The Potential of N6-Methyladenine-Targeted Editing Tools in Cancer Treatment

Xinfei Wu
Nanning No.36 Middle school, Nanning, China

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Abstract: With the continuous development of bioengineering technology, the study of m6A has become one of the core fields of epitranscription, but the development of m6A editing tools is still the focus of today's research. Increased methylation levels of m6A are highly susceptible to the development of cancer or tumors. Therefore, this review aims to discuss the development of m6A-targeting tools in cancer therapy, and summarize the role of m6A in the delivery system, gene editing modification in oncolytic viruses, and the markers of m6A in breast and lung cancer, as well as its targeted editing tools. Based on the development of targeted tools and the analysis of the defects of m6A in cancer to the prospect of the future, the new direction and vitality brought by m6A research in cancer treatment are illustrated.

1 INTRODUCTION

RNA methylation modification is an important example in epigenetics and an important link in the regulation of gene expression, the most common and abundant mRNA modification is N6-methyladenine (m6A). m6A plays a key role in stability, translation, splicing, transport and localization (Lo et al. 2022). The regulation of RNA metabolism is used to regulate the modification of m6A. Three proteins are writing proteins, read proteins and erasure proteins. The role of writing proteins is reflected in promoting methylation, including METTL3 and METTL5. Demethylases are erasing proteins, including FTO and ALKBH5, and methylated reader proteins in m6A include IGF2BP1/2/3, YTHDF1/2/3, and ELAVL (Li et al. 2017). In recent years, many studies have confirmed that the three m6A proteins mentioned above are often not normally expressed in cancer, and new therapeutic strategies can be developed by regulating the m6A modification levels of specific genes in cancer treatment research. For example, the proliferation and metastasis of tumor cells can be inhibited by m6A modifications targeting tumor-associated genes. With the development of bioengineering technology, m6A targeted editing tools have been optimized from RNA editing technology in CRISPR/Cas system to artificial editing enzymes, which provides new possibilities for regulating m6A modification, but there are still challenges in delivery efficiency and other aspects, and it is difficult to be truly applied in clinical practice (Lo et al. 2022).

According to the 2022 data from the International Agency for Research on Cancer (IARC), lung cancer was the most diagnosed cancer this year, with a high rate of 12.8%, and lung cancer is one of the important causes of cancer death, with 1.8 million cases, accounting for 18.7% of cancer deaths. Small cell lung cancer (SCLC) and non-microcellular lung cancer (NSCLC) divide lung cancer into two broad categories, and lung cancer is often not detected until an advanced stage, making it more difficult to treat. One of the prognostic factors for lung squamous cell carcinoma (LUSC) is M6A demethylase FTO, and a large number of studies have found that METTL3 and FTO play a role in lung cancer (Liu et al. 2018). Breast cancer is the most common cancer in women, accounting for 11.8%, becoming the second most incidenceous cancer. In breast cancer, the m6A programming protein plays a role by activating YAP/TAZ in the Hippo pathway. In this review, the basic mechanisms and regulatory principles of m6A modification are first summarized. The current state of development of m6A genetic engineering tools and their potential applications in cancer treatment are then discussed. Finally, the challenges and

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future prospects of m6A-targeted editing in clinical practice are analyzed.

2 MOLECULAR MECHANISMS AND DYNAMIC REGULATION OF m6A MODIFICATION

N6-methyladenosine (m6A) is an epitransc riptome modification that is ubiquitous in eukaryotic RNA molecules and is formed by the addition of a methyl group (-CH_3) at the nitrogen 6 position of adenosine (A). It is one of the most common and abundant types of RNA modifications. m6A is dynamically reversible and is carried out by three proteins, which are writers, erasers and readers, and their functions have different roles. Writers are catalyzed by methyltransferase complexes, among which METTL3, METTL14, and so on are more representative, the function of METTL3 is to catalyze the modification of m6A, and the METTL14 is to assist METTL3 in recognizing subtraction, and different regulator types make the modification of m6A more diverse. The role of erasers is the opposite of that of writers, and the more common one is FTO, which is used to remove m6A modifications and is now mostly being developed as a drug inhibitor. The m6A reader protein can recognize and bind m6A modifications that regulate gene expression by regulating a variety of processes, and different reader proteins will present different functions depending on the environment, such as HNRNPC-mediated mRNA splicing, YTHDF1 to improve translation efficiency, and IGF2BPI1/2/3 to enhance its mRNA stability. m6A methylation affects all aspects of the mRNA process. For m6A modification, it precisely affects the growth, development, and other biological functions of an individual. m6A may inhibit the cleavage of target mRNA by splicing different regulators, such as the deposition of TARBP2, which improves intron retention and promotes tumor growth (Fish et al. 2019). The more commonly known METTL16 is to induce MAT2A intron splicing to drive efficient splicing. The erasure and addition of m6A methylation require additional attention to environmental factors, which are environmentally dependent, and in the development of human hepatocytoma, the continuous expansion of the tumor leads to insufficient blood supply, resulting in a hypoxic environment. Under this condition, HIF-1α upregulates the expression of YTHDF1 and activates the transcription of YTHDF1, and the overall level of m6A is upregulated (Li et al. 2021).

3 DEVELOPMENT AND APPLICATION OF TARGETED RNA EDITING TOOLS

Through targeted editing, m6A modification has the ability to develop and intervene in the progression of cancer, neurological diseases and metabolic diseases. In the targeted modification of DNA, CRISPR/Cas9 is an extremely popular gene editing tool. In recent years, researchers have begun to explore the combination of CRISPR/Cas9 technology with m6A modification to achieve more precise gene expression regulation and disease treatment. It can complement the required sequence through the designed single guide RNA, allowing the enzyme associated with the nucleic acid to enter the target cell together. Using CRISPR/Cas9 technology to knock out METTL3, reduce global m6A levels, inhibit tumor growth, and achieve precise targeted regulation of m6A. CRISPR is also one of the programmable methylation tools. CRISPR-Cas9 is coupled with single-stranded methyltransferase and ALKBH5/FTO to form m6A writers and erasers, adjust the highly specific installation of m6A on the transcript, and program the single guide RNA. The splicing and translation efficiency of RNA have been changed, but there is still a more important problem that CRISPR/Cas9 can produce off-target modifications to non-target RNA, which needs to be solved by more researchers.

Through genetic engineering, the delivery efficiency of m6A can be improved. It has been demonstrated that METTL14 reduces osteoclast bone resorption by regulating the methylation functional site of NFATc1 upstream. In addition, in osteoclasts, EphA2 overexpression on exosomes is targeted delivery of METTL14 into osteoclasts, increasing the methylation level of m6A and inhibiting the development of osteoclasts. Exosome delivery not only improves targeting but also improves biocompatibility (Yang et al. 2023)). Singleguide RNA targeting the methyltransferase domain can reduce the rate of m6A methylation and accelerate the apoptosis of cancer cells. For example, ZCCHC4 was knocked out in HuCCT1 and RBE cell lines by targeting single-guide RNA, which led to the depletion of ZCCHC4, reduced the methylation rate of m6A, and promoted the apoptosis of ICC cells (Chen et al. 2025). Oncolytic

virus therapy is currently a hot topic in cancer treatment. Oncolytic viruses can be modified by m6A modification to improve their anti-tumor effects. Infection with herpes simplex virus 1 (HSV-1) has the function of evading innate immunity, and the overall level of m6A may be reduced during early infection. By genetically engineering oncolytic viruses (OVs), using shRNA to knock down METTL14 to reduce the expression of m6A, the anti-tumor activity of oHSV-1 was enhanced, and the effect of oncolytic viruses in treatment was better exerted (Chen et al. 2024).

4 ENGINEERING-BASED INTERVENTIONS FOR CANCER THERAPY

In breast cancer, METTL3 is one of the m6A writer proteins. Experiments have shown that the mRNA, protein, and overall m6A-methylated RNA levels of METTL3 are lower than those in normal tissues and MCF-10A cell lines, respectively, when monitored in real time by CPR. Upon knocking out METTL3 in MCF-7 and T47 cells, its deletion was found to exert an inhibitory effect on breast tumor cells. Adriamycin resistance is a significant challenge in the clinical treatment of breast cancer, as its emergence often indicates treatment failure. Elevated expression of miR-221 in the blood of breast cancer patients serves as a biomarker for predicting chemotherapy resistance. Meanwhile, METTL3 reduces the m6A-induced mRNA methylation expression mediated by miR-221, thereby improving Adriamycin resistance in breast cancer treatment. FTO dynamically regulates m6A modification to influence gene expression and holds great potential in cancer cells through genetic engineering editing. FTO inhibitors can be combined with immune checkpoint inhibitors. In their study, Su et al. reported that FTO inhibitors exhibit an inhibitory effect on breast cancer stem cells, which may enhance the anti-tumor immune response in triple-negative breast cancer (Su et al. 2020). However, m6A primarily affects cancer progression. METTL3 is often overexpressed in breast cancer, and inhibition of m6A modification of LATS1 expression can lead to tumor development. Additionally, the upregulation of METTL3 and m6A modification inhibits tumor immune surveillance. The aforementioned resistance is also attributed to the reversible presence of m6A modification in cancer, which contributes to drug resistance. This is not only seen in Adriamycin resistance but also in the upregulated m6A methylation of GPRC5A, which leads to docetaxel resistance and increases the tendency of cancer cells to metastasize to the liver (Ou et al. 2024). All these factors may contribute to a poor prognosis for breast cancer.

In lung cancer, the primary treatment modalities are chemotherapy and surgery. The p53 tumor suppressor gene, a transcription factor, regulates a variety of key cellular functions. Specifically, mRNA with m6A heterozygosity carrying the TP53 R273H mutant protein expression can modulate RNA methylation function, re-enhance the efficacy of anticancer drugs, and improve the feasibility of cancer treatment. Similarly, the knockout of METTL3, as mentioned earlier, also exerts an inhibitory effect on lung cancer progression. M6A plays a more significant role in cancer prognosis. For instance, the YTH domain family, present in m6A reader proteins, is predominantly involved in m6A methylation expression during tumorigenesis. In the treatment of small cell lung cancer (SCLC), high expression of YTHDF1 is associated with better prognosis and reduced resistance to chemotherapeutic drugs (Shi et al. 2019). The engineering intervention of M6A in cancer treatment remains to be further explored and refined, yet it holds great promise for improving lung cancer prognosis. The cleavage of the miR-143-3p/VASH1 axis by M6A promotes angiogenesis in lung cancer, a critical aspect of tumor progression, and may facilitate the metastasis of cancer cells to the brain . METTL3 is also highly likely to induce upregulation of HIF-1α via an m6A-IGF2BP2-dependent mechanism, thereby exacerbating ferroptosis, leading to acute lung injury and increased lung cancer mortality. Compared with breast cancer, drug resistance is more prevalent in lung cancer and often leads to rapid disease progression. SCLC is particularly prone to developing chemotherapy resistance, and m6A methylation negatively regulates the target gene DCP2 of METTL3, inducing chemotherapy resistance in SCLC (Sun et al. 2023). Thus, the emergence of chemotherapy resistance due to m6A methylation has become a major challenge that needs to be addressed, and it also lays the groundwork for the development of FTO inhibitors for lung cancer treatment.

5 CHALLENGES AND FUTURE DEVELOPMENT DIRECTIONS

In cancer treatment, epigenetic inhibitors have shown great advantages and good anti-cancer effects in genetic therapy in recent years, and modification of m6A is one of the features in cancer development and often appears in different types of cancer. However, only a few of the m6A modifiers can be made into drugs, but none of them have been used in clinical practice, and the inhibition of FTO activity after m6A demethylation also leads to limited activity of inhibitors, which are not suitable for clinical treatment. The difficulty of delivering m6A's gene-editing tools into tumor cells also leads to reduced drug availability. For example, Chen et al. experimentally improved the oncolytic activity of oHSV-1 in the treatment of glioma by knocking out METTL14, but at the same time enhanced the transmission of HSV-1, and a balance needs to be found between the two (Chen et al. 2024). The transformation of inhibitor drugs modified by m6A methylation into clinical applications still needs to go through many levels and obstacles and large-scale mass production needs to take into account the production cost and regulatory approval. The clinical trials of new drugs require the participation of a large number of experimenters, and the protection of the interests of experimenters and the standardization of recruiting experimenters also involves many ethical aspects, so the research and development technology and clinical application of m6A-related drugs needs to be further explored and improved.

6 CONCLUSION

In general, m6A, as a research hotspot over the years, has promoted the development of RNA dynamic regulation and revealed development. The important role of metabolism and immunity in disease plays a role through the systemic regulation of writing, erasing, and reading. In gene editing technology, it reduces the methylation rate by modifying specific sites of RNA. Gene editing m6A improves the delivery efficiency of its related substances and reversely regulates the poor prognosis of the disease caused by the original expression of m6A into a certain treatment method and future development prospects. The engineering intervention of m6A has improved the new research direction in the treatment of breast cancer and lung cancer, and the methylation of m6A has been found to lead to the occurrence of drug resistance and the development of tumor progression in cancer, which provides a new direction and progress for the development of its inhibitors and has also become the focus of cancer prognosis. Based on the current research and its development trend, m6A's gene editing tools have certain limitations in cancer treatment, there are certain risks in the uncontrollability of dynamic regulation, and the immaturity of editing tools makes m6A often become a landmark of adverse reactions in cancer treatment, and the related inhibitory drugs modified by editing technology have not been put into clinical treatment and use normally, and the long-term effects of m6A editing tools in vivo are not clear. For the development of cancer, this review can provide a direction for the diagnosis of diseases by studying more accurate biomarkers and actively carrying out experiments in phase I and phase II clinical trials of m6A targeted therapy to verify its safety. Long-term experiments in animal models can also be carried out to evaluate the potential risks of m6A editing tools, so that the m6A field can achieve its clinical transformation through technological innovation and interdisciplinary cooperation in the future, overcome related shortcomings, and promote the development of precision medicine.

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