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Review

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HENMT1: an RNA methyltransferase in biology and disease

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Abstract: HEN methyltransferase 1 (HENMT1) is an RNA methyltransferase that catalyzes 2'-*O*-methylation of piRNAs within the small RNA silencing pathway and methylates miRNAs. By stabilizing these RNA molecules, HENMT1 plays a pivotal role in regulating the biological processes they target, thereby maintaining cellular homeostasis. Mutations in *HENMT1* homologs across various species have been shown to cause altered biological traits and impaired reproduction. *HENMT1* mutations have been linked to infertility and tumor development in humans. This comprehensive review first introduces the structure and function of HENMT1 and its homologs, focusing on elucidating the piRNA methylation process. Next, we examine the aberrant expression of HENMT1 in human cancers and its relationship with immune infiltration through analysis of The Cancer Genome Atlas (TCGA) database and tumor immune infiltration profiling, providing insights into the dysregulation of HENMT1 in diseases such as infertility and cancer. Finally, we discuss current knowledge and future perspectives on the function of HENMT1 in cancer progression.

Key words: HEN methyltransferase 1 (HENMT1); RNA methyltransferase; 2'-*O*-methylation; Tumor development; Infertility

1 Introduction

Recent studies have highlighted the pivotal role of epigenetics in cancer development, with RNA modifications emerging as a crucial component of this regulatory landscape. These modifications serve as precise and efficient mechanisms for modulating RNA splicing, stability, and translation (Barbieri and Kouzarides, 2020), and occur across a wide spectrum of RNA types, including messenger RNAs (mRNAs), small interfering RNAs (siRNAs), microRNAs (miRNAs), transfer RNAs (tRNAs), ribosomal RNAs (rRNAs), and long non-coding RNAs (lncRNAs), among others. To date, more than 100 distinct RNA modifications have been identified, with methylation accounting for over half of them (Yang et al., 2021; Orsolic et al., 2023). Methylation of various RNA species has been shown to play essential roles in key biological processes, such as cell proliferation, metastasis, apoptosis, and immune responses. Consequently, exploring RNA methylation offers valuable insights into the functional significance of RNA modifications in cancer progression.

2'-*O*-methylation, a specific type of RNA methylation, enhances RNA stability by shielding molecules

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from nuclease degradation, modulating their interactions with other RNAs and proteins, and influencing various cellular processes, including epigenetic gene regulation (Dimitrova et al., 2019). HEN Methyltransferase 1 (HENMT1), the enzyme responsible for catalyzing 3'-terminal 2'-*O*-methylation within the small RNA silencing pathway, specifically targets PIWI-interacting RNAs (piRNAs) and miRNAs in humans. Notably, HENMT1 has been implicated in the regulation of human infertility and oncological disorders (Xiong and Zhang, 2023). This review will summarize HENMT1's structure, its biological functions, and its associations with infertility and cancer.

2 HENMT1 and its homologs

HENMT1 is a conserved RNA methyltransferase, responsible for methylating the 2'-OH group at the 3'-terminal nucleotide of small RNAs. Initially identified in *Arabidopsis thaliana*, this enzyme has since been found in a range of eukaryotic organisms, including mice, zebrafish, and *Drosophila* (Ji and Chen, 2012). Furthermore, a bacterial homolog, Hen1, has been linked to RNA repair processes in conjunction with polynucleotide kinase-phosphatase (Pnkp) (Chan et al., 2009).

HENMT1 and its homologs are critical for the maturation and stability of various small RNA species. In animals, HENMT1 was first identified for its essential role in catalyzing the addition of 2'-*O*-methylation to the 3'-ends of piRNAs, which are predominantly expressed in the germline. This methylation process serves as a crucial defense mechanism, protecting piRNAs from degradation and preserving their functional integrity and longevity (Lim et al., 2015; Hempfling et al., 2017). piRNAs play key roles in transposon silencing and genome defense; HENMT1-mediated methylation enhances their stability and facilitates interactions with PIWI proteins, ultimately safeguarding genome integrity (Horwich et al., 2007; Kirino and Mourelatos, 2007; Kurth and Mochizuki, 2009). Recent studies have also revealed that HENMT1 modifies mature miRNAs in animals, including humans, to promote their stability (Abe et al., 2014; Modepalli et al., 2018; Liang et al., 2020). In plants, HEN1 (Hua Enhancer 1), a homolog of HENMT1, catalyzes the 3'-terminal 2'-*O*-methylation of double-stranded siRNAs and miRNAs, a modification essential for their stability and functionality. This methylation protects these small RNAs from degradation, ensuring they play their proper roles in RNA silencing pathways (Li et al., 2005; Yu et al., 2005). In bacteria, the Hen1 homolog participates in the 3'-terminal 2'-*O*-methylation of damaged RNA in collaboration with Pnkp during RNA repair. This modification enables the recognition of repaired RNA and shields it from further damage, preserving bacterial genome fidelity and supporting proper cellular function (Chan, et al., 2009; Wang et al., 2012).

Overall, HENMT1 and its homologs are indispensable for the modification and stabilization of small RNAs across a broad spectrum of organisms. They play pivotal roles in a variety of biological processes, including gene silencing, genome defense, and RNA repair.

3 Biological function of HENMT1

3.1 Structure of HENMT1

HENMT1 and its homologs are categorized into four classes based on their domain arrangements, and all share a conserved methyltransferase (MTase) domain. Class I proteins are relatively large and contain several distinct domains, with the MTase domain located at the C terminus. Class II and III proteins feature C-terminal (CTD) and N-terminal (NTD) domains, respectively, derived from the MTase domain. Class IV proteins consist solely of the MTase domain. However, as the fourth class has not yet been reported, this discussion will focus on the other three: those found in animals (represented by humans), plants (represented by *Arabidopsis thaliana*), and bacteria (represented by *Anabaena variabilis*) (Huang, 2012).

While HENMT1 possesses a conserved MTase domain across various species, its structural features differ significantly in animals, plants, and bacteria (Peng et al., 2018). In animals, HENMT1 exhibits a compact structure primarily comprising an N-terminal MTase domain and a CTD. Notably, this structure is smaller than its counterpart in plants, reflecting distinct functional requirements and evolutionary adaptations between plant

and animal cells (Fig. 1a). The N-terminal MTase domain and the upstream FXPP motif are critical for HENMT1's catalytic activity, particularly in the methylation of small RNAs and the regulation of RNA stability. Recent studies have shown that a truncated human HENMT1 containing only the MTase domain is catalytically inactive but regains full methyltransferase activity when an FXPP motif is present at the N-terminus of the MTase domain. Mechanistically, the FXPP motif is essential for RNA substrate binding and facilitates the methylation process (Peng, et al., 2018) (Fig. 1b). However, the structure and function of the CTD in human HENMT1 remain unexplored. Insights from zebrafish suggest that the CTD plays a pivotal role in localizing Hen1 within the nuage, a perinuclear region involved in piRNA biogenesis (Kamminga et al., 2010). This raises the possibility that the CTD in human HENMT1 might regulate interactions with PIWI proteins, facilitating the generation and 3'-terminal 2'-*O*-methylation of piRNAs.

Unlike its animal homolog, HENMT1, which primarily processes single-stranded piRNAs, the plant HEN1 predominantly targets double-stranded RNAs, including siRNAs and miRNAs. This enzyme is notably larger. Its sequence in *Arabidopsis thaliana* consists of 942 amino acids, and it comprises five distinct structural regions: two double-stranded RNA-binding domains (dsRBD1 and dsRBD2), a La motif-containing domain (LCD), a peptidyl isomerase-like domain (PLD), and a C-terminal MTase domain (Huang et al., 2009) (Fig.1a). The C-terminal MTase domain is responsible for catalyzing 2'-*O*-methylation, while the four N-terminal domains are critical for recognizing and binding specific siRNAs and miRNAs, ensuring the enzyme's precise and effective activity.

In bacteria, the Hen1 protein exhibits a streamlined structure, comprising an NTD and a conserved MTase domain at its C-terminus (Jain and Shuman, 2010; Wang, et al., 2012) (Fig.1a). Unlike its eukaryotic counterpart, HENMT1, which regulates RNA silencing pathways, bacterial Hen1 plays a pivotal role in RNA repair. Specifically, it collaborates with the protein Pnkp through its NTD to facilitate the 3'-terminal 2'-*O*-methylation of repaired RNAs. Given that the NTD of Hen1 is linked to Pnkp and is required for RNA ligation, Wang et al. designated this domain the ligase-activating domain (Wang, et al., 2012). This modification safeguards the RNAs from subsequent cleavage at the same site, ensuring their stability and functionality.

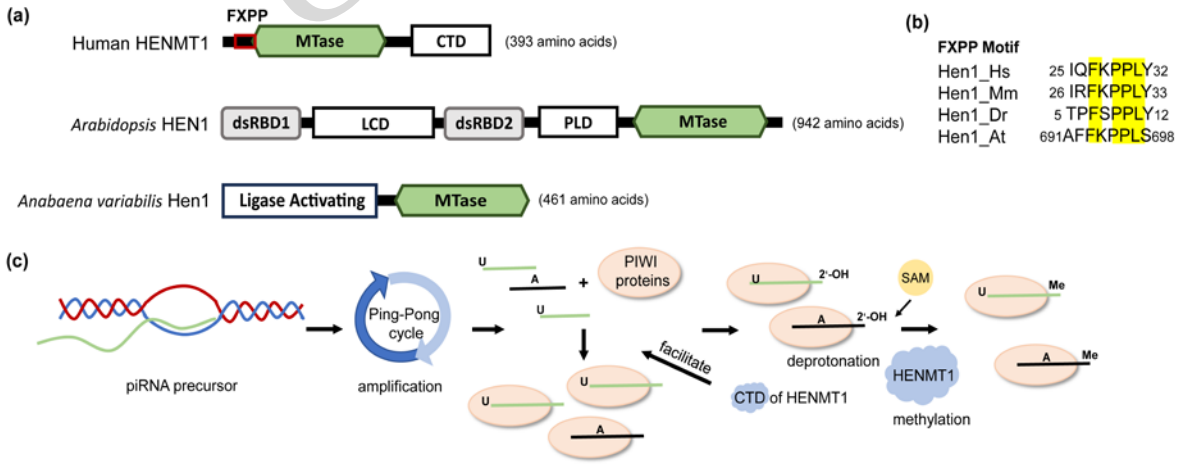

Fig. 1 Domain architecture of HENMT1 homologs and the process of 2′-*O*-methylation of piRNAs by HENMT1. (a) Schematic illustration of the domain structures of human HENMT1, *Arabidopsis* HEN1, and *Anabaena variabilis* Hen1, highlighting the FXPP motif enclosed in a red box. (b) Comparative alignment of FXPP local sequences across various species, with identical residues highlighted in yellow boxes. Abbreviations: Hs (*Homo sapiens*), Mm (*Mus musculus*), Dr (*Danio rerio*), At (*Arabidopsis thaliana*). (c) Functional depiction of human HENMT1 mediating piRNA 2′-*O*-methylation in germ cells, with ovals representing different Argonaute proteins. Me: 2′-*O*-methylation.

3.2 Process of HENMT1 methylation

In mammals, HENMT1 plays an essential role in the 3'-terminal 2'-*O*-methylation of piRNAs, a critical step in their maturation. piRNAs, processed by PIWI proteins from piRNA precursors into single-stranded RNAs of 23–31 nucleotides, undergo amplification through the ping-pong cycle, a mechanism involving reciprocal interactions between piRNAs and PIWI proteins (Wang et al., 2023). HENMT1 recognizes and methylates piRNAs at their 3'-terminal through its interaction with PIWI proteins. The 2'-*O*-methylation process at the 3'-terminal occurs in two stages: (1) the deprotonation of the 2'-OH group and (2) the transfer of a methyl group from S-adenosylmethionine (SAM) to the deprotonated 2'-OH group, as demonstrated in Kaldis's study (Kaldis and Zhao, 2024). Additionally, the CTD of HENMT1 may facilitate the interaction between piRNAs and PIWI proteins, ensuring the coordinated production and efficient methylation of piRNAs (Huang, 2012) (Fig. 1c).

Unlike other RNA 2'-*O*-methyltransferases, HENMT1 catalyzes 2'-*O*-methylation through a mechanism that depends on a metal ion at its active site. In animals, HENMT1 demonstrates a preference for Mn^{2+} over Mg^{2+}, a characteristic shared with bacterial enzymes (Huang, 2012; Peng, et al., 2018). In contrast, plants require Mg^{2+} rather than Mn^{2+} for this process (Huang, et al., 2009). HENMT1's crystal structure reveals that four specific amino acids in humans —Glu132, Glu135, His136, and His181—within the Mtase domain are critical for metal ion binding (Huang, 2012; Peng, et al., 2018).

3.3 Function of HENMT1 and its homologs

Transposable elements (TEs) are mobile DNA elements capable of relocating within the genome, which can result in mutations or disruptions in gene regulation if not properly repressed (Wang et al., 2023). A critical function of the PIWI–piRNA complex is suppressing TE expression in animal germlines. In mammals, HENMT1 plays an essential role in catalyzing the 2'-*O*-methylation of the 3'-terminal nucleotide of piRNAs. HENMT1 dysfunction destabilizes piRNAs, as evidenced by their reduced quantity and altered length. This destabilization severely compromises the ability of the PIWI–piRNA complex to silence TEs, leading to genomic instability, infertility, and even the development of cancer (Lim, et al., 2015; Wang, et al., 2023).

In plants, HEN1-mediated 3'-terminal methylation is a common process that stabilizes both miRNAs and siRNAs by protecting their 3'-ends from uridylation (Kirino and Mourelatos, 2007). Mutations in *HEN1* lead to pleiotropic developmental defects, such as reduced organ size (Chen et al., 2002) (Table 1). In animals, mutations in *HENMT1* result in shortened and reduced piRNA levels. This has been well-documented, with *Henmt1* mutations shown to cause reduced fertility (Lim, et al., 2015). The *Drosophila* Hen1 homolog (DmHen1) is involved in processing both Argonaute 2 (Ago2)-bound single-stranded siRNAs and piRNAs (Ameres et al., 2010). Loss of DmHen1 function leads to shorter piRNAs, decreased abundance, and impaired functionality (Horwich, et al., 2007). Additionally, *DmHen1* mutations, which eliminate the 2'-*O*-methylation of miRNAs, accelerate neurodegeneration and reduce lifespan (Abe, et al., 2014). Similarly, the HENMT1 homolog in zebrafish (Hen1) is essential for piRNA methylation in germline cells and plays a critical role in oocyte development. The absence of *hen1* in zebrafish causes piRNA degradation by exonucleases, leading to oocyte depletion and female infertility (Kamminga, et al., 2010). In *Caenorhabditis elegans*, the HENMT1 homolog HENN-1 specifically methylates siRNAs and piRNAs associated with the PIWI branch of Argonautes (Montgomery et al., 2012). *henn-1* mutations destabilize piRNAs and 26G RNAs, a specific class of primary endogenous siRNAs, resulting in dysregulated target mRNA expression, fertility impairments, and increased somatic RNAi activity (Billi et al., 2012; Kamminga et al., 2012; Svendsen et al., 2019). In bacteria, Hen1 functions in RNA repair by forming a complex with Pnkp and catalyzing 2'-*O*-methylation of the 3'-terminus, rendering the repaired RNA resistant to future cleavage at the same site (Wang, et al., 2012) (Table 1).

Table 1 HENMT1 homologs and their biological function

Name	Organism	Substrates	Biological consequences upon mutation	Reference
HENMT1	Human	miRNAs and piRNAs	Decreased piRNA as well as miRNA stability; transposons active and predisposed to infertility and cancer.	(Lim, et al., 2015; Wang, et al., 2023)
HEN1	*Arabidopsis*	miRNAs and siRNAs	Aberrant lengths and decreased levels of small RNAs; pleiotropic development defects.	(Chen, et al., 2002; Kirino and Mourelatos, 2007)
Henmt1	Mouse	piRNAs	piRNA instability, reductions to piRNA volume and length; developmental arrest of germ cells during the process of spermatogenesis, reduced ovarian follicular reserve, altered transcriptome in oocytes, and so on.	(Lim, et al., 2015; Hutt, et al., 2021)
DmHen1	*Drosophila*	piRNAs, Ago2-associated small RNAs	Deletion of Nm in piRNA and siRNA, accelerates neurodegeneration and shortens lifespan.	(Horwich, et al., 2007; Ameres, et al., 2010; Abe, et al., 2014)
Hen1	Zebrafish	piRNAs	A decrease in piRNA content, a shortening of exonuclease-mediated piRNAs, oocyte loss and infertility.	(Kamminga, et al., 2010)
HENN-1	C*aenorhabditis elegans*	siRNAs and piRNAs associated with the PIWI-branch Argonautes	Dysregulation of target mRNAs, compromised fertility (germline atrophy or defects in germ cell proliferation), and enhanced somatic RNAi activity.	(Billi, et al., 2012; Kamminga, et al., 2012; Montgomery, et al., 2012; Svendsen, et al., 2019)
Hen1	Bacteria	RNAs	Need to combine with Pnkp to help repair RNA against recleavage by the toxin endoribonuclease.	(Wang, et al., 2012)

HENMT1: HEN methyltransferase 1; Ago2: Argonaute 2; Nm: 2'-*O*-methylation; RNAi: RNA interference; Pnkp: polynucleotide kinase-phosphatase.

3.4 Methods for detecting 2'-*O*-methylation

Historically, the detection of 3'-terminal 2'-*O*-methylation relied on β-elimination, a method that uses alkaline periodate to remove non-methylated RNA ends. Because methylated RNA ends resist oxidation, their altered mobility during Northern blot analysis provided a visual confirmation (Yu, et al., 2005). With advancements in sequencing technologies and liquid chromatography-mass spectrometry (LC/MS), a growing array of sophisticated methods, such as RiboMethSeq (ribose methylation sequencing) and Nm-Seq (2'-*O*-methylation sequencing), have been developed for the precise detection of 2'-*O*-methylation across various samples (Helm and Motorin, 2017). RiboMethSeq leverages 2'-*O*-methylated nucleotides' resistance to alkaline cleavage, preventing the incorporation of +1 modified RNA fragments into sequencing libraries. This approach facilitates the high-throughput identification of specific 2'-*O*-methylation sites with remarkable accuracy (Marchand et al., 2016). Furthermore, Nm-Seq employs pre-treatment with periodate, selectively oxidizing and cleaving 2'-hydroxylated but not 2'-*O*-methylated nucleosides. This method enables unbiased, high-resolution mapping of Nm sites within the transcriptome at the single-nucleotide level, providing comprehensive and precise identification (Dai et al., 2017).

To overcome the challenges of large sample requirements and high sequencing costs, several simple, cost-effective methods have been developed for detecting 2'-*O*-methylation, including RTL-P and Poly(A)-tailed RT-qPCR, among others. The RTL-P method utilizes low dNTP concentrations during reverse transcription, causing the process to pause at 2'-*O*-methylation sites. This is followed by using PCR to detect Nm modifications (Dong et al., 2012). The Poly(A)-tailed RT-qPCR method exploits the observation that methylated miRNAs/piRNAs yield higher Ct values than the stem-loop RT-qPCR approach, enabling the estimation of Nm levels (Wang et al., 2018). Both RTL-P and Poly(A)-tailed RT-qPCR are foundational tech-

niques in RNA reverse transcription. By amplifying Ct differences as methylation levels increase, these methods enable the quantitative estimation of the percentage of 2'-*O*-methylation in RNA. Furthermore, the Wang group introduced a novel, label-free photoelectrochemical (PEC) biosensing technique that leverages the peroxidase-like activity of PtCu nanoframes (PtCu NFs) to amplify HENMT1 activity signals. This innovative approach not only facilitated the assessment of HENMT1 activity but also identified chlorpyrifos as an inhibitor (Wang, et al., 2018).

4 HENMT1 in human diseases

In normal human tissues, HENMT1 exhibits distinct expression profiles at both the transcriptional and protein levels (Fig. 2). At the mRNA level, HENMT1 is highly tissue-specific, with prominent expression in the testis (Fig. 2a). In contrast, at the protein level, HENMT1 is more broadly distributed, with high expression observed in the testis, as well as in the male and female reproductive systems, gastrointestinal tract, and hematopoietic/lymphoid tissues (Fig. 2b). The testis-specific mRNA expression and elevated protein levels in gonadal tissues strongly suggest that HENMT1 plays a role in human infertility. Furthermore, its widespread protein distribution hints at potential functional implications in various tumor types (discussed below).

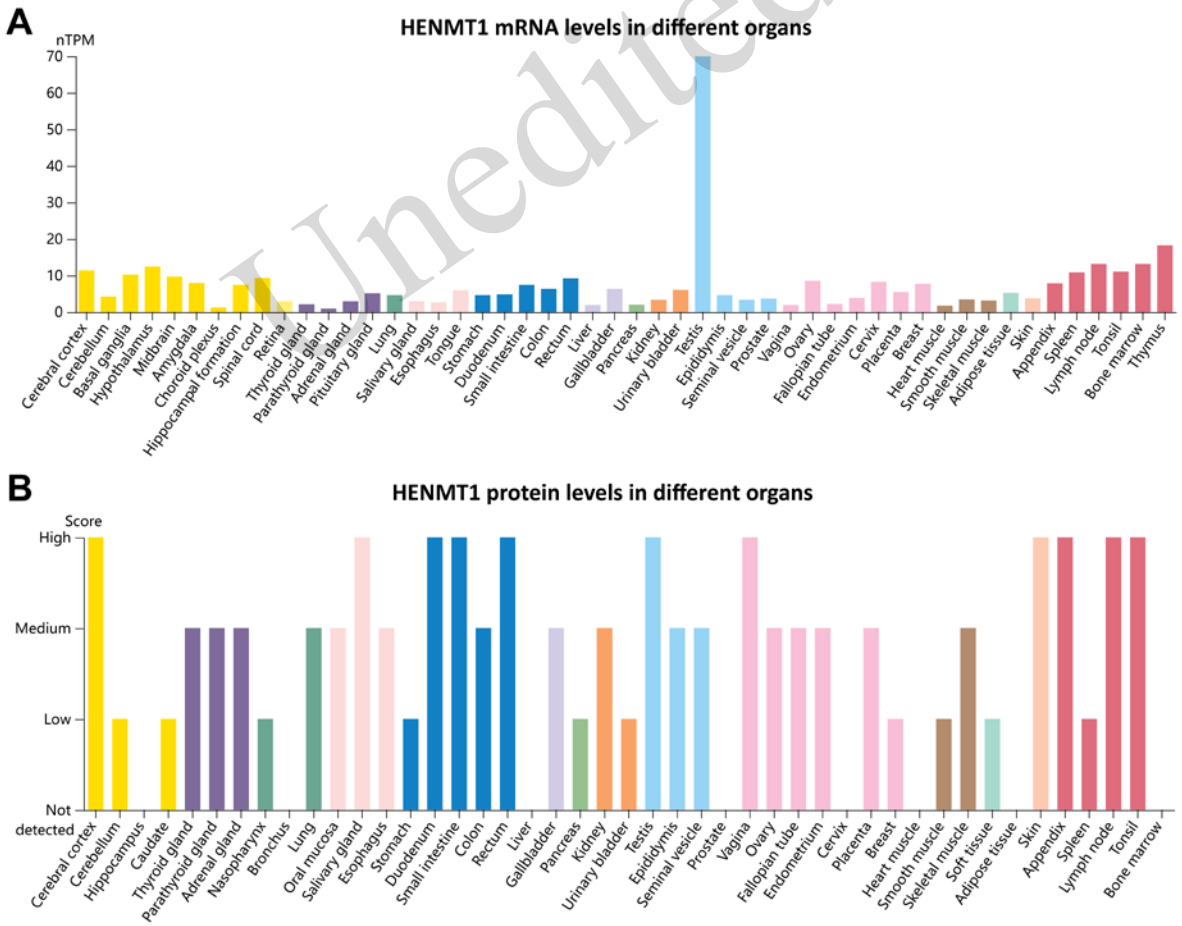

Fig. 2 HENMT1 expression in human tissues. The Human Protein Atlas (HPA) database (https://www.proteinatlas.org) is used to analyze HENMT1 expression in various human tissues. (a) Normalized transcript expression levels (nTPM) of HENMT1 across 55 human tissues, integrated from HPA and GTEx datasets. (b) Protein expression scores (High/Medium/Low/Not detected) of HENMT1 in 44 human tissues. Tissues are color-coded by functional groups.

4.1 HENMT1 and infertility

Infertility has become a significant global health issue, impacting millions of individuals and couples worldwide. An estimated 10%–25% of reproductive-age couples experience difficulties conceiving, which can lead to profound emotional, psychological, and social challenges (Thoma et al., 2021). Infertility is clinically defined as the inability to achieve pregnancy after one year of regular, unprotected intercourse, and is often attributed to hormonal imbalances, anatomical abnormalities, or specific conditions like endometriosis. In men, infertility is frequently linked to poor semen quality or functionality. Consequently, initial clinical evaluations prioritize the assessment of semen parameters, including volume, sperm concentration, viability, and morphology, to facilitate accurate diagnosis and treatment (Eisenberg et al., 2023). Men unable to produce semen are classified into two main categories: obstructive azoospermia (OA) and non-obstructive azoospermia (NOA) (Wosnitzer et al., 2014). NOA, characterized by the absence of sperm in the ejaculate even after semen sample processing and sediment analysis, affects approximately 1% of all men and 10% of infertile men (Kherraf et al., 2022). A recent study using whole-exome sequencing (WES) on individuals with NOA revealed a pronounced association between *HENMT1* mutations and subsequent impairment of the PIWI pathway, leading to meiotic abnormalities and sperm production failure (Kherraf, et al., 2022). To date, several variants of the *HENMT1* gene have been identified in infertile patients using WES. These include homozygote missense variants (c.226G > A;p.Gly76Arg and c.400A > T;p.Ile134Leu), homozygous loss-of-function variants (c.456C > G;p.Tyr152* and c.555G > A;p.Trp185*), and a novel biallelic loss-of-function variant (c.100C > T;p.Gln34* and c.456C > G;p.Tyr152*) (Kherraf, et al., 2022; Li et al., 2024; Wehbe et al., 2024). Genetic factors contribute significantly to NOA, with Klinefelter syndrome (KS) being the most prevalent genetic cause. KS affects approximately 1 in 650 newborn males and is characterized by a 47, XXY karyotype. Men with KS typically present with features such as increased height and gynecomastia, alongside azoospermia and infertility (Kanakis and Nieschlag, 2018). Site-specific differential methylation analyses in KS individuals have identified *HENMT1* as a "KS-specific" locus. KS samples exhibit higher methylation levels at the *HENMT1* locus than both male and female controls, although the underlying mechanisms remain incompletely understood (Wan et al., 2015).

In animal models, *Henmt1* knockout male mice exhibited spermatogenesis arrest due to damaged piRNAs and derepression of TEs (Lim, et al., 2015). In females, *Henmt1* mutations led to a reduced ovarian follicular reserve, altered oocyte transcriptomes, and spindle abnormalities, resulting in fewer litters (Hutt et al., 2021). The absence of HENMT1 activity in human testes triggers the activation of retrotransposons during meiosis in haploid germ cells. This premature activation disrupts normal spermatid development, ultimately leading to male infertility (Hempfling, et al., 2017).

4.2 HENMT1 and cancer

4.2.1 HENMT1 expression in human cancers

A comprehensive investigation of human RNA modification-related proteins has identified HENMT1 as the most significantly altered RNA modification-related protein (RMP) in a comparative analysis of tumor and normal human tissue samples. Elevated HENMT1 RNA and protein expression levels have been observed across various cancer types (Begik et al., 2020). Analysis of HENMT1 mRNA levels in The Cancer Genome Atlas (TCGA) database have consistently revealed significant upregulation in multiple tumor tissues compared to normal tissues. These include bladder urothelial carcinoma (BLCA), breast invasive carcinoma (BRCA), cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), cholangiocarcinoma (CHOL), colon adenocarcinoma (COAD), esophageal carcinoma (ESCA), head and neck squamous cell carcinoma (HNSC), liver hepatocellular carcinoma (LIHC), lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), prostate adenocarcinoma (PRAD), rectum adenocarcinoma (READ), sarcoma (SARC), stomach

adenocarcinoma (STAD), and uterine corpus endometrial carcinoma (UCEC) (Fig. 3). Interestingly, high HENMT1 expression in CESC, COAD, and HNSC has been associated with prolonged overall survival (Fig. 4a). Conversely, elevated HENMT1 expression in renal cancers (KICH, KIRC, and KIRP) and UCEC correlate with reduced overall survival (Fig. 4b).

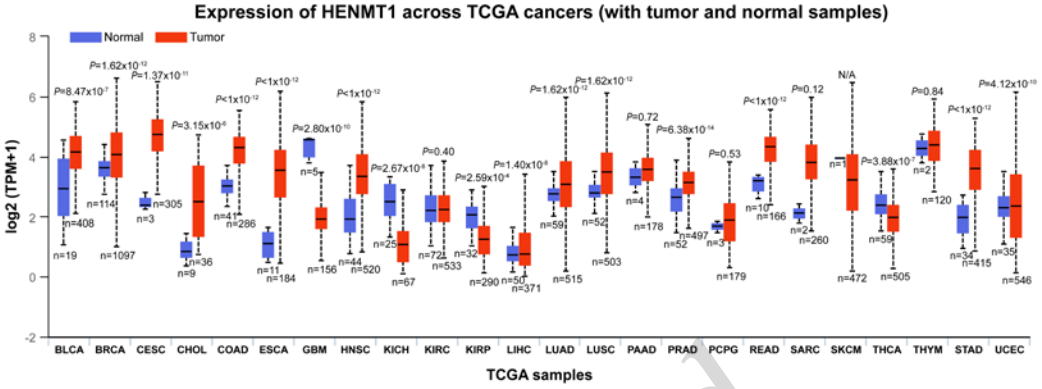

Fig. 3 HENMT1 expression in human tumor tissues and corresponding normal tissues. The mRNA expression levels of HENMT1 are significantly altered across various human cancer types compared to their respective normal tissue controls. This analysis used the University of Alabama at Birmingham Cancer Data Analysis Portal (UALCAN) database, with tumor and normal tissue samples sourced from The Cancer Genome Atlas (TCGA). The number of tumor (T) and normal (N) samples is indicated. Expression levels are measured in TPM (transcripts per million). Cancer types and abbreviations: BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL, cholangiocarcinoma; COAD, colon adenocarcinoma; ESCA, esophageal carcinoma; GBM, glioblastoma multiforme; HNSC, head and neck squamous cell carcinoma; KICH, kidney Chromophobe; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; PAAD, pancreatic adenocarcinoma; PRAD, prostate adenocarcinoma; PCPG, pheochromocytoma and paraganglioma; READ, rectum adenocarcinoma; SARC, sarcoma; SKCM, skin cutaneous melanoma; THCA, thyroid carcinoma; THYM, thymoma; STAD, stomach adenocarcinoma; UCEC, uterine corpus endometrial carcinoma.

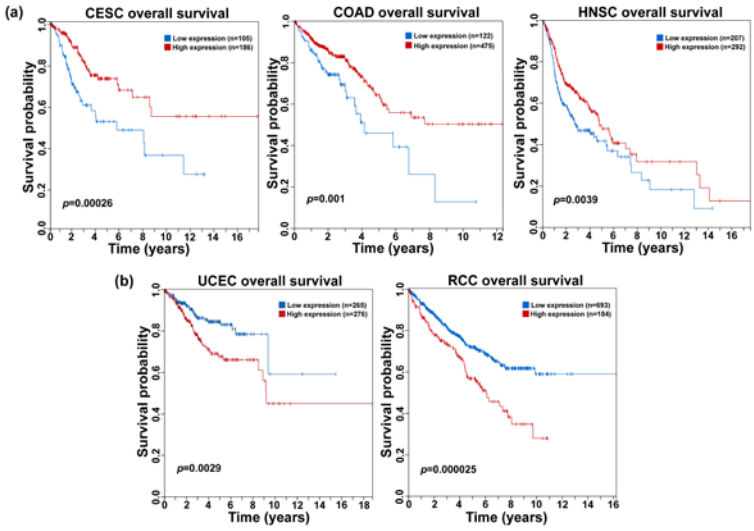

Fig. 4 Association between HENMT1 expression and patient survival across various human cancers. HENMT1 expression levels are significantly correlated with patient survival in multiple cancer types, as analyzed using data from the Human Protein Atlas database. Survival curves are stratified by HENMT1 expression levels: the blue line represents low expression, the red line high expression. (a) High HENMT1 expression is associated with improved patient survival in CESC (cervical squamous cell carcinoma), COAD (colon adenocarcinoma), and HNSC (head and neck squamous cell carcinoma). (b) High HENMT1 expression is linked to worse patient survival in UCEC (uterine corpus endometrial carcinoma), RCC [renal cancer (including KICH: Kidney chromophobe, KIRC: Kidney renal clear cell carcinoma, and KIRP: Kidney renal papillary cell carcinoma)]. Log-rank test *p*-values are indicated to reflect statistical significance.

4.2.2 Relationship between HENMT1 expression and immune cell infiltration

The tumor microenvironment (TME) involves a dynamic interplay between tumor suppressor cells and supportive cells, both of which are critical in shaping tumor progression and metastasis. Among these, tumor-infiltrating immune cells play a pivotal role in modulating tumors' immune landscapes, influencing their development and metastatic potential (Anderson and Simon, 2020). The analysis of cancer tissues from the TCGA database using the xCell algorithm revealed a positive correlation between HENMT1 expression and immune cell infiltration in most tumor types (Fig. 5a). Spearman correlation analysis further demonstrated that HENMT1 expression positively correlates with the presence of CD4+ Th2 cells, CD8+ T cells, B cells, macrophages, and mast cells across various cancers, but not with CD4+ Th1 cells (Fig. 5b). Notably, the two subtypes of CD4+ T cells—Th1 and Th2—exhibited contrasting trends, suggesting that a Th1/Th2 imbalance may be a hallmark of tumors. Specifically, CD4+ Th2 cells are often more abundant in tumors and can suppress CD4+ Th1 cell activity, thereby promoting tumor progression (Zhang et al., 2015; Frafjord et al., 2021).

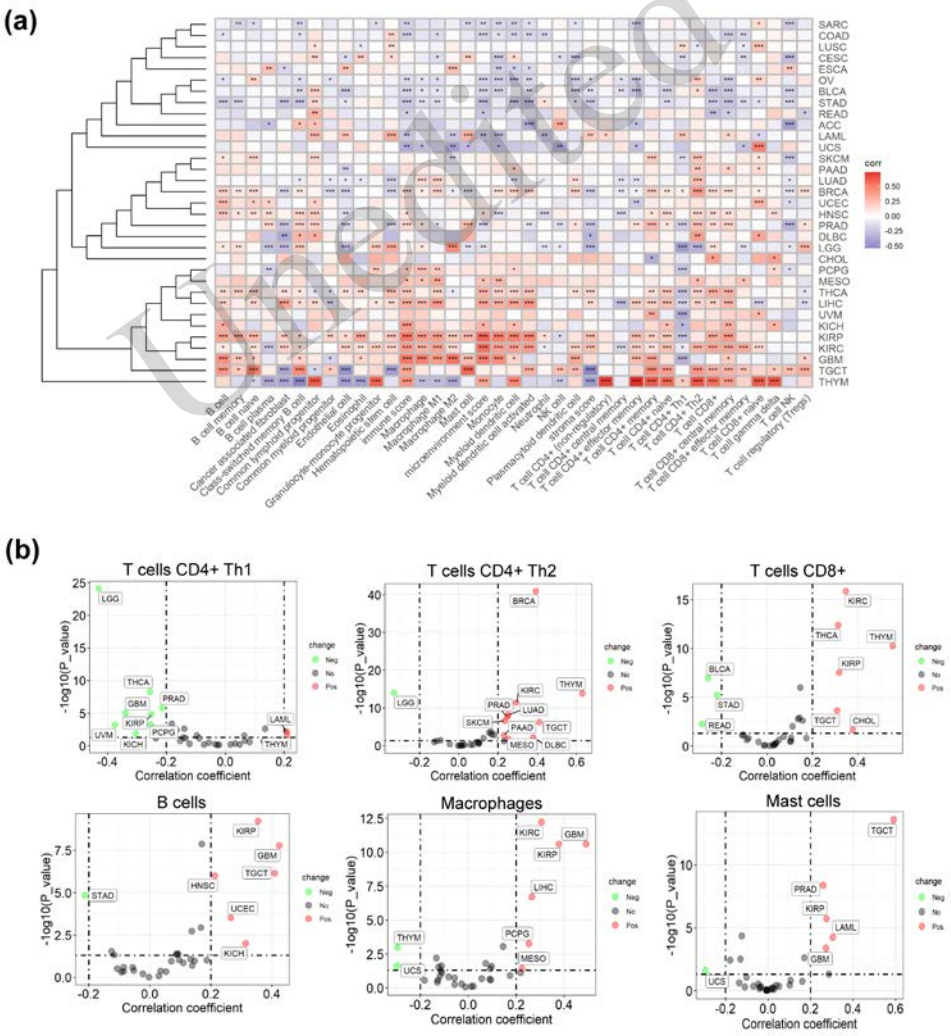

Fig 5 HENMT1 involvement in immune cell infiltration across pan-cancer types. (a) The correlation heatmap shows the relationship between HENMT1 expression and the infiltration levels of various immune cell types, analyzed using the xCELL algorithm. The color gradient from blue to red represents the range of correlation coefficients, indicating the strength and direction of the relationship. (b) Scatter plots illustrate the Spearman correlation analysis results, highlighting the association between HENMT1 expression and the infiltration of specific immune cell populations, including CD4+ Th1 cells, CD4+ Th2 cells, $CD8^+$ T cells, B cells, macrophages, and mast cells. Th: T helper cells.

4.2.3 Role of HENMT1 in human cancers

HENMT1 exhibits diverse roles across cancer types. The following section provides a concise overview of the association between HENMT1 and several cancers, particularly focusing on those affecting the reproductive system.

4.2.3.1 CESC

Cervical cancer, primarily driven by high-risk human papillomavirus (HPV) infections, remains the fourth most common cancer among women worldwide in terms of incidence and mortality, despite the availability of preventive measures such as the HPV vaccine and various treatment options (Musunuru et al., 2021; Mayadev et al., 2022). HENMT1 exhibits elevated expression levels in CESC compared to normal tissues, at both the mRNA and protein levels. Importantly, higher HENMT1 expression is associated with improved survival rates in cervical cancer patients, indicating a potential protective role (Zheng et al., 2022). This protective effect is further supported by the positive correlation between HENMT1 expression and the infiltration of immune cells such as B cells and macrophages into CESC tissues (Huang et al., 2021). Additionally, a predictive CESC model based on the competing endogenous RNAs (ceRNAs) network identified a significant relationship between HENMT1 expression and better CESC prognosis. This may be attributed to its involvement in the AKT/mTOR signaling pathway, suggesting a potential mechanism for its protective effects (Li et al., 2022).

4.2.3.2 OC

Ovarian cancer (OC) is a highly lethal gynecological malignancy with a poor prognosis, often diagnosed at advanced stages due to the absence of early symptoms. This underscores the urgent need for improved biomarkers to enhance OC research and clinical management (Xiao et al., 2022). Research on genes associated with OC and the piRNA pathway has identified HENMT1 as overexpressed in malignant compared to healthy tissues. Additionally, HENMT1 expression is significantly higher in advanced-stage patients and chemoresistant high-grade serous ovarian cancer (HGSOC) cells than in early-stage patients and chemosensitive HGSOC cells. However, no significant correlation has been observed between HENMT1 expression and patient prognosis (Lee et al., 2020). These findings suggest that HENMT1 may serve as a potential marker for monitoring OC progression and chemoresistance.

4.2.3.3 TGCT

Testicular germ cell tumors (TGCTs) are the most common solid tumors found in males aged 15–44 (Znaor et al., 2014). RNA sequencing of a subset of TGCT embryonal carcinoma (EC) cell lines and their non-malignant counterparts revealed novel fusion transcripts, with the regulator of chromosome condensation 1 (RCC1)-HENMT1 fusion transcript being particularly notable. This fusion was detected across various TGCT subtypes, as well as in intratubular germ cell neoplasia (IGCN), a precursor lesion of TGCT, and in embryonic stem (ES) cell lines (Hoff et al., 2016). A hallmark of TGCTs is the dysregulation of microRNAs (miRNAs). Research has shown that HENMT1 plays a role in regulating miRNA stability by methylating their 3'-terminal ends (Liang, et al., 2020; De Martino et al., 2021). These findings highlight the potential importance of HENMT1-mediated miRNA methylation in TGCT development, presenting a promising direction for future research.

4.2.3.4 ESCA

Esophageal cancer (ESCA) ranks as the sixth most common cancer globally and is characterized by its high mortality rate and poor survival outcomes (Tang et al., 2014). Recent bioinformatics research has identified *HENMT1* as a key gene in ESCA (Reyimu et al., 2023). The study demonstrated that HENMT1 is significantly overexpressed in ESCA, correlates with poor prognosis, and is positively associated with the expression of the tumor marker MKI67. Further analyses suggest that HENMT1 may contribute to ESCA progression by modulating the TME, highlighting its potential as both a biomarker for disease monitoring and a target for immunotherapeutic strategies in ESCA (Reyimu et al., 2023).

4.2.3.5 NSCLC

Lung cancer remains the leading cause of cancer-related mortality worldwide, with non-small cell lung cancer (NSCLC) accounting for the majority of cases (Hendriks et al., 2024). Recent studies investigating miRNA profiles in NSCLC and adjacent non-cancerous lung tissues revealed distinct 3'-terminal 2'-*O*-methylation patterns. Notably, miR-21-5p was found to be heavily methylated in NSCLC tissues but not in non-tumor tissues. HENMT1, which is overexpressed in NSCLC, catalyzes the 3'-terminal 2'-*O*-methylation of miR-21-5p, enhancing its binding to AGO2 and suppressing the translation of programmed cell death factor 4 (PDCD4) (Liang, et al., 2020). This study provides the first evidence that HENMT1, in addition to its role in piRNA methylation, can also methylate miRNAs in humans, underscoring its multifaceted functions in RNA regulation and its complex role in tumorigenesis. Additionally, it is well established that CpG island hyper-methylation leads to the epigenetic silencing of piRNAs and miRNAs in certain tumor types (Ferreira et al., 2014; Moutinho and Esteller, 2017). Thus, apart from HENMT1's regulation of piRNA and miRNA stability through methylation, another mechanism driving altered piRNA and miRNA expression in tumors involves CpG island hypermethylation-mediated silencing of the PIWI/piRNA pathway or microRNA-coding genes.

5 Conclusions and perspectives

In summary, HENMT1 acts as a methyltransferase, modifying the 3'-OH terminal of piRNAs and miRNAs. Its regulation varies across cancer types, with high HENMT1 levels being associated with improved survival outcomes in CESC, CRC, and HNSC, but correlating negatively with prognosis in KCC and UCEC (Fig. 4). Despite these findings, several key questions warrant further investigation to fully elucidate the role of HENMT1 in tumorigenesis: (1) What mechanisms underlie the association between high HENMT1 expression and favorable prognosis in certain tumor types? (2) Beyond piRNAs and miR-21-5p, what additional substrates does HENMT1 methylate in humans? Does high HENMT1 expression influence cancer progression through the regulation of different miRNAs? (3) What are the upstream regulators that control HENMT1's methylation activity? (4) Given its frequent dysregulation in cancers, could HENMT1 serve as a promising target for tumor diagnosis and therapy? Answering these questions will provide deeper insights into HENMT1's precise role in the RNA interference pathway and its involvement in pathological processes such as cancer and infertility.

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Author contributions

Danrui CUI conceived and outlined the manuscript. Shanghong JIANG performed literature search and wrote the manuscript. Danrui CUI and Yongchao ZHAO revised, and Yongchao ZHAO finalized the manuscript. All authors contributed to the discussions, and have read and approved the article.

Compliance with ethics guidelines

Shanghong JIANG, Yongchao ZHAO, and Danrui CUI declare that they have no conflict of interest.

This review does not contain any studies with human or animal subjects performed by any of the authors.

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