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REVIEW

RNA modification regulators as promising biomarkers in gynecological cancers

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Abstract This review explores the evolving landscape of gynecological oncology by focusing on emerging RNA modification signatures as promising biomarkers for assessing the risk and progression of ovarian, cervical, and uterine cancers. It provides a comprehensive overview of common RNA modifications, especially m6A, and their roles in cellular processes, emphasizing their implications in gynecological cancer development. The review meticulously examines specific m6A regulators including "writers", "readers", and "erasers" associated with three gynecological cancer types, discussing their involvement in initiation and progression. Methodologies for detecting RNA modifications are surveyed, highlighting advancements in high-throughput techniques with high sensitivity. A critical analysis of studies identifying m6A regulators as potential biomarkers is presented, addressing their diagnostic or prognostic significance. Mechanistic insights into RNA modification-mediated cancer progression are explored, shedding light on molecular pathways and potential therapeutic targets. Despite current challenges, the review discusses ongoing research efforts, future directions, and the transformative possibility of RNA modifications on early assessment and personalized therapy in gynecological oncology.

Keywords Gynecological oncology · RNA modification · Biomarkers · m6A regulators

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Introduction

Neoplasms remain the main killer worldwide (Yang et al. 2019; Hu et al. 2016; Guo et al. 2022; Zhang et al. 2023; Cheng et al. 2012). Among which, gynecological cancers encompass a diverse group of malignancies originating in the female reproductive system, presenting a formidable health challenge with significant morbidity and mortality worldwide (Borgeaud et al. 2023). This heterogeneous category includes ovarian, cervical, uterine, vaginal, and vulvar cancers, each distinguished by distinct etiologies, risk factors, and clinical manifestations (Chevalier 1954; Van Gorp et al. 2011). Ovarian cancer, often referred to as the "silent killer," tends to manifest insidiously, leading to late-stage diagnoses and limited treatment options (Penny 2020; Webb and Jordan 2017; Kossaï et al. 2018). In contrast, cervical cancer

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is closely associated with persistent human papillomavirus (HPV) infections, making effective screening and vaccination pivotal in its prevention (Burd 2003; Buskwofie et al. 2020; Olusola et al. 2019). Uterine cancer, primarily comprising endometrial carcinomas, underscores the intricate interplay of hormonal imbalances and genetic predispositions (Abu-Rustum et al. 2023; Chelmow et al. 2022; Whetstone et al. 2022). Vaginal and vulvar cancers, although rarer, pose unique challenges in early detection due to their location and often nonspecific symptoms (Buchanan et al. 2016; Gaffney et al. 2016; Bray et al. 2020).

The intricacies of gynecological cancers extend beyond their anatomical origins, involving intricate molecular mechanisms that drive initiation, progression, and metastasis. RNA modifications emerge as key players in this multifaceted landscape, contributing to the dysregulation of gene expression patterns and molecular pathways associated with cancer development (An and Duan 2022; Li et al. 2022; Yin et al. 2021). Understanding the distinctive characteristics of gynecological cancers is imperative for developing targeted therapeutic interventions and advancing personalized medicine approaches.

The importance of early detection and precision risk assessment in the realm of gynecological oncology cannot be overstated, representing a pivotal frontier in enhancing patient outcomes and overall prognosis (Hu and Ma 2018; Lheureux et al. 2019). Gynecological cancers often present latent symptoms in their early stages, underscoring the critical need for strategies that enable timely diagnosis and intervention (Rajaram and Gupta 2021; Gholiof et al. 2022). Early detection not only facilitates a more favorable response to treatment but also opens avenues for less invasive therapeutic approaches, minimizing the physical and emotional burden on patients.

Biomarkers have emerged as indispensable tools in the quest for early detection and risk assessment in gynecological cancers. These molecular indicators, ranging from genetic mutations to specific RNA modifications, provide valuable insights into the biological processes underlying cancer initiation and progression. In ovarian cancer, for instance, biomarkers such as CA-125 and HE4 have shown promise in detecting the disease at an earlier, more treatable stage (Dochez et al. 2019; Kim et al. 2019; Huy et al. 2018). Similarly, in cervical cancer, molecular markers linked to HPV infection serve as powerful tools for identifying individuals at heightened risk, guiding targeted screening efforts and vaccination initiatives (Shen et al. 2020; Zhang et al. 2018). Indeed, non-mutational epigenetic reprogramming has now been included as a new hallmark of cancer. RNA modifications, a burgeoning field within biomarker research, offer a nuanced perspective on gynecological oncology. The dynamic alterations in RNA epigenetics play a crucial role in shaping the molecular landscape of cancers (Barbieri and Kouzarides 2020; Roundtree et al. 2017). Investigating RNA modifications as biomarkers holds the potential to unveil intricate signatures associated with different stages of gynecological cancers, allowing for a more nuanced risk assessment and tailored therapeutic strategies. Precision risk assessment, enabled by biomarkers, transcends the one-size-fits-all paradigm, fostering a personalized approach to gynecological oncology. This paradigm shift holds transformative implications for treatment decision-making, enabling clinicians to tailor interventions based on the unique characteristics of each patient's cancer. In a word, the integration of emerging RNA modifications as biomarkers in the early detection and risk assessment landscape of gynecological cancers marks a paradigm shift in oncology.

RNA modifications: a molecular landscape

Decoding the landscape of common RNA modifications in gynecological cancer research

Within the intricate tapestry of cellular processes, RNA modifications play a pivotal role in orchestrating the finely tuned symphony of gene expression, and their dysregulation has emerged as a critical factor in the pathogenesis of cancers (Zhao et al. 2020; Cayir 2022). Internal modification in RNA has post-transcriptionally and extensively regulate the behaviors and biological functions of RNAs among which methylation is the most frequent. Among the diverse array of RNA chemical modifications, N6-methyladenosine (m6A) (Kou et al. 2024) N1-methyladenosine (m1A) (Bao et al. 2022), 5-methylcytosine (m5C) and pseudouridine (Ψ) stand out as key players present in eukaryotic mRNA, each contributing unique layers of complexity to the epi-transcriptomic coding in governing cellular homeostasis and disease states (Fig. 1). As depicted in Fig. 1, RNA

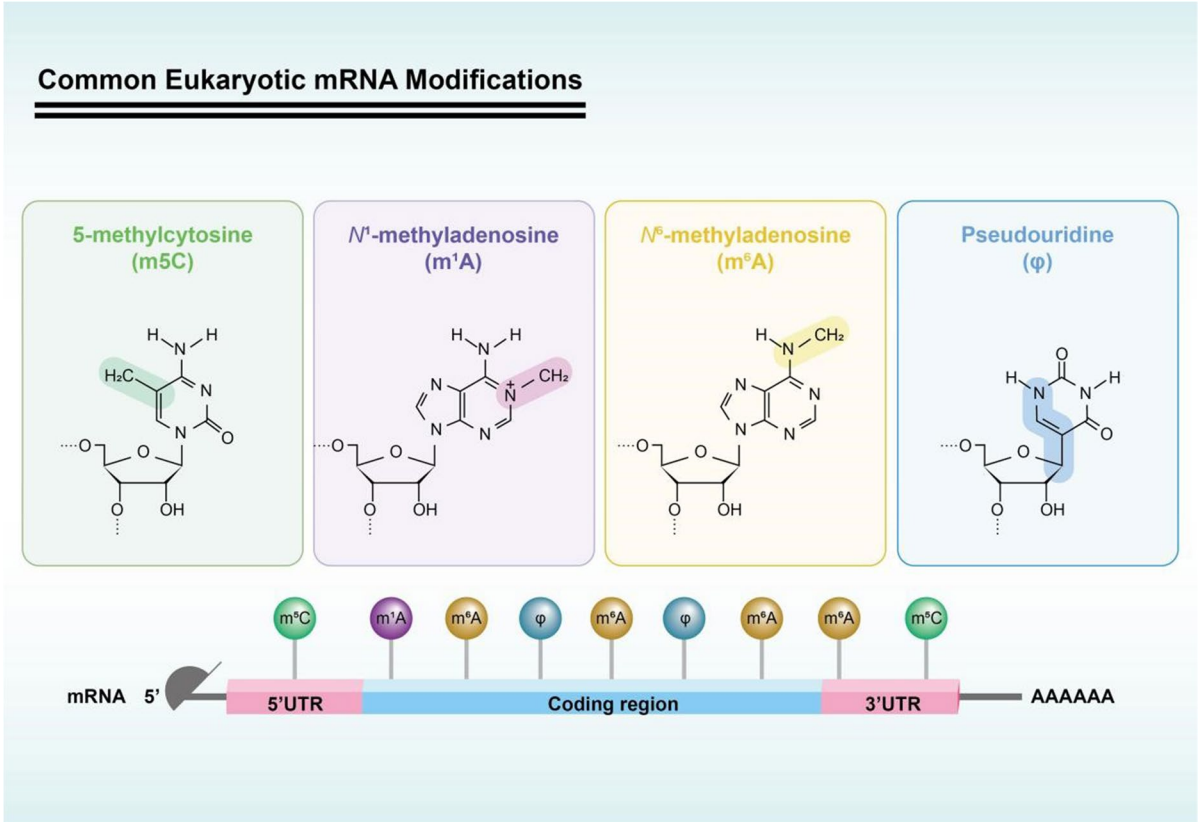

Fig. 1 Common RNA Modifications in Gynecological Cancer Research. RNA modifications like m6A, m1A and m5C occur on the distinct nitrogen or carbon atom of adenosine in RNA, while Ψ is the C5-glycoside isomer of uridine, all affecting mRNA translation fates and stability distinctively in various loci

modifications like m6A, m1A and m5C occur on the distinct nitrogen or carbon atom of adenosine in RNA, while Ψ is the C5-glycoside isomer of uridine (Li et al. 2016), all affecting mRNA translation fates and stability distinctively in various loci.

In recent years, multiple studies indicate that m6A contribute to influence the occurrence and progress of tumor by regulating tumor metabolism (Liu et al. 2018; Ma et al. 2017). Widely recognized as the most prevalent and abundant RNA modification in eukaryotes, m6A exerts profound influence on processes of RNA metabolism at a reversible manner (An and Duan 2022; Yin et al. 2021). It is demonstrated that m6A is involved in RNA translation, degradation, splicing, exporting and folding. Notably, m6A alterations have been implicated in the dysregulation of pathways governing cell cycle progression and proliferation. Aberrant m6A patterns influence the translation of key transcripts involved in cell division, contributing to the uncontrolled growth characteristic of gynecological malignancies. Therefore, in the context of cancers, especially gynecological cancers, alterations in m6A patterns can impact critical processes such as mRNA stability, splicing, and translation initiation to regulate a series of physiological processes, such as self-renewal, invasion and proliferation (Liu et al. 2020; Zhang and Liu 2022). The basic processes of m6A modification are that mRNA is installed by m6A methyltransferase, removed by m6A demethylase and recognized by m6A reading molecules. It means that distinct sets of transcriptomic objects introduce (methyltransferases as writers), recognize (methylation reading proteins as readers) and remove (demethylases as erasers) the RNA modification, allowing it to exert reversible regulation of mRNA metabolism. Previous evidence found that m6A modification facilitated the proliferation of endometrial cancer by regulating Akt activity (Liu

et al. 2018). Other research demonstrated that m6A reader YTHDF1 boosted the development of OC via enhancing EIF3C translation.

Although less explored compared to m6A, m1A is gaining prominence as a key modifier with regulatory functions in cellular processes (Wang et al. 2023a). This modification has been linked to the modulation of mRNA stability and translation efficiency. Previous study verified that silencing m1A writer TRMT10C inhibited the proliferation and migration of ovarian cancer and cervical cancer cells suggesting close association of m1A modification and its regulators with the occurrence and development of gynecological malignancies (Wang et al. 2020a; Ye et al. 2023). It is found that m1A reader YTHDF3 can reduce the invasion and migration of trophoblast by facilitating the mRNA decay of IGF1R (Zheng et al. 2020).

In addition, m5C stands out for its involvement in maintaining RNA structure and stability with its prevalence in tRNAs, rRNAs, and mRNAs. In common cancers, disruptions in m5C patterns could influence mRNA splicing and translation, potentially contributing to the dysregulation of key oncogenic pathways (Zhang and Liu 2022; Hu et al. 2021). Exploring the impact of m5C-related lncRNA provides a nuanced perspective based on the epi-transcriptomic alterations for the prognosis of ovarian cancer (Wang et al. 2023b). It has also reported that the expression of m5C writer NSUN2 is associated with the clinical stage, tumor classification, and pathological differentiation of breast cancer, and its overexpression can increase the proliferation, migration, and invasion of breast cancer cells (Yi et al. 2017). The correlation of m5C modifications with other gynecological disease remains further exploration.

As for Ψ, studies concerning pseudouridylation on mRNA are little in female tumors. In eukaryotes, two prominent processes of pseudouracilylation function in RNA substrates. One is an RNA-independent mechanism based on pseudouridine synthases (PUSs) directly recognizing and catalyzing substrates. The other is an RNA-dependent mechanism that requires catalysis by box H/ACA RNPs based on DKC1. Nobody discovered specific "readers" and "erasers" in Ψ due to the C–C bond formed between the base and the ribose sugar is more inert than the C–N bond, making this process irreversible (Xue et al. 2022). Existing research found it can be distinctively detected in diverse human fluids due to the lack of enzymes to metabolize C-glycosyl compound in human cells. It demonstrated that excess plasma Ψ levels in ovarian cancer patients before diagnosis, suggesting that Ψ dysregulation may be associated with preclinical ovarian cancer progression (Zeleznik et al. 2020). Another findings have identified PUS7 (PUS7) as a candidate diagnostic biomarker and therapeutic target for ovarian cancer (Li et al. 2021). Excess DKC1 protein may function in RNA biosynthesis and telomerase activity in the progression of breast cancer (Montanaro et al. 2008).

The functions of these RNA modifications extend beyond mere structural changes, which intricately modulate cellular processes in tumor development and progression. From influencing mRNA fate to regulating the translation machinery, these modifications act as dynamic regulators, orchestrating the delicate balance between cell proliferation, differentiation, and survival. In gynecological cancers, it is essential to understand RNA modification for unraveling the molecular mechanisms of these diseases, and ultimately benefit for identifying novel therapeutic targets of gynecological oncology (Guo et al. 2021; Xu et al. 2023).

Exploring the intricate interplay of RNA modifications regulators in gynecological cancer

The post-transcriptional modification (PTCM) of RNA primarily involves three effectors: (i) writers for writing specific chemical groups into mRNA, which subsequently mediates mRNA modifications; (ii) readers for reading the information contained in these mRNA modifications to maintain mRNA stability and participate in RNA translation and splicing; and (iii) erasers for erasing mRNA modification signals, mediating mRNA modifications, and converting them back into unmodified nucleosides. The summary of writers, erasers, or readers of various RNA modifications was listed in Table 1. Functioning as epigenetic regulators of gene expression, dysregulation of writers, erasers, or readers exert profound influence over the complex signaling networks and molecular processes in the female reproductive system, making it a prime focus in understanding the molecular intricacies of gynecological malignancies.

m6A, the foremost and extensively investigated RNA modification, intricately intertwines with the etiology of gynecological cancers (Liu et al. 2020;

Table 1 Common writers, erasers, or readers of RNA modifications in human gynecological cancers

Category	m6A	m5C	m1A	Ψ
writers	METTL3/14/16; WTAP; VIRMA	NSUN; DNMT2	TRMT10C/61A/61B; RRP8	PUS7; DKC1
readers	YTHDF1/2/3;YTHDC1/2;IGF2BP1/2/3;HNRNPA2B1	ALYREF; YBX1; FMR1	YTHDF1/2/3; YTHDC1	unknown
erasers	ALKBH5; FTO	TET1; ALKBH1	ALKBH1/3/7; FTO	unknown

Liang et al. 2022). In general, METTL3 methyltransferase is the key catalytic subunit acting as m6A writers (Liu et al. 2014). Previous findings suggest that upregulated METTL3 lays oncogenic impact in ovarian carcinoma progression by stimulating AXL translation and EMT (Hua et al. 2018). The key mammalian demethylase ALKBH5 is regarded as m6A erasers on modification of mRNA. This demethylation activity of ALKBH5 significantly affects mRNA export and RNA metabolism as well as the assembly of mRNA processing factors in nuclear speckles (Zheng et al. 2013). As a result of its ability to stabilize BCL2 mRNA as well as promote its binding to BECN1 in ovarian cancer cells, ALKBH5 inhibits autophagy and promotes epithelial ovarian malignancy (Zhu et al. 2019). Besides, Ovarian cancer cells become aggressive as a result of ALKBH5-mediated mRNA demethylation (Jiang et al. 2020). Another mammalian demethylase FTO also keeps the dynamic balance of m6A modification. Oncogenic function of FTO is based on directly regulating the overexpression of E2F1 or Myc to promote the proliferation and migration of cervical cancer cells (Zou et al. 2019). The well-recognized m6A reader YTHDF1 is demonstrated to facilitate tumorigenesis and metastasis of ovarian cancer cells by binding to m6A-modified EIF3C mRNA (Liu et al. 2020). Things like that indicated the intricate molecular processes and interaction of writers, readers, and erasers realize the reversible feature of m6A modification, which intricately linked to the dysregulation of pivotal oncogenes and tumor suppressors, impacting diverse facets of cancer biology (Zhang and Liu 2022; Liu et al. 2023). From facilitating the translation of crucial oncogenic transcripts to modulating mRNA stability, alterations in m6A contribute intricately to the complex molecular events steering cells toward malignancy (Ma et al. 2021; Su et al. 2018).

m1A, although less explored, emerges as a regulatory nexus in the gynecological cancer development landscape. This modification is implicated in fine-tuning mRNA stability and translation efficiency, processes integral to the controlled proliferation and survival of cells. M1A and RNA modification writer-related lncRNAs function in prognosing ovarian malignancy and in reforming the immune microenvironment (Liu et al. 2021a; Ye et al. 2022). The cooperation of m1A readers, including YTH domain-containing proteins (YTHDF1, YTHDF2, YTHDF3, and YTHDC1), combined with m1A writers (TRMT10C, TRMT61B, and TRMT6/61A) and m1A erasers (ALKBH1, ALKBH3) regulate the post-transcriptional process of mRNA and ncRNAs (Dai et al. 2018). Perturbations in m1A levels are posited to disrupt these delicate balances, potentially contributing to the uncontrolled growth observed in malignancies. For example, the demethylation of m1A erasers ALKBH3 functioned in promoting the invasion of breast and ovarian cancer cells via increasing the expression and half-life of CSF-1 mRNA (Woo and Chambers 2019). m1A writer TRMT61B at 2p23.2 is a susceptibility gene in ER-negative breast cancer (Martín et al. 2023). Understanding the nuanced regulatory roles of m1A in the context of cancer development unveils novel avenues for unraveling the intricacies of disease progression (Wang et al. 2023a; Zeng et al. 2023).

M5C, ubiquitous in various RNA species, renowned for its involvement in RNA export and ribosome translation, alterations of m5C in eukaryotic tRNAs and rRNAs could influence the splicing and translation of mRNAs implicated in critical oncogenic pathways (Fang et al. 2022). m5C writers contain DNMT2 and NSUN family members. ALYREF

and YBX1 were considered as key m5C readers. The TET family and ALKBH1 are composed of the m5C erasers. Recent study investigates that m5C reader YBX1 can discern CHD3 mRNA and hold mRNA stability in ovarian cancer (Meng et al. 2024). It provides an additional layer of intricate relationship between RNA modifications and gynecological cancer development. Patients with high-expressed m5C writer NSUN2 and low-expressed IGF-II exhibited the best overall survival of ovarian cancer, suggesting the upregulation of m5C writer NSUN2 may affect ovarian cancer progress (Yang et al. 2017). Disruptions in m5C patterns may thus contribute to the dysregulation of key cellular processes, providing a molecular foothold for the development and progression of gynecological cancers.

The pseudouridylation process of Ψ refers to two routes in eukaryotes with few relative reports in gynecological cancers. RNA-independent mechanism is conducted independently by the PUSs. RNA-dependent one is closely bound up with the DKC1 gene-encoding protein. In ovarian cancer, PUS7 links to promote proliferation of tumor cells as a potential diagnostic marker (Li et al. 2021).

In essence, the nexus between RNA modifications and gynecological cancer development represents a dynamic interplay where these molecular modifications serve as pivotal regulators of gene expression networks. In a word, we will focus on exploring roles of m6A, m1A, and m5C in gynecological malignancies in this review.

Common RNA modification in the initiation and progression of specific gynecological cancers

Gynecological cancers, comprising ovarian, cervical, and uterine/endometrial malignancies, manifest as intricate interplays of epigenetic modifications such as RNA alterations. Dysregulation of RNA modifications disrupted the finely tuned balance of gene expression, necessitating a meticulous examination of specific RNA modification patterns involving in their pathogenesis.

Ovarian cancer is diagnosed at advanced stages due to the absence of specific symptoms at its early phases. The epi-transcriptomic landscape of ovarian cancer is characterized by modifications in m6A patterns. In ovarian cancer, alterations in m6A patterns have been implicated in the dysregulation of key genes involved in cell proliferation, DNA repair, and apoptosis (Liu et al. 2020; Ye et al. 2022; Xu et al. 2021). Dysfunctional m6A regulators contribute to the unchecked cell growth characteristic of ovarian malignancies. Previous sequencing results indicated that knockdown of m6A writer METTL3 decreases the m6A reader YTHDF1-mediated translation of SPRED2, contributing to increased tumor growth and metastasis (Yin et al. 2021). FBW7 induces proteasomal degradation and reverses the tumor-promoting effect of m6A reader YTHDF2 in ovarian cancer (Xu et al. 2021). Exploration of m6A dynamics in ovarian cancer holds promise for identifying molecular markers conducive to early detection and guiding tailored therapeutic interventions.

Cervical cancer, primarily associated with persistent HPV infections, manifests unique RNA modification patterns influenced by viral interactions (Hu and Ma 2018; Yuan et al. 2021). Cervical cancer mortality can be drastically reduced if a woman is tested for human papillomavirus (HPV) and cervical dysplasia (CD) by periodic inspection (Bedell et al. 2020; Goodman 2015). Specific m6A modifications may play a role in the regulation of viral oncogenes and host cell factors. Understanding the interplay between HPV infection and RNA modifications is crucial for deciphering the molecular intricacies of cervical carcinogenesis. Evidence revealed that E6/E7 proteins enhanced the proliferation and metastasis of cervical cancer cells by mediating MYC mRNA m6A modifications and activating IGF2BP2 (Hu et al. 2022). m6A eraser ALKBH5-mediated regulated the expression of PAK5 under a m6A-dependent way of m6A reader YTHDF2 promoted tumorigenesis and metastasis of cervical cancer (Huo et al. 2023). The m5C reader ALYREF bound explicitly to the m5C-labeled NDRG1 mRNA to improve stability of NDRG1 mRNA, which increased homologous recombination-mediated DNA repair in cervical cancer (Yu et al. 2024).

Uterine cancer, predominantly comprising endometrial carcinomas, is intricately linked to hormonal imbalances. Within this context, m5C modifications emerge as key players influencing mRNA stability and splicing. Diverse m5C patterns may contribute to the altered expression of genes and display cell adhesion properties in uterine cancer with a correlation with prognosis (Yang et al. 2023). m5C modification

writer NSUN2 function in stimulating the m5C modification of SLC7A11 mRNA which recognized by m5C reader YBX1, boosting lipid peroxidation and ferroptosis of endometrial cancer cells (Chen et al. 2024). Exploring the specific m5C signatures associated with uterine cancer sheds light on the epigenetic factors contributing to disease progression and potential therapeutic vulnerabilities.

As we navigate these epi-transcriptomic intricacies, we pave the way for precision medicine strategies that could revolutionize the diagnosis and treatment of ovarian, cervical, and uterine cancers.

High-throughput methodologies for detecting RNA modifications with high sensitivity

The comprehensive exploration of RNA modifications in gynecological cancer mandates a sophisticated array of experimental techniques capable of discerning subtle epi-transcriptomic alterations. High-throughput sequencing techniques, exemplified by RNA bisulfite sequencing (RNA-BS-seq), have arisen as indispensable tools for detecting RNA modifications at a transcriptome-wide scale with high sensitivity. Adapted from DNA bisulfite sequencing, RNA-BS-seq identifies methylated cytosines, like m5C, offering insights into modification patterns associated with gynecological cancers (Amort and Lusser 2017; Jian et al. 2021; Amort et al. 2017; Schaefer 2015). MeRIP-seq (methylated RNA immunoprecipitation sequencing), the other powerful technique to view the m6A location transcriptome-wide, facilitate the enrichment and sequencing of methylated RNA fragments (Bao et al. 2023; Wan et al. 2022; Zeng et al. 2018). Nevertheless, this method typically has a demand of total RNA 300 μg, limiting its application to tumors. Advancements in mass spectrometry techniques offer promising solutions to these challenges. Mass spectrometry (MS) enables the direct measurement of modified nucleotides, providing both qualitative and quantitative information (Clark et al. 2022; Amalric et al. 2022; Giessing and Kirpekar 2012; Patel and Clark 2023). Integration with liquid chromatography enhances sensitivity, enabling the discernment of modifications within complex RNA mixtures. GC–MS/MS and LC–MS/MS overcome the limitation of detecting and quantifying one more type of RNA modification at a time and simultaneously detect modified nucleosides by multiple reaction monitoring (Amalric et al. 2022). Additionally, advanced computational algorithms aid in accurately interpreting MS data, addressing challenges associated with identifying specific modification types. Due to prohibitively expensive expense for screening purposes, MS is not applicable for detecting Ψ in human biological fluids. Ψ molecularly imprinted polymer (Ψ-MIP) emerged as a straightforward Ψ detection tool to distinguish Ψ from U (Krstulja et al. 2017). These techniques afford a global perspective on RNA modification landscapes, aiding in identifying modification hotspots and alterations specific to gynecological cancers. With the development of technology, liquid biopsy will be a novel and innovative technique to replace the invasive examination. This is a vision for the future. As noted by Professor Karama, current research efforts are increasingly focusing on the development of liquid biopsy-based biomarkers derived from patients throughout the progression of their disease. Liquid biopsy offers a distinctive opportunity to monitor intercellular communication within the tumor microenvironment. Similarly, Professor Syeda Maheen Batool has highlighted that the emerging field of liquid biopsy is at the forefront of innovative diagnostic strategies for cancer and other diseases. This approach enables minimally invasive molecular characterization of cancers, facilitating diagnosis, patient stratification for therapy and long-term monitoring.

As depicted in Fig. 2, we provide an overview of RNA-seq across the transcriptome scale. In brief, researchers purify cell populations isolated from reference tissue samples and define reference gene signature matrix. Subsequently, they combine testing tumor samples with reference gene signature matrix for estimates based on deconvolution machine learning algorithm and then deconvolve cell proportions from tumor samples. MeRIP-m6A-seq provides a global view of ubiquitous RNA peaks (Liu et al. 2022), identifying more than 12,000 m6A sites in more than 7,000 mammal genes. The ability to capture modification patterns at a single-nucleotide resolution allows for a detailed exploration of the epitranscriptomic code with high sensitivity, revealing nuances that might be overlooked by traditional methods. This is also particularly critical in gynecological cancer research.

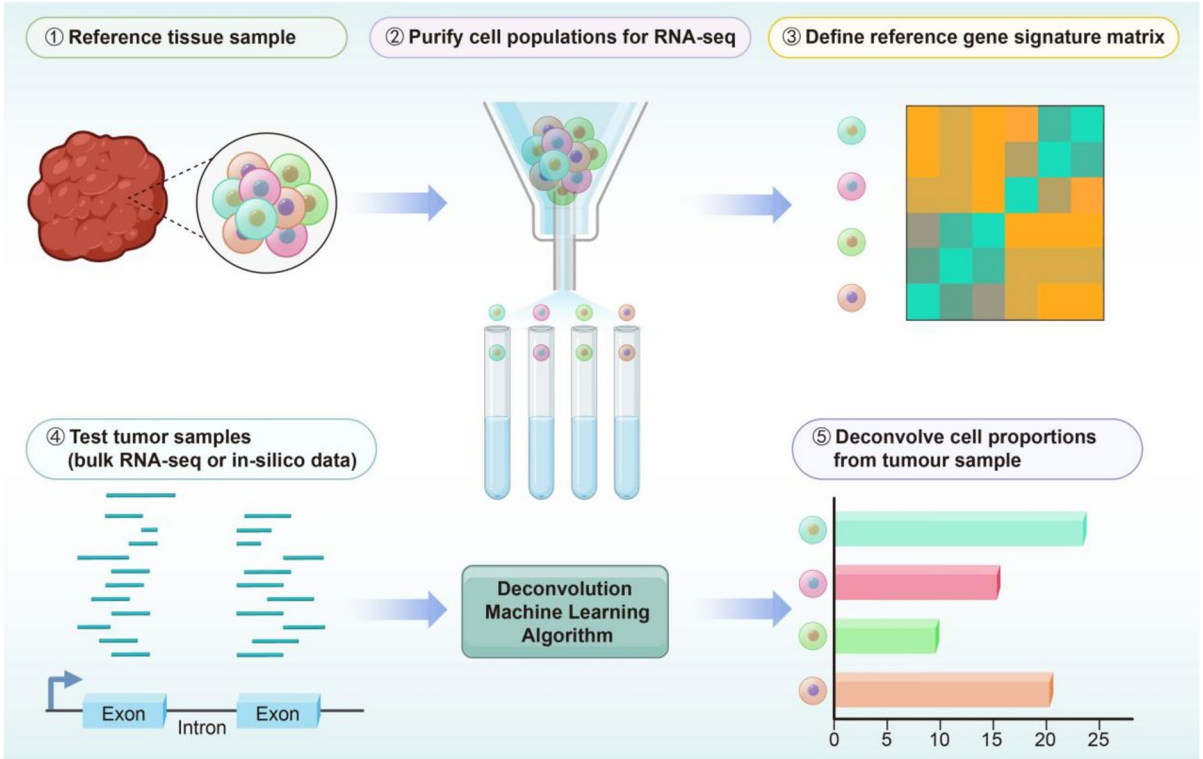

Fig. 2 Advancing Gynecological Oncology Through High-Throughput RNA Sequencing Technique. It is an overview of RNA-seq across the transcriptome scale. The first, second and third steps indicate that researchers purify cell populations isolated from reference tissue samples and define reference gene signature matrix. Fourth, combine testing tumor samples with reference gene signature matrix for estimates based on deconvolution machine learning algorithm. Fifth, deconvolve cell proportions from tumor samples

Despite notable progress in RNA modification profiling, challenges persist in accurately detecting and quantifying modification signatures. Fundamental challenges lie in the heterogeneity of RNA samples, demanding techniques capable of capturing modification patterns at a single-nucleotide resolution, particularly crucial in the context of gynecological cancers where subtle alterations may bear diagnostic or prognostic significance. The chemical diversity of RNA modifications adds complexity, requiring specific treatment and detection strategies for each modification (Ontiveros et al. 2019; Sharma and Entian 2022; He and He 2021). Distinguishing between similar modifications, such as m6A and m1A, demands high specificity in experimental approaches. In essence, the adoption of high-throughput methods marks a transformative era in gynecological cancer research.

Exploration of m6A biomarkers in gynecological cancers

Emerging m6A writers, erasers, or readers as biomarkers with diagnostic and prognostic significance

Recently, epitranscriptomic markers associated with cancers have emerged as a promising source of diagnostic and prognostic biomarkers. In the pursuit of precise prognosis or diagnosis in gynecological oncology, it is imperative to identify RNA modifications poised to serve as potential biomarkers. This endeavor is underpinned by the recognition that RNA modifications, acting as dynamic regulators of gene expression, constitute a rich source of molecular signatures for establishing an initial

diagnosis, monitoring disease evolution, and predicting response to treatment.

Notably, as the RNA modification implicated in gynecological cancers, m6A takes a prominent role in unveiling its potential as a diagnostic biomarker (Nie et al. 2021). To redefine the precision and efficacy of gynecological cancer management, we visualized the interplay among m6A writers, erasers, or readers in Fig. 3 and Table 2. In brief, the m6A writers are mainly composed of METTL3, METTL14 and their cofactor WTAP, etc. We generally considered FTO and ALKBH5 as mammalian RNA demethylases that catalyze the removal of the m6A modification on mRNA. Besides m6A writers and erasers, another indispensable group in m6A is called "readers". YTH domain-containing proteins such as YTHDC1, YTHDC2, YTHDF1, YTHDF2 were prone to bind directly to m6A and to stimulate RNA translation or increase mRNA stability as mRNA readers. m6A reader HNRNPC and HNRNPA2B1 are considered

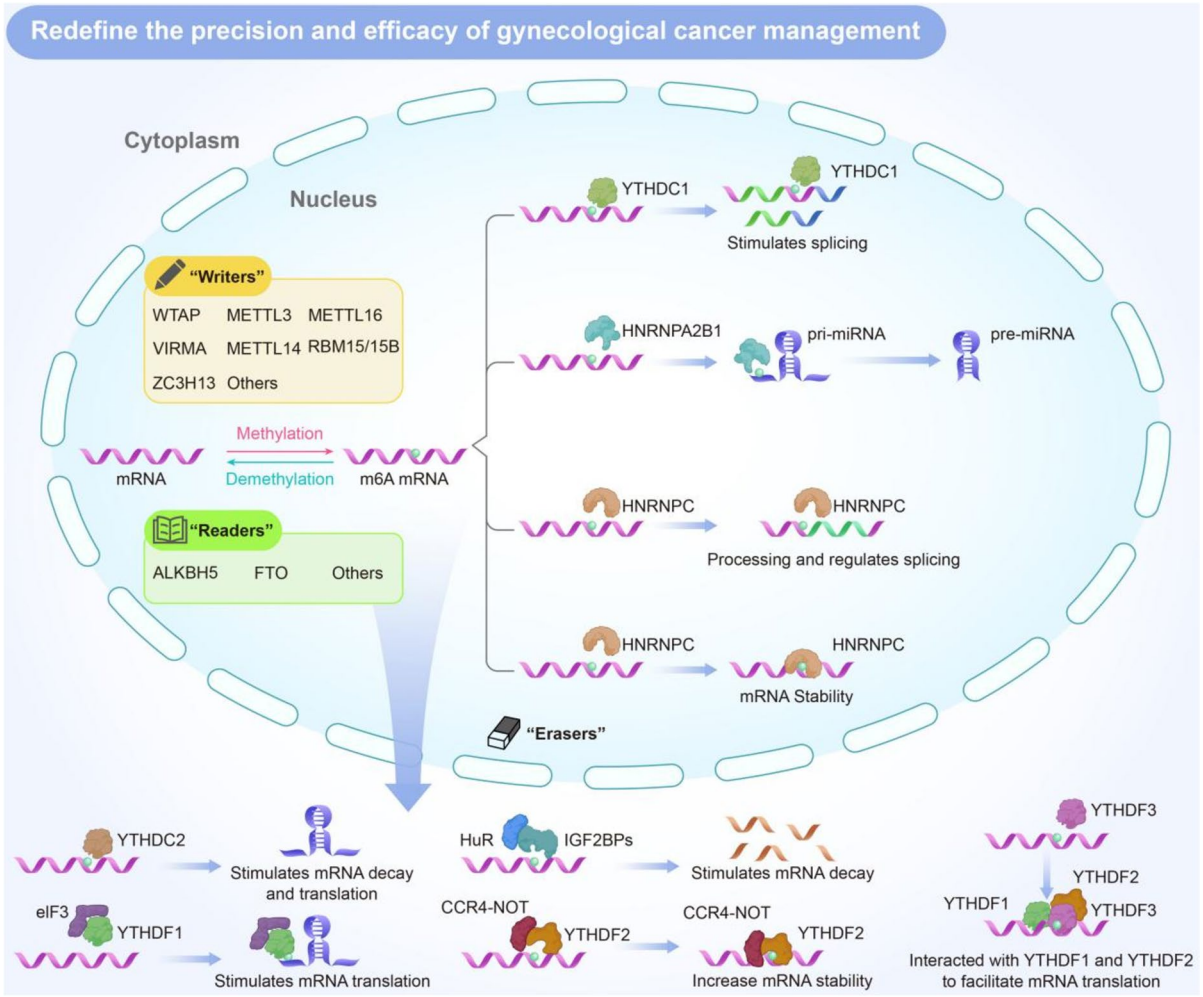

Fig. 3 Redefine the Precision and Efficacy of Gynecological Cancer Management. m6A mRNA methylation is orchestrated by three main classes of proteins: methyltransferases ("writers"), demethylases ("erasers"), and m6A-binding proteins ("readers"). Methyltransferases, such as METTL3/14, WTAP, VIRMA, ZC3H13, and RBM15/15B, predominantly catalyze the addition of m6A modifications to mRNA. Conversely, demethylases, including FTO and ALKBH5, facilitate the removal of m6A modifications from bases. The primary role of m6A-binding proteins is to recognize m6A-modified sites and subsequently activate downstream regulatory pathways, including RNA degradation and microRNA (miRNA) processing. The binding of m6A sites to different readers mediates distinct functional outcomes

Table 2 The main function of common m6A readers

m6A readers	Function
YTHDC1	stimulate mRNA splicing
YTHDC2	stimulate mRNA decay and translation
HNRNPC	impact processing and regulate splicing
HNRNPC	combined with mRNA to promote stability
HNRNPA2B1	promote the generation of pre-miRNA
YTHDF1	interacted with eIF3 to stimulate mRNA translation
YTHDF2	interacted with CCR4-NOT to increase mRNA stability
IGF2BPs	interacted with HuR to stimulate mRNA decay
YTHDF3	interacted with YTHDF1 and YTHDF2 to facilitate mRNA translation

as RNA-binding proteins, involving in pre-mRNA splicing (Sun et al. 2022). Another reader IGF2BP2 was demonstrated to stabilize MEIS2 and GATA6 mRNA, thereby facilitating the proliferation, migration, and invasion of ectopic endometrial stromal cells (Zhao et al. 2022). In humans, they widely function in multiple processes including precursor mRNA (pre-mRNA) splicing, mRNA translation, stability, and decay. The detail interplay of common m6A regulators in gynecological oncology was summarized in Table 2.

The dysregulation of m6A writers, erasers, or readers has been correlated with the aberrant expression of key genes linked to initiation and progression of gynecological cancer (Pang et al. 2021). As a result, three gene signatures concerning VIRMA, YTHDF3, and IGF2BP1 serve as robust prognostic indicators for predicting survival outcome of endometrial cancer with high accuracy ($p < 0.05$), and consistently increasing expression of IGF2BP2 was found through stage I to stage IV of endometrial cancer (Feng et al. 2021). IGF2BP3, as a potential oncogene, reduced disease-specific survival of endometrial and ovarian clear cell carcinoma with prognostic significance (Köbel et al. 2009; Fadare et al. 2013). Recent studies revealed that m5C-modified gene signature functions well in prognosis of cervical cancer (Yu et al. 2021). The presence of these modifications has been linked to distinct clinical outcomes, offering insights into disease progression and patient survival. Future perspectives encompass the validation of these reliable biomarkers in larger patient cohorts, the development of standardized detection methodologies, and the exploration of targeted therapeutic interventions guided by the distinct epitranscriptomic profiles of individual patients.

Specificity of m6A biomarkers for predicting different gynecological cancers

As dynamic regulators of gene expression, RNA modifications exhibit the potential to capture subtle alterations indicative of early disease stages. m6A regulators have demonstrated remarkable specificity as a diagnostic biomarker, discerning nuanced modification patterns associated with diverse cancer subtypes. In Fig. 4, we illustrated vital m6A writers, erasers, and readers in the progress of ovarian cancer, cervical cancer, and endometrial cancer, respectively, combining with their diverse molecular pathways. The specificity of RNA modification biomarkers based on distinctive substates and corresponding signal pathways ensures their precision in delineating between different gynecological cancer types and distinguishing malignant from benign conditions (Liu et al. 2021b), allowing for the development of biomarker panels.

Challenges persist in achieving optimal sensitivity and specificity, including variability in sample types and the need for standardized detection methodologies. Ongoing efforts in refining experimental techniques, such as RNA sequencing and mass spectrometry, aim to enhance the specificity of biomarkers by reducing false-positive rates. Additionally, the integration of multi-omics approaches, combining RNA modifications with genetic and proteomic data, holds promise in refining the specificity of biomarkers (Luo et al. 2022; Wang et al. 2020b).

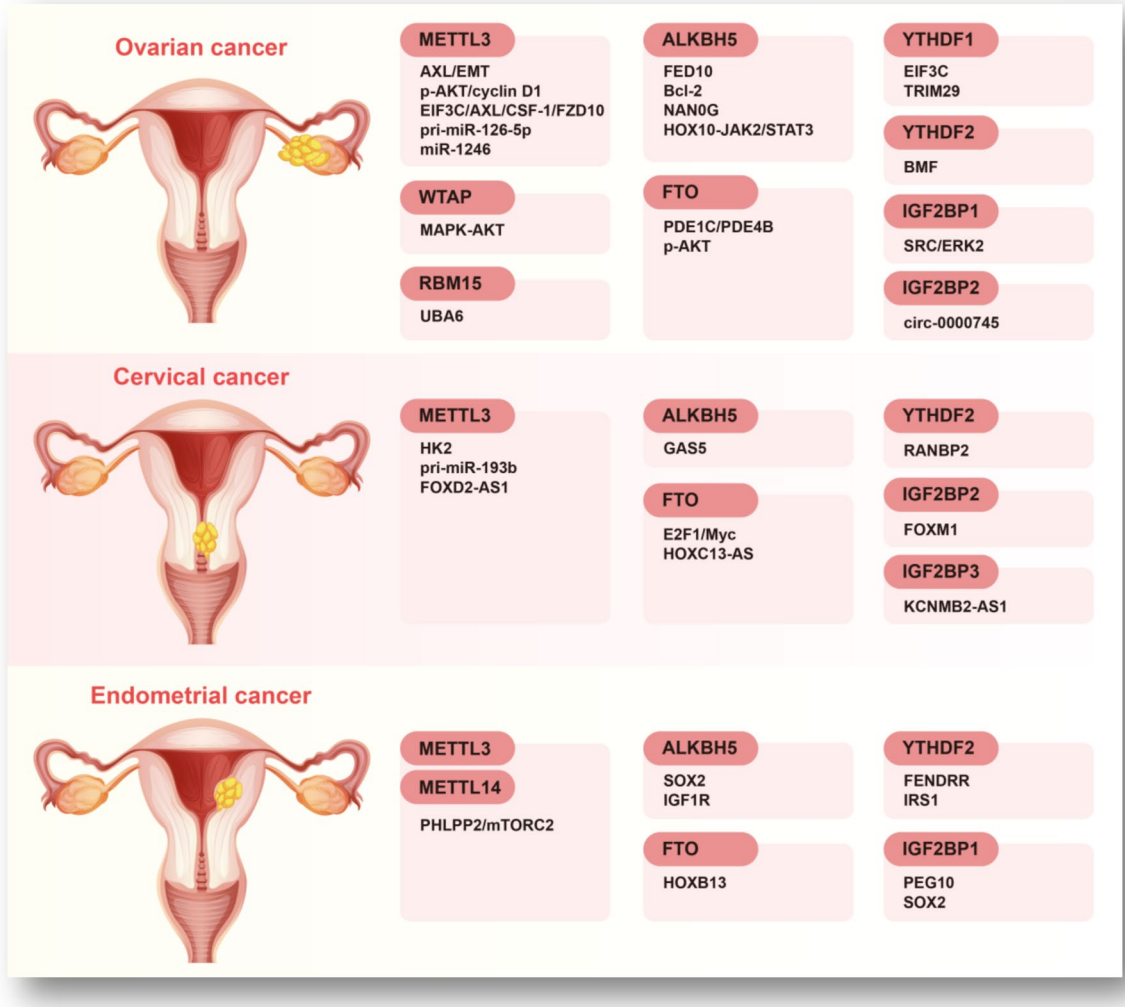

Fig. 4 Unraveling m6A Regulators in Specific Gynecological Cancer, Comprising of Ovarian, Cervical, and Endometrial cancer. m6A writers (the left column), erasers (the middle column), and readers (the right column) functioned in diverse signal pathways in specific gynecological cancers

Mechanistic insights into m6A regulators-mediated in gynecological cancer progression

In the intricate landscape of gynecological cancers, RNA modifications, particularly m6A, exert significant influence over key molecular pathways governing disease initiation and progression. The dysregulation of m6A modifications intricately affects critical signaling cascades. Differences in molecular signaling pathways are shown in Fig. 4. For example, in ovarian cancer, the maturation of miR-126-5p mediated by METTL3 motivate cellular proliferation of ovarian cancer cells by PTEN-related PI3K/Akt/mTOR pathway (Bi et al. 2021). Yet, in cervical cancers, m6A writer METTL3 upregulated the proliferation and aerobic glycolysis of tumor cells by targeting HK2 mRNA region, contributing to the molecular landscape governing carcinogenesis (Wang et al. 2020c). As for endometrial cancer, either mutation of METTL14 or the low expression of METTL3 led to the reduction of m6A methylation in most endometrial cancer patients, which induced downtranslation of negative regulator PHLPP2, and upregulation of the positive regulator mTORC2, finally

enhancing the proliferation of cells by the AKT signaling pathway (Liu et al. 2018). Additionally, in metastatic endometrial carcinoma, FTO catalyzes the demethylation of the 3'-UTR region of HOXB13 mRNA, thereby abolishing the recognition of m6A methylation by YTHDF2. This reduction in mRNA attenuation leads to increased expression of HOXB13, which subsequently activates the Wnt signaling pathway, thereby facilitating tumor invasion and metastasis (Zhang et al. 2021). In summary, the exploration of these epitranscriptomic regulators within the context of gynecological cancers not only deepens our understanding of disease mechanisms but also opens new frontiers for the development of targeted therapeutic strategies (Ni et al. 2020).

Challenges and future directions

The incorporation of RNA modifications as biomarkers in gynecological cancer diagnostics confronts multifaceted challenges and limitations. A formidable hurdle arises from the inherent heterogeneity within tumor samples, where distinct RNA modification patterns across various cancer subtypes and individual patients introduce complexities in biomarker identification. The absence of standardized methodologies for RNA modification detection exacerbates this issue, fostering variability among studies and impeding result reproducibility. The dynamic nature of RNA modifications necessitates advanced techniques capable of capturing real-time changes, thereby adding an additional layer of complexity to the quest for biomarker discovery. Moreover, the potential crosstalk between different modifications and the intricate interplay between genetic and epitranscriptomic factors further contribute to the challenge of accurately interpreting biomarker signatures.

Concerted research initiatives are currently underway to surmount these challenges and facilitate the effective utilization of RNA modifications as reliable biomarkers in gynecological cancers. Methodological standardization efforts are actively employing sophisticated techniques, such as high-throughput sequencing and mass spectrometry. Collaborative endeavors are addressing heterogeneity by expanding sample cohorts and conducting multi-omics analyses, integrating RNA modification data with genetic and proteomic information. Furthermore, the development of computational algorithms capable of deciphering complex modification patterns and discerning subtle differences between similar modifications shows promise in refining biomarker specificity. Ongoing research endeavors are exploring innovative approaches to capture real-time changes in RNA modifications, providing dynamic insights into disease progression and treatment response.

Notwithstanding prevailing challenges, the future prospects of harnessing RNA modifications as biomarkers in gynecological cancers are auspicious. As ongoing research refines methodologies with high sensitivity and unveils the intricate dynamics of epitranscriptomic regulation, the identification of robust biomarker panels exhibiting high specificity looms on the horizon. These biomarkers carry potential clinical applications encompassing early detection, precise diagnosis, prognostic stratification, and monitoring treatment response. The evolution of non-invasive techniques, exemplified by liquid biopsy-based assays, further amplifies the translational potential of RNA modification biomarkers in routine clinical practice. Precision medicine strategies, guided by the distinctive epitranscriptomic profiles of individual patients, are poised to revolutionize the gynecological cancer management landscape, offering bespoke interventions that optimize therapeutic efficacy and enhance patient outcomes.

Discussion and conclusion

The investigation into dysregulation of RNA modifications such as m6A, m5C, and m1A has emerged as a transformative frontier, providing profound insights into the intricate landscape of gynecological cancers (Fig. 5). The intricate interplay between RNA modifications and key molecular pathways governing cancer cell proliferation, invasion, and metastasis underscores their pivotal role in shaping the dynamics of disease. Extensively studied m6A regulators showcases obvious diagnostic specificity in distinguishing between different cancer subtypes and providing a molecular fingerprint for precise diagnosis. As indicated in Fig. 5, the interaction of m6A patterns like writers, erasers, and readers, are notably linked to aberrant signaling cascades, such as Wnt and PI3K/Akt/mTOR, thus furnishing a molecular foundation for therapeutic exploration of ovarian cancer, cervical

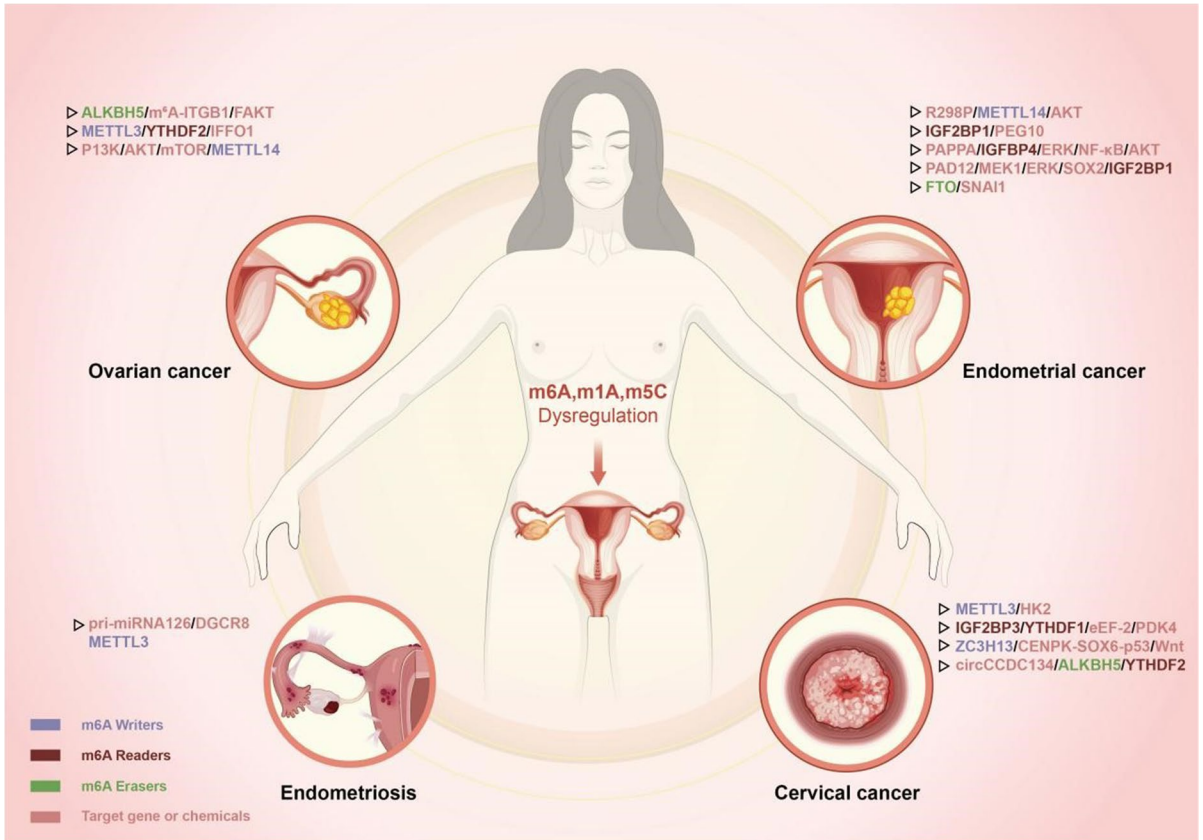

Fig. 5 Aberrant signaling cascades of m6A Writers, Erasers, and Readers in Gynecological Cancers. The interaction of m6A patterns like writers, erasers, and readers, are notably linked to aberrant signaling cascades, such as Wnt and PI3K/Akt/mTOR, thus furnishing a molecular foundation for therapeutic exploration of ovarian cancer, cervical cancer, and endometrial cancer

cancer, and endometrial cancer. These dynamic epitranscriptomic alterations with priority of m6A, demonstrate a multifaceted impact on diverse facts of cancer biology, thereby unraveling their potential as promising biomarkers in gynecological oncology. Additionally, m1A and m5C demonstrate prognostic significance, also impacting clinical outcomes and shedding light on disease severity.

The biomarkers for early detection are crucial, particularly in gynecological cancers where timely intervention significantly influences patient outcomes. We listed common liquid biopsy-derived biomarkers for prognosis of ovarian cancer in Table 3. It is well-known that CA-125 is the most representative tumor marker for early detection for ovarian cancer patients which could detect 66.5% ovarian cancers (95% CI 49.5–58.4) under 22 U/mL (Burki 2015). However, CA-125 is demonstrated to have relatively low sensitivity (50%-62%) for detecting early-stage ovarian cancer and limited by a low specificity (around 75%) (Funston et al. 2021; Ghaemmaghami and Akhavan 2011). Overexpression of HE4 as another diagnosis biomarker is detected in ovarian tumors with a specificity of 96% and a sensitivity of 67% (Li et al. 2009). CA125 has been demonstrated to have a higher sensitivity than HE4 in a late-stage ovarian tumors (90.8% vs. 56.9%), but HE4 performed better than CA125 with respect to the specificity (96.9% vs. 67.1%) (Hamed et al. 2013). It has been reported that ovarian cancer would require a sensitivity of greater than 75% and a specificity of at least 99.6%, suggesting the significance of serum-derived biomarkers and liquid biopsy for detecting gynecological cancers. Liquid biopsy strategies referred to detecting and monitoring

Table 3 Diagnostic performance of common biomarkers for ovarian cancer cited in this article

Biomarkers	Specificity (%)	Sensitivity (%)	AUC
CA125	~75	50~62	/
HE4	96	67	/
miR-200a-3p	83	84	0.89
miR-200c-3p	66	75	0.77
miR-205	78	66	0.72
methylated HOXA9 and HIC1	100	88.9	0.95
circulating ctDNA	/	/	0.89

of circulating tumor cells, cell-free genetic molecules, and extracellular vesicles. Except for CA125 and HE4, other genetic biomarkers emerged with high specificity and sensitivity for detecting ovarian cancer. Peer researchers conducted an analysis of the DNA methylation status of HOXA9 and HIC1 in ovarian cancer, revealing that the combination panel exhibited a sensitivity of 88.9%, a specificity of 100%, and AUC of 0.95 for distinguishing ovarian cancer from healthy individuals (Singh et al. 2020). Furthermore, it was determined that circulating ctDNA demonstrated a performance comparable to the CA125 and HE4 biomarkers, with AUCs of 0.8958, 0.883, and 0.899, respectively (Li et al. 2019). A recent study also identified that miR-200a-3p (sensitivity: 84%, specificity: 83%) and miR-200c-3p (sensitivity: 75%, specificity: 66%) exhibited relatively high diagnostic efficacy for ovarian cancer (Teng et al. 2016). miR-205 possessed an AUC of 0.715, a sensitivity of 66.7%, and a specificity of 78.1% for predicting ovarian cancer. Once miR-205 combined with CA125 or HE4, it found that an AUC of 0.951 and a sensitivity and specificity of 100% and 86.1% in early detection for ovarian cancer (Zhu et al. 2022). In this article, we reviewed and discussed aberrant m6A RNA modification patterns in OC tissues, which contributed to ovarian tumorigenesis and emerged as promising potential diagnostic biomarkers for OC. The detection of specific m6A-modified RNA transcripts in blood or tissue samples may enhance early detection of OC, which helps to improve patient outcomes through timely intervention. m6A regulators holds promise for representing subtle molecular alterations indicative of early disease stages, which mainly focus on laboratory level and fail to apply into clinical practice due to an indefinite predictive sensitivity and specificity value at present. As for cervical cancer, Pap test has been the gold standard for several decades due to its high specificity, but it also has poor reproducibility due to cytological alterations (Chantziantoniou et al. 2017). HPV testing relies on the detection of the virus or effects of the viral infection avoiding against morphologic interpretation bias (Bhatla and Singhal 2020). Compared to Pap test and HPV testing, DNA methylation tests show higher specificity than cytology and higher sensitivity than tests relying on HPV16/18 genotyping, constituting important candidates for triage tests due to its objectivity and capabilities for risk stratification (Chrysostomou and Kostrikis 2020). Hence, there is an urgent need to conduct much more experiments to apply RNA modification to early detection of gynecological cancers.

The identification of specific modification patterns associated with different cancer types offers a tool for risk assessment, enabling clinicians to stratify patients based on their epi-transcriptomic profiles. The potential impact of RNA modifications extends to the realm of personalized medicine in gynecological oncology. The dysregulation of m6A machinery, particularly, offers a target-rich environment for the development of precision medicine strategies. In the future, small molecules designed to modulate m6A levels or inhibit specific components of the m6A regulatory network hold promise for disrupting cancer progression. The integration of RNA modification data into the broader landscape of patient-specific omics information heralds a new era in personalized treatment planning.

Despite the promising potential, challenges and limitations in utilizing RNA modifications as biomarkers in gynecological cancers persist. Heterogeneity within tumor samples, lack of standardized detection methodologies, and the dynamic nature of RNA modifications pose challenges in achieving optimal sensitivity and specificity. Ongoing research endeavors are actively addressing these challenges.

Standardization efforts, advanced detection methodologies, and collaborative initiatives leveraging multi-omics approaches aim to refine biomarker identification and interpretation. Computational algorithms capable of deciphering complex modification patterns further contribute to overcoming existing limitations, paving the way for the clinical translation of RNA modification biomarkers.

The future prospects of RNA modifications as biomarkers in gynecological cancers are promising and transformative. As ongoing research refines methodologies with high sensitivity and elucidates the intricate dynamics of epi-transcriptomic regulation, robust biomarker panels with high specificity are on the horizon. The potential impact on early detection, risk assessment, and personalized medicine positions RNA modifications at the forefront of gynecological cancer research. Precise medicine strategies, guided by the unique epi-transcriptomic profiles of individual patients, hold the potential to revolutionize the landscape of gynecological cancer care. The integration of RNA modifications into routine clinical practice is not merely a possibility but a promising reality that will redefine diagnostic accuracy, prognostic precision, and therapeutic efficacy in the personalized management of gynecological cancers. The epitranscriptomic revolution is underway, charting a course towards a new era in the understanding and treatment of gynecological malignancies.

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Declarations

None.

Competing interests The authors declare no competing interests.

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