced by it. Abnormal m6A modification can lead to cell death and uncontrolled cell proliferation, leading to the occurrence and development of tumors (Wang et al. 2017, Li et al. 2024). These discoveries have not only made it possible to utilize m6A-targeted therapies but have also led to a significant increase in the success rate of m6A-targeted therapies for human cancers. Given the critical role of m6A in cancer, summarizing and presenting the latest research findings on its role in cancer is essential. This can provide researchers with valuable insights into the

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latest progress in m6A-related cancer research (Pu et al. 2023).

2 CRISPR/CAS9 GENE-EDITING TECHNOLOGY

The gene-editing technique CRISPR/Cas9 is an effective method for locating genes that are linked to chemoresistance, invasion, migration, and cell proliferation (Yang et al. 2019, Cai et al. 2020, Kang et al. 2021). The Mature CRISPR RNA (crRNA), as well as complementary trans-activating crRNA (tracrRNA), which are both capable of forming stable double-RNA complex structures, are components of the CRISPR/Cas9 system. These structures guide the CRISPR-associated protein Cas9 to precisely target and split the particular DNA sequences (Cong et al. 2013). When single-guide RNA (sgRNA) is complementary to the particular DNA and contains PAM, which is called appropriate protospacer adjacent motif, the Cas9 protein cleaves the double-stranded DNA at that location (Potts et al. 2020). Cells have two different ways to fix these breaks: homologous recombination (HR), a more accurate process that uses a homologous template for error-free repair, and non-homologous end joining (NHEJ), which quickly ligates the broken DNA ends. By leveraging these mechanisms, CRISPR/Cas9 technology can be used to delete, replace, or insert specific gene sequences (Chiou et al. 2015). CRISPR/Cas9 is not limited to modifying one genomic site, and a group of sgRNAs to simultaneously will also be employed for modifying several different genomic sites, a process known as multiplex editing (Gaj et al. 2013, Cai et al. 2020, Potts et al. 2020, Kang et al. 2021). Initially, CRISPR/Cas9 technology relied on the combination of Cas9 ribonuclease and sgRNAs to precisely eliminate specific genes. In 2013, researchers successfully developed a Cas9 mutant, dCas9, which lacks nuclease activity but retains endonuclease activity to perform its function (Qi et al. 2021).

3 M6A MODIFICATION

One of the most prevalent internal alterations on mRNAs in eukaryotes is m6A (Wang et al. 2020). It regulates mRNA transcription, translation, splicing, and degradation processes, as well as the overall mRNA lifecycle. Additionally, m6A modulates RNA stability and participates in various physiological and pathophysiological processes (Pu et al. 2023, Jonas & Frank 2024). The majority of m6A alterations take place in RRACH sequences, where H stands for adenine (A), cytosine (C), or uracil (U), and R for adenine (A) or guanine (G). These alterations are mostly seen in intronic regions and close to the 3' untranslated region (UTR) and termination codon of mRNAs (Wang et al. 2017, Wu et al. 2018). The frequency of m6A distribution ranges from 0.15% to 0.6%, with approximately 25% to 60% of transcripts carrying the modification (Pu et al. 2023). The regulation of m6A modifications involves a diverse set of proteins classified into three functional groups including writers, erasers, and readers. The “writers,” responsible for catalyzing m6A methylation, include ZC3H13, METTL3, METTL14, WTAP, VIRMA, and RBM15/15B. The “erasers,” which remove m6A marks, comprise FTO, ALKBH5, and ALKBH3. Meanwhile, the “readers,” which recognize and interpret m6A modifications to mediate downstream effects, consist of YTHDF1/2/3, YTHDC1/2, IGF2BP1/2/3, HNRNP, and eIF3 (Wang et al. 2020).

The m6A mutation modifies several facets of RNA metabolism, such as translation, alternative splicing, nuclear export, stability, degradation, and the processing of microRNA in order to govern and control gene expression. These actions influence cellular functions and physiological processes (Wu et al. 2018, Jonas & Frank 2024). The modification of m6A is dynamic and reversible. Specifically, 'writers' add m6A modifications, 'erasers' remove them, and 'readers' recognize and act on the modified RNA (Pu et al. 2023). m6A functions are typically mediated by m6A-recognizing proteins, such as those in the YTH family. For example, YTHDC1 is primarily involved in pre-mRNA processing, while YTHDF1-3 are mainly associated with mRNA degradation (Jonas & Frank 2024). The researchers studied m6A regulation, showing that m6A regulation has a tight relationship to type 2 diabetes and cancer. Recent research has also found that m6A modifications can influence tumor progression, participate in tumor metabolism, regulate ferroptosis (tumor cell iron death), and alter the tumor immune microenvironment, thereby affecting tumor immunotherapy (Pu et al. 2023). Due to its dynamic regulatory function, m6A also plays a key role in various disease processes, including neurodegenerative diseases, cardiovascular diseases, and metabolic diseases (Pu et al. 2023). Traditional m6A detection methods rely on antibodies, which can suffer from cross-

reactivity issues. In comparison, LC-MS/MS technology offers a more accurate method for m6A quantification, allowing the detection of even extremely subtle changes in m6A levels (Jonas & Frank 2024). Single-base resolution detection of m6A is an emerging, antibody-independent technology that can directly detect m6A modifications in RNA molecules, enabling single-base resolution detection of m6A (Jonas & Frank 2024).

4 THE MECHANISMS OF M6A ACTION

The m6A methyltransferase complex's essential elements, METTL3 and METTL14, combine to create a stable heterodimer. METTL3 functions as the catalytic subunit, and at the same time, METTL14 primarily is responsible for being an RNA-binding structure. METTL14 as well as METTL3 will react with WTAP to regulate the m6A RNA methylation process (Wang et al. 2017). The m6A 'reader' family includes YTHDF and hnRNP proteins. Among these, YTHDF2 was the first identified m6A-binding protein that mediates m6A-dependent RNA degradation by targeting RNA to P-bodies (Wang et al. 2017). YTHDF and hnRNP proteins are members of the m6A'reader' family. Of them, YTHDF2 was the first m6A-binding protein to be discovered. It marks P-bodies for RNA and facilitates m6A-dependent RNA degradation (Wang et al. 2017). m6A modifications regulate gene expression through multiple processes, including mRNA splicing, transport, stability, and translation. YTH family proteins function as key 'readers,' with YTHDC1 primarily involved in pre-mRNA processing and YTHDF1-3 mediating mRNA degradation. Additionally, IGF2BPs can bind to m6A-modified mRNAs, enhancing their stability and translation efficiency (Jonas & Frank 2024). m6A-modified mRNAs can form condensates through phase separation, which can further differentiate to rather stress granules or P-bodies. This process introduces a new dimension of m6A-dependent post-transcriptional regulation (Jonas & Frank 2024).

5 THE ASSOCIATION BETWEEN M6A AND CANCER

(1) The relationship between m6A modification and cancer: Dysregulation of m6A modification has a significant relationship with the procedure of tumorigenesis, the development of tumor, its metastasis, as well as prognosis in a myriad of cancer symptoms. The breast cancer, where increased expression of METTL3 has a positive relationship with PIN1 expression, can be served as an example. PIN1 stabilizes METTL3 by preventing its ubiquitination and degradation, thereby promoting tumorigenesis. **(2) m6A modification and tumor metabolism:** m6A modification influences tumor cell energy metabolism by regulating processes such as glycolysis, fatty acid metabolism, and glutamine metabolism. For example, METTL3 enhances glycolysis in tumor cells by promoting the expression of PDK4 and stabilizing GLUT1. **(3) m6A modification and tumor immunotherapy:** m6A modification affects the tumor immune microenvironment by regulating the expression of immune checkpoint proteins, such as PD-1/PD-L1. For example, METTL3 inhibits anti-tumor T-cell activation by enhancing PD-L1 mRNA stability and expression in an IGF2BP3-dependent manner (Pu et al. 2023).

6 THE ROLE OF M6A IN HUMAN CANCER

(1) Glioblastoma: Tumorigenesis and cell proliferation in glioblastoma are linked to the presence of m6A in RNA. METTL3 or METTL14 knockdown increases the expression of certain oncogenes, including EPHA3, ADAM19, and KLF4. Glioblastoma stem cells (GSCs) have high levels of ALKBH5, and GSC growth is inhibited by its silencing. **(2) Acute Myeloid Leukemia (AML):** By controlling the expression of target genes (such as RARA and ASB2), FTO, a m6A demethylase, promotes leukemogenesis as well as leukemia oncogene-mediated cell transformation. ATRA-induced AML cell differentiation is also inhibited by it. **(3) Lung Adenocarcinoma (LUAD):** EGFR and TAZ are two mRNAs that METTL3 stimulates to translate in LUAD. Target mRNA translation is improved by METTL3 through the recruitment of eIF3 to the translation initiation complex. **(4) Breast Cancer (BRC):** Knocking down METTL14 in HCC

can increase tumor metastasis and lower the amount of m6A in RNA. Hypoxia-dependent overexpression of ALKBH5 decreases m6A levels in NANOG mRNA, which in turn increases the stability of NANOG mRNA and the amounts of NANOG protein in breast cancer stem cells (BCSCs).

7 CANCER-SPECIFIC M6A DYNAMICS

(1) Glioblastoma: METTL3 promotes glioma stem cell maintenance and radiation resistance by enhancing SOX2 stability. ALKBH5 promotes glioma stem cell proliferation by demethylating FOXM1 mRNA. **(2) Cancers of the Female Reproductive System:** In endometrial cancer, reduced m6A methylation correlates with METTL14 mutations or decreased METTL3 expression, and AKT pathway activation drives tumorigenesis. In cervical cancer, FTO promotes chemoresistance and radiation resistance by demethylating β-catenin mRNA. **(3) Pancreatic Cancer:** Expression changes in FTO and ALKBH5 correlate with pancreatic cancer invasion and chemotherapy resistance. **(4) Nasopharyngeal Carcinoma:** METTL3 promotes the development of nasopharyngeal carcinoma by inhibiting ZNF750 and FGF14 expression. **(5) Lung Cancer:** Through the enhancement of the MALAT1-miR-1914-3p-YAP axis and the promotion of YAP mRNA translation, METTL3 causes treatment resistance and metastasis in non-small cell lung cancer (NSCLC). **(6) Hepatocellular Carcinoma:** the defective prognosis in hepatocellular carcinoma is related to high levels of METTL3 and YTHDF1 expression. By controlling SOCS2 m6A alteration, METTL14 prevents the advancement of hepatocellular carcinoma. **(7) Colorectal Cancer:** METTL3 stimulates the proliferation of colorectal cancer and its migration via IGF2BP2-dependent mechanism. METTL14 inhibits the growth and migration of colorectal cancer cells by regulating the miR-375/SP1 and miR-375/YAP1 signaling cascade. **(8) Bladder Cancer:** METTL3 stimulates the AFF4/NF-κB/MYC signaling network, which accelerates the development of bladder cancer. **(9) Prostate Cancer:** METTL3 promotes prostate cancer progression by regulating GLI1 expression. **(10) Breast Cancer:** METTL3 promotes breast cancer progression by enhancing m6A modification of BCL-2 mRNA. **(11) Renal Cancer:** Changes in m6A modification levels are significantly correlated with the deterioration of clinical parameters in renal cancer. **(12) Gastric Cancer:** Decreased m6A modification levels activate the WNT/PI3K-AKT signaling pathway, thereby promoting the development of gastric cancer.

8 PROSPECTS FOR CANCER THERAPY TARGETING M6A EDITING

(1) FTO Inhibitors: Rhein and Meclofenamic Acid (MA) are FTO inhibitors that increase m6A levels in mRNAs by pairing with the active site of FTO, thereby inhibiting it from interacting with m6A substrates. **(2) ALKBH5 Inhibitors:** Citrate and IOX3 can inhibit ALKBH5, thereby maintaining the tumorigenicity of glioblastoma stem cells. **(3) SAM-Dependent Methyltransferase Inhibitors:** S-Adenosylhomocysteine (SAH) in this reaction is the competitive inhibitor of SAM-dependent methyltransferases. Additionally, 3-deazoadenosine (DAA), an inhibitor of SAH hydrolase, prevents the incorporation of m6A into mRNA substrates (Wang et al. 2017).

9 THE POTENTIAL OF M6A MODIFICATION AS A CANCER THERAPEUTIC TARGET

(1) FTO Inhibitors: Inhibitors targeting FTO, such as R-2HG, have been developed to inhibit AML cell proliferation and promote apoptosis. **(2) Melanoma and FTO Knockdown:** In melanoma, knockdown of FTO increases m6A modification, decreases the expression of tumor-associated genes, and increases sensitivity to immune checkpoint inhibitors (Wang et al. 2020). **(3) Changes in m6A in Cancer:** According to recent research, m6A quantity is significantly reduced in bladder cancer tissues, possibly due to an imbalance in the composition of the chemical complex of m6A methyltransferase. **(4) METTL3 Therapeutic Target:** METTL3, the main "writer" of m6A, is investigated as a possible drug target in various cancers. In acute myeloid leukemia (AML), STM2457, a small molecule inhibitor of METTL3, which can decrease tumor phenotype of leukemia cells, performs functions to increase the life of mice. Similar researches show that METTL3 inhibitors have therapeutic influences on a variety of

solid tumors. **(5) Dual Roles of METTL3:** In many tumors, METTL3 has a variety of functions, functioning as a tumor suppressor to stop tumor growth and as an oncogene to encourage tumor development. **(6) m6A-Dependent Condensates as Therapeutic Targets:** Targeting m6A-dependent pathophysiological condensates may provide a more tumor-selective therapeutic strategy. For example, inhibiting the interaction of m6A-reading proteins with modified targets through small molecules or peptides may prevent the formation of oncogenic condensates or promote their dissociation. **(7) Potential of METTL3 Inhibition in Cancer Immunotherapy:** Recent studies have shown that METTL3 inhibitors can enhance anti-tumor immune responses. For example, STM3006 inhibitors increase the anti-tumor immunity through removing m6A modification levels, inducing double-stranded RNA (dsRNA) formation, and activating intracellular interferon responses (Jonas & Frank 2024).

10 CONCLUSION

In this review, as a new way of therapy, the editing m6A shows its multiple functions. m6A modifications regulate gene expression through multiple processes, including mRNA splicing, transport, stability, and translation. In the meantime, CRISPR gene editing technology is a powerful tool. For identifying genes associated with cell proliferation, migration, invasion, and chemoresistance. In 2013, the researchers successfully developed a Cas9 mutant, dCas9, which lacks nuclease activity but retains endonuclease activity to perform its function. This will greatly promote the subsequent application of gene editing in various aspects, especially in gene editing m6A, which will greatly improve its efficiency and accuracy. m6A modification influences tumor cell energy metabolism by regulating processes such as glycolysis, fatty acid metabolism, and glutamine metabolism. m6A modification affects the microenvironment of tumor immune by controlling the gene of immune checkpoint proteins expressions. According to current research, m6A is served as a factor which is responsible for a number of different cancers. Although it is not clear whether gene editing m6A is more efficient enough to treat tumors, according to the current development, gene editing m6A as a potential regulatory mechanism will play a significant role in the future.

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