The Role and Clinical Significance of N6-Methyladenosine Methylation in Ovarian Cancer: Research Progress and Future Prospects

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Abstract: Ovarian cancer (OC), often diagnosed late due to asymptomatic early stages, remains highly lethal despite treatment advances. N6-methyladenosine (m6A) RNA methylation, regulated by writers, erasers, and readers, plays a key role in OC progression, metastasis, and chemoresistance by modulating RNA stability and translation. Dysregulated m6A modifications influence oncogenic pathways like PI3K/AKT and EMT, while m6A-related proteins serve as diagnostic and prognostic biomarkers. Preclinical studies highlight the therapeutic potential of targeting m6A regulators, though challenges like tumor heterogeneity hinder clinical translation. Future research should clarify m6A's roles, develop diagnostics, and advance m6A-targeted therapies. m6A methylation offers promising avenues for improving OC diagnosis, prognosis, and treatment.

1 INTRODUCTION

Among malignancies affecting the female reproductive system, ovarian cancer (OC) is highly prevalent, standing as the 18th most frequently occurring cancer and the 14th leading cause of cancer-related deaths (Stewart et al. 2019). Of all the organs in the body, the ovary has the greatest range of primary tumor types, and the composition of ovarian tumor tissue is extremely complicated. The biological behavior and histological structure of various ovarian cancer types differ noticeably. Sex cord-stromal tumors, germ cell tumors, and epithelial ovarian cancer (EOC) are the three main histological categories of OC (Siegel et al. 2023). EOC is the most prevalent of these, making up between 50% and 70% of all cases. EOC is further subdivided into serous (52%), endometrioid (10%), mucinous (6%), clear cell (6%), and other kinds according to histological features. [3]. Most patients are diagnosed at an advanced stage, which leads to a dismal prognosis because of the disease's subtle early signs and the absence of efficient screening techniques. Common symptoms include abdominal distension, abdominal pain, indigestion, and frequent urination, but these symptoms are often misdiagnosed as gastrointestinal disorders. Diagnosis mainly relies on imaging examinations (such as ultrasound, CT/MRI), tumor markers (such as CA-125), and pathological examinations. Treatment methods include surgery, chemotherapy, targeted therapy, and immunotherapy. However, the recurrence rate is high in advanced patients, and the 5-year survival rate remains low.

Post-synthetic chemical modifications of DNA, RNA, and proteins regulate gene expression and cellular functions at the transcriptional, post-transcriptional, and post-translational levels, respectively. Specifically, DNA modifications primarily affect the transcriptional level, RNA modifications act at the post-transcriptional level, and protein modifications regulate post-translational processes. Among these, one of the core mechanisms of post-transcriptional regulation is RNA modification and its interaction with non-coding RNAs (ncRNAs), commonly referred to epitranscriptomics. More than 100 post-synthetic RNA modifications have been discovered in the last half-century, and they are present in almost all known RNAs, with transfer RNA (tRNA) and ribosomal RNA (rRNA) containing the great bulk of these modifications (Sun et al. 2019). According to recent research, RNA alterations have a significant impact on a variety of molecular processes, including splicing, translation, stability, and RNA metabolism, which in turn affects gene

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expression and cellular functioning. RNA modifications predominantly consist of methylation modifications, which represent both the most prevalent and thoroughly investigated class. Eukaryotic RNAs predominantly feature four major internal methylation modifications: N6-methyladenosine (m6A), 5-methylcytosine (m5C), 7-methylguanosine (m7G), and N1-methyladenosine (m1A). Of these, m6A represents the predominant internal modification found in messenger RNA molecules (mRNA) and one of the most widely studied RNA modifications (Sun et al. 2019). m6A modification is achieved through methylation of the adenosine nitrogen at position 6, a process catalyzed by methyltransferases and reversible by demethylases, forming a dynamic and reversible regulatory network. m6A modification has multiple functions in RNA metabolism. It can regulate RNA stability, splicing, nuclear export, translation efficiency, and degradation processes, thereby profoundly influencing gene expression. Additionally, m6A modification plays a critical role in various biological processes, particularly in cancer-related mechanisms.

2 THE MECHANISMS OF M6A METHYLATION MODIFICATION

N6-methyladenosine (m6A) constitutes a predominant RNA modification in eukaryotes, occurring extensively across diverse RNA species including messenger RNA (mRNA), long non-coding RNA (lncRNA), ribosomal RNA (rRNA), and microRNA (miRNA) (Zhang et al. 2020). m6A modification is achieved by adding a methyl group (-CH3) to the nitrogen atom at the sixth position of adenosine (A). This process is dynamically regulated by three types of regulatory factors: writers, erasers, and readers, which play a critical role in RNA metabolism and function (Fang et al. 2022). Writers are a group of methyltransferase complexes, with core components including METTL3 (the main catalytic enzyme), METTL14 (assisting in substrate RNA recognition), WTAP (localizing the complex to nuclear speckles), VIRMA (guiding the complex to specific RNA regions), and RBM15 and ZC3H13 (regulating the stability and specificity of the complex) (Yang et al. 2023). Together, they recognize specific RNA sequences (such as RRACH) and catalyze methylation reactions. Erasers are a group of demethylases, primarily including FTO and ALKBH5, which remove methyl groups through oxidation reactions, making the m6A modification process reversible and thereby regulating mRNA stability, translation efficiency, and nuclear-cytoplasmic transport. Readers are a class of proteins that recognize and bind to m6A modifications, including the YTH domain protein family (e.g., YTHDF1 promotes translation, YTHDF2 mediates degradation, YTHDC1 regulates splicing and nuclear-cytoplasmic transport), the IGF2BP family (enhancing mRNA stability and translation efficiency), and the HNRNP family (regulating pre-mRNA processing and miRNA processing). These readers mediate the functions of m6A modifications, regulating RNA metabolic processes such as RNA stability, splicing, translation efficiency, nuclear-cytoplasmic transport, and the functions of non-coding RNAs (Yang et al. 2023).

Through this dynamic and reversible regulatory network, m6A modifications play a key role in gene expression, cellular functions, and various biological processes. They regulate stem cell differentiation and embryonic development during development and differentiation, influence immune cell activation and function in immune responses, and modulate malignant cell growth, migration, invasion, and chemoresistance in cancer (Zhao et al. 2020). For example, in ovarian cancer, the abnormal expression of writers such as METTL3, WTAP, and VIRMA, as well as erasers such as FTO and ALKBH5, is closely related to tumorigenesis and prognosis. METTL3 demonstrates promoter hypomethylation and upregulated expression in OC, with extensive evidence supporting its oncogenic role in disease pathogenesis. Low expression of FTO and ALKBH5 is associated with shorter overall survival and progression-free survival (PFS) in patients. Additionally, readers such as YTHDF1, IGF2BP1, and HNRNPA2B1 regulate RNA metabolism, affecting cancer cell proliferation, invasion, and chemoresistance. YTHDF1 and YTHDF2 show elevated expression in ovarian cancer with potential prognostic value. IGF2BP1 promotes invasion by counteracting miRNA-mediated gene suppression, while HNRNPA2B1 enhances malignancy through Lin28B upregulation (Zhu et al. 2022).

3 THE ROLES OF m6A METHYLATION IN OVARIAN CANCER

3.1 m6A RNA Modification Modulating mRNA to Influence the Progression of Ovarian Cancer

Elevated expression levels of METTL3 demonstrate a significant and independent correlation with diminished survival outcomes and enhanced malignant characteristics in endometrioid epithelial ovarian cancer (EEOC) patients. Experimental evidence reveals that targeted suppression of METTL3 through knockdown methodologies effectively curbs cellular proliferation and migratory capacity while simultaneously inducing programmed cell death (apoptosis) in both CRL-11731D and TOV-112D ovarian cancer cell models (Fan et al. 2020). Notably, the observed phenotypic alterations following METTL3 depletion exhibit substantially greater magnitude than those resulting from comparable knockdown interventions targeting either WTAP or METTL14 proteins. At the molecular level, experimental reduction of METTL3 expression leads to a significant decrease in m6A methylation patterns specifically within ovarian cancer-associated genes including CSF-1, AXL, EIF3C, and FZD10, thereby demonstrating that the m6A modification profile mediated by METTL3 exhibits unique characteristics that differentiate it from those modifications catalyzed by either WTAP or METTL14 (Zhang et al. 2021). Furthermore, in the COV362 ovarian cancer cell line model, genetic silencing of METTL3 expression results in a pronounced accumulation of cells in the G0/G1 phase of the cell cycle accompanied by a marked increase in cellular mortality through apoptotic pathways. In vivo investigations employing METTL3 conditional knockout (cKO) murine models demonstrate enhanced ovarian cancer cell proliferation concurrent with altered macrophage polarization from the pro-inflammatory M1 phenotype to the tumor-promoting M2 phenotype, collectively indicative of accelerated neoplastic progression. Therapeutically, sulforaphene (Sul) reverses METTL3 overexpression, reducing OC cell viability and promoting apoptosis via the FAS/FADD and Bax/Bcl-2 pathways (Zhang et al. 2021). METTL14 exhibits a dual role in OC. Molecular analyses reveal context-dependent roles for RNA methylation regulators in epithelial ovarian cancer (EOC). In specific tumor subsets, METTL14 demonstrates reduced expression accompanied by decreased global m6A levels relative to normal ovarian tissues, where it exhibits tumor suppressive activity through m6A-mediated downregulation of TROAP expression, thereby inhibiting EOC cell proliferation. Paradoxically, distinct EOC populations display METTL14 overexpression, which functionally promotes malignant behaviors including enhanced proliferation, migration and invasion in A2780 and SKOV3 cellular models. Similarly, ALKBH5 shows consistent overexpression in ovarian carcinoma specimens, where it drives aggressive tumor phenotypes via TLR4/NF-κB pathway-mediated transcriptional activation of the stemness factor NANOG. Hypoxia-inducible factor (HIF)-1α induces ALKBH5 expression, which stabilizes RMRP and promotes OC growth and migration (Zhang et al. 2021).

Additionally, ALKBH5 suppression in SKOV3 cells enhances autophagy and inhibits proliferation and invasion by modulating the EGFR-PIK3CA-AKT-mTOR axis. FTO expression is decreased in OC and OC stem cells (OCSCs). Overexpression of FTO inhibits OCSC self-renewal and tumorigenesis by demethylating PDE4B and PDE1C, leading to cAMP accumulation. The RNA demethylase FTO demonstrates additional tumor-suppressive effects in ovarian cancer through induction of reactive oxygen species accumulation and programmed cell death pathways, ultimately inhibiting xenograft tumor growth in immunocompromised murine models. Concurrently, the m6A reader protein YTHDF1 exhibits significant upregulation in ovarian carcinomas and shows strong clinical correlation with adverse patient outcomes. Mechanistically, YTHDF1 facilitates malignant progression by selectively enhancing the translational efficiency of EIF3C through recognition of m6A-modified EIF3C transcripts (Han et al. 2020). Similarly, elevated expression of IGF2BP2 promotes multiple oncogenic phenotypes including tumor expansion, migratory capacity and invasive potential via its m6A-dependent regulation of CKAP2L protein synthesis. YTHDC1 is downregulated in OC, and its overexpression inhibits OC development by stabilizing PIK3R1 and downregulating GANAB via the STAT3 pathway.

3.2 m6A RNA Modification Modulating Non-Coding RNA to Influence the Progression of Ovarian Cancer

Non-coding RNAs (ncRNAs) are broadly categorized into housekeeping ncRNAs (rRNA, tRNA, snRNA) and regulatory ncRNAs (miRNA, circRNA, lncRNA). m6A modification critically regulates ncRNA metabolism by controlling RNA stability, splicing processes, and degradation rates. METTL3 promotes OC progression by targeting miR-1246, leading to the suppression of CCNG2. It also enhances miR-126-5p maturation via m6A methylation, activating the PI3K/Akt/mTOR pathway by targeting PTEN. m6A writer METTL3 mediates RHPN1-AS1 stabilization to trap miR-596 and stimulate FAK/PI3K/Akt signaling (Tan et al. 2023). The METTL3-IGF2BP1 axis maintains circASXL1 stability through m6A modification, driving ovarian cancer development by modulating the miR-320/RACGAP1 pathway. In EOC, reduced METTL16 expression promotes MALAT1 decay and blocks β-catenin nuclear translocation, inhibiting tumorigenesis. WTAP is upregulated under hypoxia and promotes OC proliferation and invasion by modulating miR-200 expression in an m6A-dependent manner. YTHDF2 is upregulated in EOC and inversely correlated with miR-145 levels. It enhances EOC growth and migration by reducing the level of m6A mRNA. CACNA1G-AS1 promotes OC growth and migration by upregulating FTH1 via the IGF2BP1 axis, inhibiting ferroptosis. UBA6-AS1 inhibits OC growth and invasion by enhancing UBA6 mRNA stability via RBM15-mediated m6A modification. MEG3, which is downregulated in OC, inhibits VASH1 degradation by suppressing miR-885-5p. YTHDF2 promotes MEG3 RNA decay in a METTL3-dependent manner. circRAB11FIP1 promotes OC progression by sponging miR-129 and regulating ATG14 and ATG7 expression in an FTO-dependent m6A modification manner (Tan et al. 2023).

4 THE CLINICAL SIGNIFICANCE OF m6A METHYLATION IN OVARIAN CANCER

Ovarian cancer continues to pose significant diagnostic and therapeutic challenges in clinical oncology. Current treatment modalities, including surgical intervention and cytotoxic chemotherapy, have shown limited efficacy in improving patient outcomes, underscoring the urgent demand for novel biomarkers and molecular targets. Recent studies have identified

Epitranscriptomic alterations, especially m6A RNA methylation, as pivotal gene expression modulators that are increasingly investigated in ovarian cancer studies (Zhu et al. 2022). Dysregulated m6A modifications in OC tissues compared to normal ovaries alter gene expression profiles, contributing to ovarian tumorigenesis. These modifications influence key oncogenic pathways, including PI3K/AKT, Wnt/β-catenin, and EMT (epithelial-mesenchymal transition), promoting tumor growth, invasion, and metastasis. Additionally, m6A methylation modulates the expression of oncogenes (MYC, EGFR) and tumor suppressors (PTEN), driving cancer progression. Furthermore, m6A modifications play a role in maintaining cancer stem cells (CSCs), which are associated with tumor initiation, drug resistance, and recurrence. m6A-altered RNA molecules in blood/tissue demonstrate potential as minimally invasive OC diagnostic markers (Zhu et al. 2022). Early detection of these modifications could improve patient outcomes through timely intervention. Abnormal expression of m6A-related proteins (FTO, METTL14, METTL3, ALKBH5) in OC tissues compared to normal ovaries provides potential diagnostic markers. Increased expression of m6A methyltransferases (METTL14, METTL3) correlates with higher tumor grades, lymphatic spread, and unfavorable clinical outcomes, while decreased levels of demethylases (FTO) are linked to better patient survival. Additionally, high expression of KIAA1429 and YTHDC2 is associated with poorer prognoses, while IGF2BP1 enhances invasive growth and is linked to unfavorable outcomes. m6A modifications also regulate miRNAs (miR-145, miR-126-5p) and lncRNAs, influencing OC cell proliferation, apoptosis, and migration (Tan et al. 2023).

Pharmacological agents directed against m6A regulatory machinery (METTL3, FTO, ALKBH5) demonstrate therapeutic potential in experimental models, effectively suppressing ovarian cancer cell growth, motility and metastatic capacity. These RNA epigenetic alterations additionally modulate chemotherapeutic sensitivity to platinum-based agents and PARP inhibitors. Notably, YTHDF1 binding to m6A-marked TRIM29 enhances protein synthesis in platinum-resistant ovarian cancer cells,

underscoring its clinical relevance, whereas FZD10 m6A methylation mediates PARP inhibitor responsiveness (Han et al. 2020). Modulating m6A levels can enhance OC cell sensitivity to existing therapies, offering new avenues for combination treatments. Additionally, m6A modifications modulate the tumor microenvironment and immune response, suggesting potential synergies with immunotherapy (Fan et al. 2020). m6A methylation affects mRNA stability, translation efficiency, and degradation, influencing key signaling pathways in OC. It also regulates the function of miRNAs and lncRNAs, which play critical roles in OC progression and metastasis. Furthermore, m6A modifications interact with other epigenetic mechanisms, contributing to the complexity of OC biology. Detection of m6A-modified RNAs in circulating tumor cells or exosomes offers a non-invasive method for OC diagnosis and monitoring (Tan et al. 2023). Understanding the m6A landscape in individual tumors could guide the development of personalized therapeutic strategies. Ongoing research aims to translate m6A-targeted therapies into clinical practice, with promising results in preclinical models.

5 CONCLUSION

The research on m6A RNA methylation has revealed its critical role in the occurrence, progression, metastasis, and therapeutic resistance of ovarian cancer (OC). The dynamic regulation of m6A modifications by writers, erasers, and readers influences gene expression, offering new opportunities for the diagnosis, prognosis, and treatment of ovarian cancer. However, challenges such as tumor heterogeneity, contradictory findings, and methodological limitations still need to be addressed. m6A modifications affect the occurrence, progression, and drug resistance of ovarian cancer by regulating the stability, translation, and degradation of mRNA and non-coding RNA (ncRNA). m6A-related genes (METTL3, FTO, YTHDF1) exhibit significant expression abnormalities in ovarian cancer and are closely associated with tumor progression, metastasis, and patient prognosis. m6A modifications promote the progression and chemoresistance of ovarian cancer by regulating key signaling pathways (PI3K/AKT, Wnt/β -catenin) and epithelial-mesenchymal transition (EMT). m6A-modified RNA transcripts and related regulatory proteins (METTL3, FTO, YTHDF1) can serve as early diagnostic and prognostic markers for ovarian cancer. High expression of m6A writers (METTL3, METTL14) is associated with poor prognosis, while low expression of erasers (FTO) is linked to better survival rates. Small molecule inhibitors targeting m6A regulators (METTL3, FTO, ALKBH5) have shown potential in preclinical studies to suppress tumor growth and overcome chemoresistance. m6A modifications influence treatment response by regulating chemoresistance-related pathways, such as the FTO-mediated RP5-991G20.1/hsa-miR-1976/MEIS1 axis.

Tumor heterogeneity and the diversity of m6A modifications complicate the development of universal diagnostic and therapeutic strategies. Contradictory findings regarding certain m6A regulators (FTO, ALKBH5, METTL14) in different studies require further validation. The lack of standardized methods for detecting m6A modifications hinders clinical translation. Developing high-throughput, standardized techniques for detecting m6A modifications will promote research reproducibility and clinical application. Integrating multi-omics data (genomics, transcriptomics, proteomics) can provide a comprehensive understanding of the role of m6A modifications in ovarian cancer. In-depth research is needed to elucidate the specific roles of m6A modifications in different ovarian cancer subtypes and clarify their functions in various molecular contexts. Developing novel inhibitors targeting m6A regulators (METTL3, FTO, ALKBH5) and evaluating their efficacy in preclinical and clinical trials is essential. Exploring the mechanisms by which m6A modifications contribute to chemoresistance and developing strategies to reverse resistance, such as targeting YTHDF1 or FTO, is crucial. Investigating the interactions between m6A modifications and other molecular pathways (immune checkpoints, DNA repair pathways) can lead to the development of synergistic treatment strategies.

Using m6A modification profiles to guide personalized treatment and tailoring therapies based on patients' molecular characteristics is a promising approach. Developing liquid biopsy techniques to detect m6A-modified RNA in circulating tumor cells (CTCs) or exosomes enables non-invasive diagnosis and dynamic monitoring. Studying the interactions between m6A modifications, DNA methylation, and histone modifications can reveal their synergistic regulatory mechanisms in ovarian cancer. Exploring the regulatory effects of histone modifications on

m6A modifications, such as H3K27me3 influencing m6A levels by regulating METTL14 expression, is also important. Advancing clinical trials of m6A-targeted therapies to evaluate their safety and efficacy in ovarian cancer patients is critical. Combining epigenetic therapies (DNMT inhibitors, HDAC inhibitors) with m6A-targeted therapies offers potential for combination treatments. The role of m6A methylation in ovarian cancer is increasingly recognized, and its potential in diagnosis, prognosis, and treatment provides new hope for improving patient outcomes. Despite challenges such as tumor heterogeneity, contradictory findings, and methodological limitations, the advancement of standardized techniques, mechanistic research, and clinical trials holds promise for establishing m6A modifications as a key target for precision therapy in ovarian cancer. Future research should focus on elucidating the molecular mechanisms of m6A modifications, developing novel targeted drugs, and exploring their synergistic effects with other epigenetic modifications, thereby providing more effective treatment strategies for ovarian cancer patients.

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