N6-methyladenosine Modifications in Cervical Cancer: The Molecular and Clinical Perspective

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Abstract: N6-methyladenosine (m6A) is closely associated with cervical cancer. m6A is responsible for the stability, transport, and splicing of RNA, which regulates apoptosis, cell proliferation, RNA metabolism, the tumor immune microenvironment, and metastasis. The m6A modification process is found in both mRNA and non-coding RNAs (ncRNAs). A group of enzymes called writers, erasers, and readers are involved in controlling m6A.These enzymes act as oncogene promoters in cervical cancer by upregulating or downregulating oncogenes. m6A modifications hold future potential to be used as biomarkers. Several drugs and inhibitors targeting m6A modulators have been discovered. This review explores the molecular mechanisms of m6A modifications, as well as their perspectives and potentials.

1 INTRODUCTION

Cervical cancer (CC) ranks as one of the most common cancers and leading cause of cancer-related death among women, with approximately 660,000 new cases and 350,000 deaths reported globally in 2022. One major contributing factor to CC is the persistent infection with human papillomavirus (HPV). Typically, it takes 15 to 20 years for cells to become abnormal and progress to cervical cancer. Prevention strategies for CC include vaccination between the ages of 9 and 14, as well as cervical screening every 5 to 10 years beginning at age 30. CC can be treated with radical hysterectomy, radiotherapy, chemoradiation, or excisional cone biopsy, depending on the disease's stage. Epigenetics is a field of study that regulates gene expression without altering the underlying DNA sequence. One form of epigenetic modification is RNA modification. N6-methyladenosine (m6A) is a subtype of RNA modification (Jiang et al. 2021). The most widely accepted consensus sequence for m6A modification is RRACH (where R = A or G, and H = A, C, or U). m6A plays a crucial role in RNA stability, transport, and splicing. The m6A modification process is catalyzed by a set of enzymes categorized as “writers” (e.g., METTL3, METTL14), “erasers” (e.g., FTO, ALKBH5), and “readers” (e.g., YTHDF1, YTHDC1).

m6A is closely associated with cervical cancer. Studies have shown that m6A regulatory factors are abnormally overexpressed in cervical cancer (CC), such as methyltransferase-like 13 (METTL3). Additionally, m6A regulates key pathways involved in cervical cancer, including immune response, cell proliferation, and cancer cell survival. One example is through modifying the stability and translation of mRNAs that encode immune checkpoint proteins (e.g., PD-1, PD-L1) (Mao et al. 2023). Despite growing interest in the field of m6A research and significant progress made by scientists, the exact mechanisms and functions of m6A remain poorly understood. In this review, the biological processes and enzyme functions involved in m6A modification are elucidated. Next, the link between m6A and cervical cancer is explored, including the role of m6A dysregulation in cervical cancer (CC). Moreover, the clinical relevance and therapeutic potential of m6A are discussed, such as its use as a biomarker and the potential for m6A modulation in CC treatment. Finally, recent advances in m6A research and future directions are examined.

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2 ENZYMES INVOLVED IN M6A RNA METHYLATION

There are three methyltransferase complexes (MTCs) involved in the regulation of m6A modifications on RNA: writers, erasers, and readers.

Writers promote methylation. Some key enzymes include METTL3, METTL14, and WTAP. Methyltransferase-like 3 (METTL3), the first identified m6A methyltransferase, plays dual roles as both an oncogene and a tumor suppressor in cancer (Fang et al. 2022). METTL3 is involved in numerous physiological processes, such as embryonic and brain development (Jiang et al. 2021). METTL3 primarily functions together with METTL14 as a heterodimeric complex. METTL14 aids METTL3 in identifying and interacting with RNA substrates. WTAP guides the METTL3/14 heterodimer core complex into nuclear speckles via a nuclear localization signal (Huang et al. 2022).

Erasers are demethylases that remove m6A modifications from RNA (Jiang et al. 2021). The two major erasers are FTO and ALKBH5. FTO functions by adding a hydroxyl group (–OH) to the N6 position, converting m6A to hm6A, f6A, and ultimately to adenosine. This process requires cofactors such as iron (Fe^{2+}) and α-ketoglutarate (α-KG) (Mao et al. 2023). FTO has been shown to remove m6A modifications from the mRNA of specific genes, thereby controlling the expression of targets such as ASB2 and RARA. The second m6A demethylase, ALKBH5, removes m6A marks from PD-L1 mRNA, stabilizing the mRNA and preventing its degradation (Fang et al. 2022). ALKBH5 also modulates mRNA export, RNA metabolism (Jiang et al. 2021), and the tumor immune microenvironment (Fang et al. 2022). Like METTL3, ALKBH5 can function as both an oncogene and a tumor suppressor (Qu et al. 2022). ALKBH5 is important for acquired immunity and natural immunity. ALKBH5 demethylase gene transcripts that encode for molecules of the natural immune system, such as TRAF3 and TRAF6. Thus, it affects viral infection and antiviral immune responses. ALKBH5 removes the m6A modifications on NR4A1 mRNA, increasing the expression of NR4A1 protein. NR4A1 is required for gut immunity and homeostasis of ILC3s. Moreover, ALKBH5 regulates the function of CD4+ T cells during induced neuroinflammation by enhancing interferon-γ (IFN-γ) and C-X-C motif chemokine ligand 2 (CXCL2) mRNA stability. Therefore, there is an increased activation of CD4+ T cells, and more neutrophils to the central nervous system (CNS) (Qu et al. 2022).

Readers are proteins that bind to and recognize m6A-modified RNA molecules (Fang et al. 2022). They control gene expression by influencing various aspects of RNA metabolism, such as mRNA stability and translation efficiency. Some examples of readers include IGF2BP1 and YTHDC1/2 (Jiang et al. 2021, Mao et al. 2023). IGF2BP1 is highly expressed in tumor cells but downregulated in adult tissues (Huang et al. 2021). One of its functions is to stabilize specific mRNAs necessary for cell proliferation and metastasis. YTHDC1/2 are readers containing a YTH domain. They affect cancer progression by regulating multiple genes (Fang et al. 2022). YTHDC1 promotes the splicing of m6A-modified RNA by interacting with splicing factors to facilitate exon inclusion in target mRNAs (Mao et al. 2023). The helicase domain of YTHDC2, an RNA helicase, aids in RNA binding and controls mRNA translation (Fang et al. 2022).

3 THE ROLE OF M6A DYSREGULATION IN CERVICAL CANCER

Studies have shown that METTL3, WTAP, FTO, ALKBH5, IGF2BP1/2/3, and several other enzymes act as oncogenic drivers in cervical cancer (Fang et al. 2022). These enzymes promote tumor growth by downregulating tumor suppressor genes or upregulating oncogenes. For instance, METTL3 adds m6A marks to FOXD2-AS1, thereby promoting the progression of cervical cancer. FOXD2-AS1, stabilized by m6A modification, inhibits the expression of p21 (Gao et al. 2024). One study found that METTL3 downregulates the expression of RAGE in cervical cancer. RAGE signaling promotes carcinogenesis by causing abnormal activation of cell survival pathways, chronic inflammation, and impaired cell communication. RAGE is also reported to inhibit apoptosis in cervical cells (Li et al. 2021). Abnormal expression of WTAP is closely associated with cell cycle regulation, metabolic vulnerabilities, and drug resistance, all of which may contribute to cancer development. In a WTAP knockdown experiment, reduced WTAP expression in nasopharyngeal carcinoma (NPC) cells induced G1 phase cell cycle arrest and apoptosis. Convincing evidence indicates that WTAP targets HK2 in multiple cancer cell types. Induction of HK2 enables aerobic glycolysis in tumor cells (Ju et al. 2023).

FTO promotes the progression of cervical cancer, as higher expression levels of FTO are observed in late-stage patients (stages III and IV) compared to early-stage patients (stages I and II) and normal cervical tissues. Overexpression of FTO enhances the migration and proliferation of cervical cancer cells by targeting the translation of E2F1 and Myc. Overexpression of E2F1 and Myc, in turn, encourages cell division. ALKBH5 inhibits cell apoptosis, increases the migration and invasion capabilities of cervical cancer (CC) cells, promotes cell division in HeLa and SiHa cells, and encourages angiogenesis. ALKBH5 enhances the expression of PAK5, a protein kinase linked to chemoresistance and cancer aggressiveness. Additionally, PAK5 is associated with HPV infection. In a study where ALKBH5 was silenced, ectopic expression of PAK5 partially restored the inhibited malignant characteristics, thereby promoting tumorigenesis and metastasis of cervical cancer (Huo et al. 2023). IGF2BP2 interacts with circARHGAP12 at exon 3. Overexpression of IGF2BP2 may stabilize circARHGAP12 and FOXM1 when binding to them. circARHGAP12 is an unfavorable circular RNA for cervical cancer, while FOXM1 is identified as a driver of oncogenesis in this disease. circARHGAP12 promotes cell proliferation, migration, colony formation, and in vivo tumor growth of cervical cancer cells (Ji et al. 2021).

4 M6A ASSOCIATING WITH NONCODING RNA

RNA sequences that do not encode proteins are known as noncoding RNAs (ncRNAs). Research has demonstrated that m6A methylation was present on ncRNA as well. Circular RNAs (circRNAs), microRNAs (miRNAs), and long non-coding RNAs (lncRNAs) are the three most prevalent forms of non-coding RNAs (ncRNAs) (Mao et al. 2023). Long non-coding RNAs (lncRNAs) control a large percentage expression of genes by interactions between DNA/RNA/protein, and involves in cancer development (Modi et al.2024). LncRNA has four subtypes of molecules that are involved in the development or occurrence of tumors: signal, bait, guide, or scaffold (Mao et al. 2023). lncRNA DARS-AS1 is an oncogene in cervical cancer. Cytoprotective autophagy in the hypoxic tumor microenvironment is regulated by DARS-AS1. Hypoxia-inducible factor 1-alpha (HIF1α) transcriptionally upregulates DARS-AS1 expression in CC cells. DARS-AS1 recruits METLL3 and METTL14 to promote the translation of the DARS mRNA in CC cells, and it binds with DARS mRNA to make it more stable. FOXD2-AS1 is linked to cancer cell migration, proliferation, and a bad prognosis. METTL3 is in charge of FOXD2-AS1. Through the attraction and promotion of lysine-specific demethylase 1 (LSD1), FOXD2-AS1 can decrease p21 mRNA expression. CC cell migration and proliferation are inhibited by FOXD2-AS1 knockdown, which also encourages CC cell death (Modi et al. 2024).

MicroRNAs (miRNAs) are another small non-coding RNAs with approximate 22 nucleotides long. It specifically prevents target mRNA molecules from translating by attaching to their 3′ UTR, so then the mRNA molecules will break down. Studies on METTL3 and recognition protein HNRNPA2B1 manifests m6A participate in synthesis of miRNA through pri-miRNA modification. Similarly other m6A methyltransferases, for example METTL14 affects miRNA synthesis. It has been discovered an abnormal amount of miRNA is expressed in cervical cancer, inducing miR-139-3p, and miR-532- 5p (Mao et al. 2023). High levels of the lncRNA ZNFX1 anti-sense RNA 1 (ZF-AS1) in CC are indicative of advanced FIGO stage, increased risk of metastasis, and poor patient survival. ZF-AS1 suppresses miR-647 in a METTL3-mediated way to encourage CC growth and metastasis. A poor prognosis for patients with CC is linked to the highly expressed lncRNA KCNMB2-AS1, although CC cells undergo apoptosis and proliferation suppression when KCNMB2-AS1 is inhibited. MiR-130b-5p and miR-4294 are silenced by KCNMB2-AS1. As a result, IGF2BP3 is unregulated. It improves stability and expression by interacting with m6A mutations on KCNMB2-AS1. Thus, tumorigenicity of CC cells increases (Modi et al. 2024).

Circular RNAs (circRNAs) have a closed-loop structure. CircRNA participates in transcription, splicing, gene expression, sequestering miRNA, and RNA-binding proteins (Deng et al. 2022). When circRNA is formed, it is very stable and increases in the cytoplasm by moving out of the nucleus. Additionally, circRNA can be found in extracellular vesicles. Recent studies showed some circRNA is able to encode mRNA, and be modified by m6A (Mao et al. 2023). In CC, low levels of ALKBH5 allow for m6A alterations on circCCDC134, which significantly improves its

expression and stability in a way that is reliant on YTHDF2. Through its interactions with miR-503-5p and p65, circCCDC134 regulates MYB expression. Increased HIF1α transcription or overexpression of ALKBH5 as a result of this regulation promotes the growth, survival, and metastasis of cancer cells. Additionally, stability is increased and CXC motif chemokine ligand 1 (CXCL1) is overexpressed when circRNF13 is overexpressed, which leads to radiation resistance. Overexpression of METTL3 leads to more m6A modifications on circRNF13. Then YTHDF2 binds to circRNF13 and promotes breakdown. The degradation of circRNF13 leads to a decrease in its expression. As a result, radiosensitivity in CC cells improved (Modi et al. 2024).

5 CLINICAL RELEVANCE AND THERAPEUTIC POTENTIAL

m6A regulators have garnered increasing attention in recent years. Although research in this area is still limited, there have been some notable breakthroughs. Selberg et al. conducted a structure-based virtual screening of compound databases and identified four small molecules capable of enhancing the activity of the METTL3-METTL14 complex. Additionally, Bedi et al. discovered a promising adenosine analog that exhibited significant inhibitory activity against METTL3. Inhibiting METTL3 reduces the expression of CXCL1 by stabilizing circRNF13, thereby decreasing radioresistance in cervical cancer (Shen et al. 2025). Similarly, ALKBH5 modulates immune responses in the tumor microenvironment through chemokine signaling. Inhibiting ALKBH5 results in decreased recruitment of tumor-associated macrophages (TAMs), making tumors more susceptible to immune surveillance.

m6A modifications hold potential as biomarkers, including prognostic, diagnostic, and predictive biomarkers (Mao et al. 2023). One potential prognostic biomarker is the METTL3/YTHDF1/HK2 axis. HK2 is a key enzyme involved in the Warburg effect, a hallmark of cancer characterized by the preferential use of aerobic glycolysis over mitochondrial oxidative phosphorylation for energy production. Measuring the activity of the METTL3/YTHDF1/HK2 axis can help determine tumor aggressiveness and cancer prognosis. FTO levels can also serve as prognostic markers to predict disease progression. Patients with late-stage cervical cancer (as classified by the FIGO staging system) typically exhibit higher FTO levels (Mao et al. 2023). Several prediction models integrating multiple m6A regulators have been developed. For instance, Ji et al. constructed a risk score system based on METTL16, YTHDF1, and ZC3H13, which demonstrated high predictive performance for the prognosis of cervical cancer patients (Ji et al. 2021). Wang et al. analyzed 33 m6A regulators and developed a diagnostic and prognostic model for cervical cancer. They calculated an m6A score and identified seven diagnostic elements and one prognostic factor from 20 pairs of population tissues. RBM15, NSUN2, METTL3, CBLL1, RBMX, and ZC3H13 were found to be upregulated in cervical cancer, while HNRNPAB and YTHDF3 were downregulated (Wang et al. 2022). Regarding predictive biomarkers, overexpression of FTO in the β-catenin pathway leads to chemoradiotherapy resistance both in vitro and in vivo. YTHDF3 is also upregulated in cervical cancer and positively correlates with radioresistance in cancer cells (Gao et al. 2024). The identification of these biomarkers could help predict the overall efficacy of treatments.

6 FUTURE RESEARCH DIRECTIONS

Recently, there have been multiple drug discoveries targeting m6A modulators. Curcumin is used to decrease ALKBH5 expression, leading to an increase in methylation of TRAF4's mRNA. Thus YTHDF1 will bind to TRAF, and results in better translation of the TRAF4 protein. Quercetin bind with FTO through hydrophobic interactions and hydrogen bonds. Quercetin inhibits the expression of METTL3, leading to decreased cancer cell proliferation, migration, and invasion. When Quercetin is used with a chemotherapeutic drug cisplatin, it’s more effective in targeting cervical cancer cells like HeLa and SiHa (Deng et al. 2022). Researchers have been studying screening numerous 2OG analogues and linked molecules as inhibitors of FTO and ALKBH5. Cofactors 2OG and Fe2+ regulates the demethylation activity of FTO and ALKBH5. Numerous 2OG oxygenases generic inhibitors inhibit FTO demethylation. The inhibitors are based on compounds like pyridyl, hydroxyquinoline, and isoquinoline. A high-throughput fluorescence polarization (FP) assay was carried out for FTO/ALKBH5. Meclofenamic acid (MA) can function as a selective inhibitor of FTO over ALKBH5. Combining data from ligand-protein

complex crystal structures with structure-based drug designs was another effective method for finding inhibitors with chemical scaffolds (You et al. 2022). Rhein, a Rheum rhabarbarum-rich anthraquinone, was discovered to be one of the most competitive, cell-active, reversible inhibitors of FTO. Rhein inhibited FTO by either competitively binding to the 2-oxoglutarate (2-OG) cofactor at the active site, directly binding to nucleic acids, or both.

Traditional medicine sources have recently been a new way of finding new agents targeting m6A, with the assistant of AI and traditional medicines databases. For instance, the TCM Systems Pharmacology Database and Analysis Platform (TCMSP) has approximately 30 thousand ingredients of five hundred kinds of Chinese herbal medicine, and more than three thousand targets. Moreover, with the combination of AI, it could collect and analyze data of different demethylases and methylases, such as the site of modification, and the antigen it downregulates, the side effect it exhibits, etc. Then AI will give a prediction of the targeted drugs based on this information. Scientists are able to use these designs and perhaps make changes based on the models. Thus the costs of developing drugs and inhibitors of such methylates and demethylases will significantly decrease, and the time will shorten. More and more effective m6A inhibitors and drugs targeting them will be approved in the future (Deng et al. 2022).

7 CONCLUSION

m6A modification is actively involved in the development of cervical cancer, through regulating RNA stability, translation, and degradation. Methyltransferase complexes (MTC) such as writers (METTL3, METTL14, WTAP), erasers (FTO, ALKBH5), and readers (IGF2BP1, YTHDC1/2) promote tumor growth, translation, immune response, cell proliferation, metastasis, and drug resistance. These enzymes promote tumor growth by downregulating tumor suppressor genes or upregulating oncogenes. For instance, METTL3 stabilizes FOXD2-AS1, inhibiting the expression of p21, while FTO and ALKBH5 enhances tumorgenesis by controlling cell apoptosis, proliferation, and metastasis. m6A modifications exist on noncoding RNAs (ncRNAs), such as lncRNAs, circRNAs, and miRNAs. Oncogenic lncRNAs like DARS-AS1 and FOXD2-AS1 promote tumor growth, knockdown of these genes inhibit proliferation and induces apoptosis. Abnormal miRNA expression linked to cervical cancer. circRNAs contribute to regulation of genes and therapeutic resistance.

m6A regulators offers a promising therapeutic strategy. For instance, small molecules enhancing METTL3-METTL14 activity, and METTL3 inhibitors are being discovered. Furthermore, m6A modifications serve as prognostic, diagnostic, and predictive biomarkers. The METTL3/YTHDF1/HK2 axis links m6A to cancer metabolism, and FTO levels correlate with advanced-stage cervical cancer. Discoveries of drugs targeting m6A modulators include Curcumin, Quercetin, Meclofenamic acid and Rhein. Curcumin reduces ALKBH5 expression. Quercetin inhibits expression of METTL3, leading to decreased cancer cell proliferation. Meclofenamic acid and Rhein are inhibitors of FTO. AI and traditional medicine databases aid in finding new inhibitors through analyzing demethylases, methylases, and drug interactions, reducing costs and accelerating drug development. The continued research in this field holds great promise for improving diagnosis, prognosis, and treatment strategies for cervical cancer patients.

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