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The role of RNA 2′-O-methylation in prostate cancer

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ABSTRACT

2′-O-methylation (Nm) represents a pervasive RNA modification occurring in multiple RNA species, including messenger RNA (mRNA), ribosomal RNA (rRNA), transfer RNA (tRNA), microRNA (miRNA), and PIWI-interacting RNA (piRNA). This chemical mark exerts critical regulatory functions in RNA stability, transcriptional control, and translational efficiency. Recent advances in high-throughput sequencing technologies have enabled transcriptome-wide mapping of Nm sites, uncovering increasing evidence that aberrant Nm modifications contribute to oncogenesis and tumor progression. Prostate cancer, the second most diagnosed malignancy in men in the United States, has emerged as a disease context in which dysregulated Nm-related pathways are of particular significance. As sequencing-based investigations continue to expand, delineating the activities of Nm-modifying enzymes across distinct RNA classes in prostate cancer is anticipated to provide mechanistic insights into disease biology, facilitate the discovery of novel therapeutic targets, and ultimately guide the development of Nm-centered anticancer strategies.

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1. Introduction

Prostate cancer (PCa) remains the second leading cause of cancer-related death among men in the United States, following lung cancer [1]. In 2025, approximately 313,780 new PCa cases are expected to be diagnosed, accounting for 15.4% of all new cancer cases, with an estimated 35,770 deaths attributed to this disease. Although androgen receptor (AR) inhibitors such as enzalutamide and apalutamide have demonstrated therapeutic efficacy in androgen-dependent prostate cancer (ADPC), treatment options for castration-resistant prostate cancer (CRPC) remain limited [2-4]. Moreover, prolonged suppression of AR signaling often drives tumor progression, leading to the transition from ADPC to CRPC[5]. This highlights the urgent need to identify novel therapeutic targets and alternative treatment strategies to improve patient outcomes.

More than 170 types of chemical modifications have been identified on RNA molecules, with N6-methyladenosine (m^6A) [6, 7], 5-methylcytosine (m^5C) [8, 9], N7-methylguanosine (m^7G) [10, 11], and N1-methyladenosine (m^1A) accounting for the majority [12]. Dysregulation of these RNA modifications has been increasingly linked to the initiation and progression of PCa [13]. Among these, m^6A is the most extensively studied. Yao et al. demonstrated that m^6A modification promotes the biogenesis of circDDIT4, a circular RNA that is downregulated in PCa and functions as a tumor suppressor [14]. Similarly, Mao et al. revealed that the m^6A-modified circRBM33 forms a complex with FMR1 to stabilize PDHA1 mRNA, enhancing mitochondrial metabolism and promoting PCa progression. Notably, this pathway also reduces the therapeutic efficacy of ARSI, suggesting a role in treatment resistance [15]. Another study reported that m^6A-mediated upregulation of NFIB triggers epithelial-mesenchymal transition (EMT) and metastasis in AR-negative CRPC, identifying the m^6A/NFIB axis as a potential therapeutic target for AR-negative PCa metastasis [16].

In addition to m^6A, m^7G modification has also gained attention. SP1, in complex with P300, binds to the promoter region of METTL1 and activates its transcription in CRPC. Functional experiments showed that METTL1 stabilizes CDK14 mRNA via

internal m^7G modification, thereby promoting CRPC progression [17].

Emerging evidence also implicates m^5C modification in lipid metabolism dysregulation in PCa. Mechanistically, CDK13 interacts with the RNA methyltransferase NSUN5 and promotes its phosphorylation at Ser327. Phosphorylated NSUN5 catalyzes m^5C modification of ACC1 mRNA, which subsequently binds to ALYREF, enhancing mRNA stability and nuclear export. This results in elevated ACC1 expression and increased lipid deposition in PCa cells [18]. Furthermore, several m^5C regulatory genes, such as: TET1, TET3, DNMT3B, YBX1, NSUN2, NSUN6, and NOP2-exhibit aberrant expression in PCa [19]. Among these, NSUN2 has been shown to enhance the stability of TRIM28 mRNA via m^5C modification, thereby promoting its expression. Depletion of NSUN2 leads to reduced expression and activity of AR and its variant AR-V7. Mechanistic studies have indicated that NSUN2 post-transcriptionally stabilizes AR mRNA through clustered m^5C modifications in a YBX1-dependent manner. Interestingly, treatment with the AR inhibitor enzalutamide suppresses NSUN2 expression and reduces m^5C levels in PCa cells, suggesting a feedback regulatory loop wherein AR further transcriptionally regulates NSUN2 [20].

Moreover, m^1A modification and its regulatory factors have also been implicated in PCa. Several regulators-including ALKBH3, TRMT61A, TRMT10C, YTHDF1, and YTHDF2-are overexpressed in PCa [21]. In particular, TRMT61A may promote PCa progression by enhancing mitochondrial β-oxidation via activation of the PI3K/AKT signaling pathway [21].

RNA species are broadly categorized as coding RNAs-such as mRNAs, and non-coding RNAs, including rRNA, tRNA, miRNA, snoRNA, lncRNA, and circRNA [22]. Nm plays critical regulatory roles in both coding and non-coding RNAs, contributing to the regulation of transcription, splicing, and translation [23, 24]. Nm modifications are predominantly enriched in rRNA, tRNA, and small nuclear RNA (snRNA) [25-27]. Recent studies have shown that Nm also occurs at internal sites within mRNA transcripts, beyond the canonical 5′ cap region, indicating broader functional significance in mRNA metabolism [28-30]. Growing evidence suggests that RNA modifications, including Nm, are involved in PCa initiation and progression by modulating RNA stability, splicing, and translation [24, 31]. These findings underscore the therapeutic potential of targeting

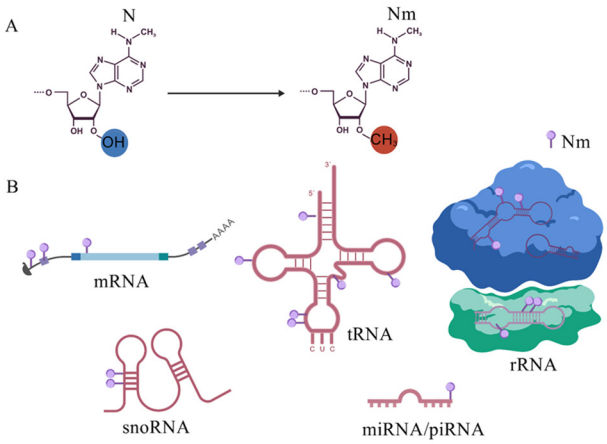


Figure 1: Nm modification in cellular RNAs. (A) Nm modification refers to 2′-O-methylation of the ribose moiety of a ribonucleotide N. (B) Nm modification occurs in noncoding RNAs, including rRNA, tRNA, and snRNA, at the 3′ end of miRNAs and piRNAs, as well as coding RNA: mRNA. Created with BioGDP.com.

RNA modifications, including Nm are now recognized as critical regulators of gene expression and cellular function in a wide range of diseases, including PCa [13, 24]. Enzymes that are involved in Nm installation and removal have emerged as promising drug targets. This review aims to provide a comprehensive overview of RNA 2′-O modifications in PCa, with an emphasis on identifying new molecular mechanisms and therapeutic opportunities, particularly in the context of CRPC.

2. 2′-O-methylation

Nm refers to the methylation of the 2′-hydroxyl group on the ribose sugar of RNA nucleotides, which could occur on all four nucleobase types [32-34](**Figure 1A and 1B**). This modification is prevalent across various RNA species[35]. Yet no specific consensus motif for Nm deposition has been identified; nearly all RNA classes appear to be 2′-O-modified by distinct 2′-O-methyltransferases [31, 36]. For instance, rRNA is 2′-O-methylated by Fibrillarin (FBL) as part of the C/D box small nucleolar ribonucleoprotein (snoRNP) complex [37, 38]. tRNA modifications are catalyzed by enzymes such as FTSJ1, TRMT13, TARBP1, TRMT44, and the C/D box snoRNP [27, 39-42]. Cap-proximal Nm sites are installed by CMTR1 and CMTR2 [43, 44], while HENMT1 modifies the 3′ ends of small RNAs, such as miRNA and piRNA [45-47].

Interestingly, depletion of FBL in PCa cells results in a globally reduced Nm level on mRNAs, suggesting that FBL may also function as an mRNA 2′-O-meth-

yltransferase [31, 48]. FBLL1, a neuron-specific methyltransferase, has also been shown to modify mRNA and regulate neuronal development. However, its expression does not appear altered in PCa, indicating a context-specific role [49]. Currently, no demethylases ("erasers") for Nm have been identified. Nonetheless, with the ongoing advancement of sequencing technologies, it is anticipated that additional enzymes and RNA-binding proteins involved in Nm regulation will be discovered.

Nm enhances RNA stability by increasing the hydrophobicity of the ribose sugar, thereby protecting the molecule from hydrolytic degradation [50]. This effect is particularly important under conditions of cellular stress or viral infection [51]. Beyond its role in stabilization, Nm can influence RNA secondary structure and alter interactions with proteins or other RNAs, ultimately affecting transcriptional regulation and translation efficiency [32, 52]. However, the hydrophobic nature of Nm also poses challenges during RNA sequencing. Nm modifications can hinder reverse transcription, leading to premature termination at modified sites and reducing both the accuracy and efficiency of sequencing-based detection [53].

2.1 2′-O-methyltransferases

Enzymes that catalyze RNA Nm can be broadly divided into two classes: snoRNA-dependent and snoRNA-independent methyltransferases [54, 55].

snoRNA-dependent methyltransferases:

The Box C/D snoRNP complex is the canonical machinery for snoRNA-dependent methylation. It comprises a guiding C/D box snoRNA and four core proteins: FBL, NOP56, NOP58, and SNU13 [54, 56]. Assembly begins when SNU13 binds the kink-turn motif of the snoRNA [56]. NOP56 and NOP58 form a structural scaffold that positions FBL near the D/D′ boxes of the snoRNA [57]. The antisense element of the snoRNA base-pairs with the target RNA, positioning the modification site five nucleotides upstream of the D/D′ box, an arrangement known as the "+5 rule" [58, 59]. FBL then uses S-adenosyl-L-methionine (SAM) as the methyl donor to catalyze Nm modification at the targeted ribose [60]. This mechanism primarily modifies rRNA and snRNA, with more limited activity on tRNA and mRNA [30, 48]. Currently, it remains unclear whether FBL can act as a standalone methyltransferase to deposit Nm. Notably, our recent findings in PCa cells show that most mRNA Nm sites affected by FBL suppression lack complementary snoRNA binding sequences [31]. This observation suggests that FBL may confer Nm marks through a mechanism distinct from the canonical Box C/D snoRNP-dependent rRNA Nm methylation.

snoRNA-independent methyltransferases:

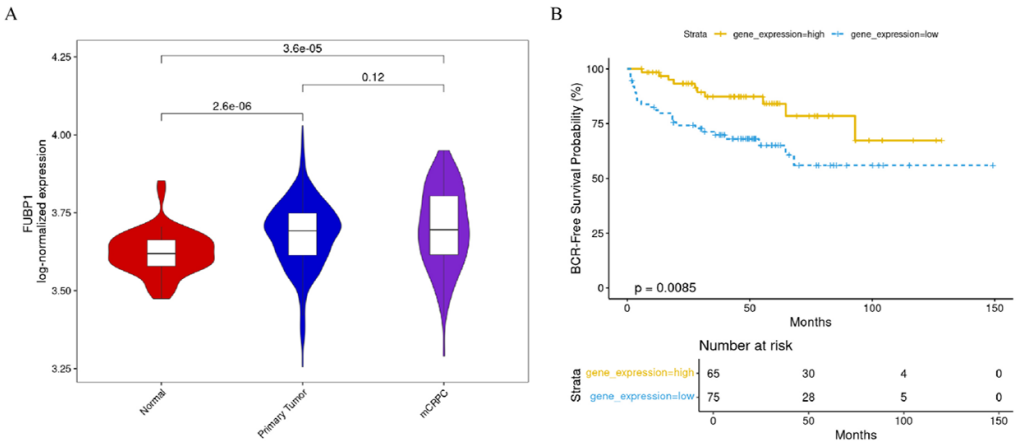
snoRNA-independent enzymes fall into two major structural families: the SPOUT and RFM (RrmJ) families [61, 62]. SPOUT family enzymes catalyze Nm via a metal-independent SN2-like reaction. They are characterized by: first, a lack of strongly conserved catalytic residues, relying instead on precise RNA positioning and the electrostatic properties of SAM; second, a rigid, pre-formed active site formed by the hallmark SPOUT fold; third, substrate recognition largely based on RNA structure rather than strict sequence specificity, with a strong preference for tRNA [63-65]. Notable SPOUT family members include TRMT13, TRMT44, and TARBP1. TARBP1 specifically catalyzes Gm18 modification in tRNA^Gln and tRNA^Ser, affecting tRNA stability and promoting the progression of hepatocellular carcinoma [41]. TRMT44 catalyzes Um44 modification in yeast tRNA^Ser, although its role in mammals remains unclear [42]. TRMT13 introduces Nm in specific tRNAs and is upregulated in multiple cancers, including breast, liver, and papillary thyroid cancers [40, 66].

RFM/RrmJ family enzymes share a Rossmann-like α/β fold that binds SAM, stabilized by a conserved GxGxG motif [67, 68]. Some members operate with snoRNAs (e.g., FBL), while others recognize RNA substrates directly. FTSJ3, which installs Nm modifications on viral RNA (e.g., HIV) to evade immune detection and is upregulated in hepatocellular carcinoma [69-71]. FTSJ1, which modifies residues in the tRNA anticodon loop and is implicated in X-linked intellectual disability [72]. MRM1, MRM2, and MRM3, which introduce Nm at defined sites within mitochondrial 16S rRNA[73-75]. HENMT1, which uniquely catalyzes 3′-terminal Nm on small RNAs such as miRNAs and piRNAs, independent of snoRNA guidance [76, 77]. Of note, Nm does not have a known demethylase ("eraser"), possibly due to the inert chemistry of 2′-O-methyl [30].

2.2 Nm reader

Research on proteins that recognize Nm sites, commonly referred to as "readers", remains limited. Recently, a study by Chuan He's group employed an

Figure 2: FUBP1 plays a potential oncogenic role in PCa. (A) FUBP1 is significantly upregulated in prostate tumors. (B) FUBP1 correlates with shorter BCR-free survival probability.



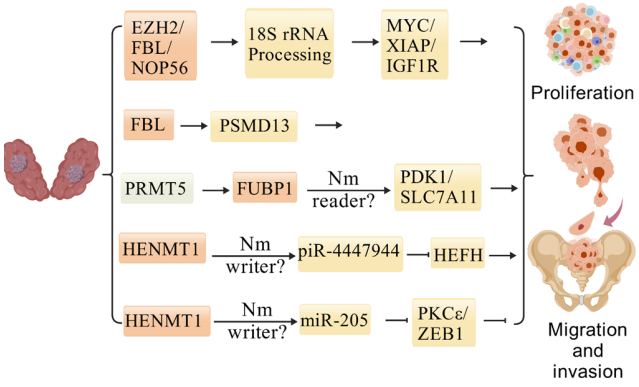
integrative approach combining RNA affinity purification, mass spectrometry, electrophoretic mobility shift assays (EMSA), and PAR-CLIP. This effort identified FUBP1 as a bona fide Nm-binding protein involved in regulating RNA splicing [78]. Mechanistically, FUBP1 recognizes Nm-modified intronic regions or U-rich sequences, where it recruits spliceosomal components and promotes splice-site pairing. This activity suppresses exon skipping and enhances splicing efficiency [79]. Beyond its role in RNA processing, FUBP1 is overexpressed in several cancer types, including PCa [80-82]. Elevated FUBP1 levels are associated with reduced disease-free survival, underscoring its oncogenic potential. In PCa C4-2 xenograft models, FUBP1 knockdown significantly impaired tumor growth. Moreover, arginine methylation of FUBP1 at residues R359, R361, and R363 by PRMT5 is required for its oncogenic activity [80]. Disrupting this interaction using competitive peptides-such as the PUBLISH peptide-effectively suppressed tumor progression, highlighting a potential therapeutic target. Consistent with these findings, data from the SU2C PCa cohort confirm that FUBP1 is significantly upregulated in prostate tumors and correlates with shorter biochemical recurrence-free survival [83] (**Figure 2A and 2B**). These studies provide the first direct evidence for an Nm "reader" protein and establish a link between Nm-sensitive splicing regulation and cancer progression. Additional candidate Nm readers have been proposed, including FUBP3, KHSRP, and IGF2BP1/3; however, these require further experimental validation [78].

3. Functions of Nm in PCa

3.1 rRNA Nm in PCa

The rRNA forms the structural and catalytic core of the ribosome and is essential for mRNA translation. Among its numerous post-transcriptional modifications, Nm plays a critical role in promoting proper rRNA folding and stability, facilitating ribosome biogenesis, and regulating translational fidelity and efficiency [84]. FBL, the key catalytic subunit of the C/D box snoRNP complex, is responsible for installing Nm modifications on rRNA [85]. Notably, mounting evidence, including ours, demonstrated that both FBL and its associated scaffolding protein NOP56 are highly expressed in PCa and correlate with poor clinical outcomes [86-88]. Mechanistically, histone methyltransferase and prostatic oncogene EZH2 were found to interact directly with FBL and NOP56, forming a trimeric complex that enhances rRNA 2′-O-methylation. As a result, depletion of EZH2 significantly reduced rRNA Nm levels, revealing a non-canonical role for EZH2 in modulating ribosomal RNA modification. These findings suggest that targeting the EZH2-FBL-NOP56 axis may offer a novel therapeutic strategy in cancers characterized by elevated EZH2 expression [88]. Further analysis of the SU2C PCa dataset revealed that, in addition to FBL and NOP56, several other rRNA Nm-associated proteins exhibit dynamic changes in expression throughout disease progression [83]. For instance, SNU13, FBL, and MRM1/3 are upregulated in primary PCa but revert to near-normal expression levels in CRPC [34] (Figure 3 and Table 1). Conversely,

Figure 3: Molecular mechanisms by which Nm regulates the biological functions of PCa. Nm-related proteins FBL and NOP56 promote PCa proliferation, invasion, and migration. Similarly, FUBP1 and HENMT1 may contribute to these processes by regulating Nm. Created with BioGDP.com.



NOP56, NOP58, and MRM2 display progressively increased mRNA expression from primary tumors to CRPC [83]. This stage-specific regulation suggests that different Nm-associated factors may play distinct roles during PCa evolution. However, apart from FBL, the contributions of other Nm regulators remain poorly characterized, warranting further investigation into their functional relevance and therapeutic potential in PCa.

3.2 tRNA Nm in PCa

The tRNAs decode mRNA codons via their anticodon loops, ensuring the accurate incorporation of amino acids during protein synthesis. Most tRNAs undergo extensive post-transcriptional modifications, which are essential for their structural integrity and biological function [89]. Among these, Nm plays a pivotal role in maintaining tRNA stability and optimizing translational fidelity [90, 91]. Several Nm methyltransferases that function independently of snoRNAs have been implicated in tRNA Nm regulation [92]. TARBP1 catalyzes the Gm18 modification on tRNA, modulating mRNA stability and affecting glutamine metabolism in hepatocellular carcinoma [41, 93]. Although direct evidence for TARBP1 involvement in PCa is currently lacking, transcriptomic analyses from the SU2C dataset indicate that TARBP1 expression progressively increases during PCa progression [83]. TRMT13 and TRMT44 are additional tRNA-specific methyltransferases that contribute to Nm installation and tRNA stabilization [40, 42]. Interestingly, these enzymes display opposing expression patterns in PCa: TRMT13 is significantly upregulated in CRPC, whereas TRMT44 is downregulated [83]. This divergence raises the possibility that reduced TRMT44 levels may compromise the translation of tumor-suppressive transcripts, while elevated TRMT13 expression could promote oncogenic translation-hypotheses warranting experimental validation. FTSJ1 catalyzes Nm at positions Cm32/Gm32 and Am34 within the tRNA anticodon loop, enhancing translational fidelity and efficiency [72, 94]. FTSJ1 is also upregulated during PCa progression and is associated with poor clinical outcomes, suggesting a potential oncogenic role [83]. Collectively, these findings highlight a complex regulatory landscape in which distinct tRNA Nm methyltransferases may exert tumor-promoting or tumor-suppressive functions. Notably, TARBP1, TRMT13, TRMT44, and FTSJ1 all belong to the SPOUT superfamily, sharing a conserved catalytic fold [65, 95]. This structural homology could facilitate the rational design of broad-spectrum inhibitors targeting multiple Nm-associated enzymes, offering a promising avenue for therapeutic intervention [34] (**Figure 3 and Table 1**).

3.3 mRNA Nm in PCa

Emerging evidence suggests that Nm modification on mRNA modulates transcript stability, translational efficiency, and immune recognition, with its functional consequences highly dependent on the modification's positional context [48, 96-98]. For example, cap-proximal Nm at the 5′ end of mRNA shields transcripts from innate immune sensors such as MDA5 and RIG-I, thereby suppressing type I interferon responses [29, 99, 100]. In contrast, mRNAs lacking cap Nm modifications are recognized as "non-self" and activate antiviral signaling through MDA5 and RIG-I [101, 102]. Within coding sequences, Nm modifications have been shown to disrupt translation [96]. The Puglisi group demonstrated that internal Nm residues within codons impair ribosomal decoding, leading to translational stalling in a position- and sequence-dependent manner [103]. Mechanistically, Nm modification interferes with codon-anticodon interactions, reduces EF-Tu GTP hydrolysis efficiency, and hampers tRNA accommodation, ultimately affecting translational dynamics and fidelity [103-105].

Nm modification also influences mRNA stability when located in untranslated regions [31]. Our recent study found that Nm-modified mRNAs tend to have shorter 3′ untranslated regions (UTRs), fewer miRNA-binding sites, and reduced AU-rich ele-

ments, collectively contributing to transcript stabilization [31, 106]. Moreover, the RNA-binding protein CPSF7 has been identified as a potential co-regulator that may cooperate with Nm modifications to influence alternative polyadenylation, thereby promoting 3′UTR shortening [31, 107, 108]. In PCa cell lines, FBL not only catalyzes Nm modifications on rRNA but also directly binds to and modifies mRNA [31, 88]. These FBL-associated mRNAs are more stable, whereas FBL knockdown markedly decreases their Nm levels, half-life, and expression. Notably, FBL-bound mRNAs are significantly enriched in cancer-related pathways [31]. Together, these findings suggest that therapeutically targeting the FBL–Nm axis may represent a promising strategy for PCa treatment.

In addition to FBL, FTSJ3 has been identified as a key mRNA Nm modification methyltransferase [70, 109]. It modifies cytoplasmic mRNAs and participates in pre-rRNA processing through its Spb1-C domain, which interacts with proteins such as PAR14, RPS19, and nucleolin [110]. In mammalian cells, mRNA Nm modification helps distinguish endogenous RNAs from exogenous ones. Yamina et al. demonstrated that FTSJ3, recruited by TARBP1, installs Nm on HIV RNAs to evade MDA5-mediated immune detection [70]. Similarly, work by Xiaolong Liu et al. showed that FTSJ3 is upregulated in hepatocellular carcinoma, where it modifies endogenous double-stranded RNAs to suppress RIG-I recognition and interferon-β (IFN-β) production, promoting immune evasion [69]. FTSJ3 has also been reported as an oncogenic factor in lung and breast cancers [109, 111]. Although FTSJ3 has not been extensively studied in PCa, analysis of the SU2C dataset indicates that its mRNA levels are significantly elevated in primary prostate tumors but return to near-normal levels in CRPC [83]. This dynamic pattern implies stage-specific regulatory roles that merit further investigation.

3.4 miRNA/piRNA Nm in PCa

3.4.1 piRNA Nm in PCa

The piRNAs are a class of 24–32 nucleotide small RNAs whose 3′ termini undergo Nm modification catalyzed by HENMT1, which enhances their stability [46, 112]. In germ cells, piRNAs complex PIWI proteins to silence transposable elements through transcript cleavage or epigenetic repression. This silencing prevents mobilization of transposons, limits DNA damage and mutations, and maintains genome integrity [113, 114]. Accumulating evidence indicates that piRNAs also contribute to tumorigenesis [115, 116]. For instance, piR-4447944 is upregulated in CRPC, where it promotes castration resistance, tumor growth, and metastasis [117]. Mechanistically, piR-4447944 associates with PIWIL2 to repress the tumor suppressor NEFH [117, 118]. Its overexpression accelerates tumor growth in xenograft models, while reintroducing NEFH partially reverses this effect. These findings reveal a novel oncogenic pathway mediated by piRNAs in CRPC and suggest new therapeutic strategies. Notably, HENMT1 is also significantly upregulated in PCa [83]. However, whether HENMT1 stabilizes oncogenic piRNAs such as piR-4447944 via 3′-end Nm modification and contributes to disease progression remains unknown (**Figure 3 and Table 1**).

3.4.2 miRNA Nm in PCa

miRNAs are approximately 22-nucleotide non-coding RNAs that regulate gene expression post-transcriptionally by binding to target mRNAs. They function as either tumor suppressors or oncogenes depending on the cellular context [119]. A meta-analysis by Declan J. McKenna's group showed that elevated miR-21 expression in PCa correlates with poor prognosis, highlighting its potential as a biomarker [120]. In addition to expression changes, chemical modifications of miRNAs also influence their stability and function. In non-small cell lung cancer (NSCLC), Hongwei Liang et al. reported that HENMT1-mediated Nm modification at the 3′ end of miR-21-5p increases its stability and enhances AGO2 binding. This modification augments miR-21's ability to repress the tumor suppressor PDCD4, thereby promoting tumorigenesis [47]. Whether this mechanism operates similarly in PCa is still unclear. For instance, miR-205, frequently downregulated in PCa, functions as a tumor suppressor. Its overexpression enhances radiosensitivity by suppressing PKCε and ZEB1, impairing both non-homologous end joining (NHEJ) and homologous recombination (HR) DNA repair pathways [121]. Additional tumor-suppressive miRNAs in PCa include miR-27b-3p [122], miR-130b [123], miR-124 [124], and miR-1231 [125]. Oncogenic miRNAs include miR-98-5p [126], miR-152-3p [127], miR-4289 [128], miR-9-5p [129] and miR-148-3p [127]. These findings underscore the value of miRNAs as potential biomarkers for diagnosis and disease monitoring in PCa. However, no studies have provided evidence that HENMT1 influences PCa initiation or progression by modulating miRNA stability.

Given its role in stabilizing both piRNAs and miRNAs, the upregulation of HENMT1 in PCa is of considerable interest [83]. To date, no specific HENMT1 inhibitors have been developed. Haiyan Wang et al. established an in vitro assay to evaluate HENMT1's Nm modification methyltransferase activity and identified chlorpyrifos as a candidate inhibitor [77]. However, its ability to modulate HENMT1-dependent Nm modification of small RNAs has not yet been validated in cellular or in vivo models. These findings highlight the importance of HENMT1 in small RNA biology and the need for further investigation into its therapeutic potential (**Figure 3 and Table 1**).

4 Potential drug development based on Nm-modified regulators

Emerging evidence indicates that many 2′-O methyltransferases are aberrantly expressed in PCa [31, 83, 88], suggesting that broad-spectrum inhibitors targeting these enzymes may offer a promising therapeutic approach for tumors driven by dysregulated Nm modification pathways. However, drug development in this area remains in its infancy, with only a limited number of chemical probes or inhibitors currently available.

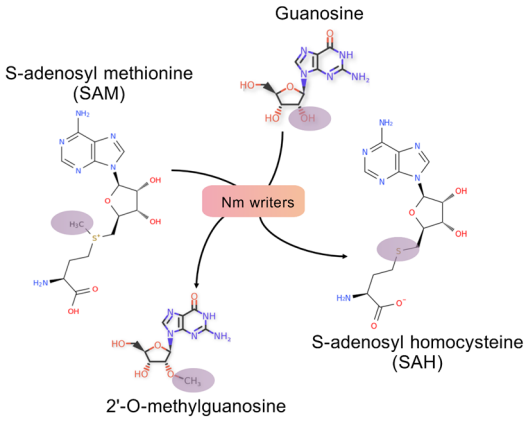
Recent research has focused on FBL, the catalytic core of the C/D box snoRNP complex. Using a fragment-based drug discovery (FBDD) approach, Thomas Ve et al. identified small-molecule fragments (PS-6655 and FS-2818) that competitively bind the SAM pocket of FBL [130]. Co-crystal structural analysis revealed conformational flexibility in the SAM-binding site, providing a foundation for future structure-guided inhibitor development. Similarly, research by Kangdong Liu et al. demonstrated that fludarabine phosphate inhibits the FBL-YY1-CAD transcriptional axis and suppresses hepatocellular carcinoma progression [131]. Notably, this compound disrupts the FBL-YY1 protein–protein interaction without impairing FBL's canonical rRNA Nm methyltransferase activity.

Beyond SAM-competitive inhibition, an alternative strategy involves disrupting protein–protein interactions (PPIs) within the snoRNP complex [132]. For example, interfering with the assembly of NOP56/NOP58 and SNU13 with FBL and associated snoRNAs has been proposed [133, 134]. While theoretically promising, no effective PPI-targeting chemical probes have been reported to date.

Most other Nm methyltransferases belong to the SPOUT family, characterized by a conserved SPOUT domain that functions as the catalytic center for SAM binding and methyl transfer. This structural fold represents a viable druggable target [135, 136]. For instance, Vitor Mendes and colleagues used FBDD, coupled with fragment merging and structure-based optimization, to develop inhibitors that bind the SAM-binding pocket of the SPOUT domain in *Mycobacterium abscessus TrmD*. These compounds effectively inhibited methyl transfer and suppressed tRNA m^1G37 modification [137].

Another strategy under consideration is the use of synthetic SAM analogs as competitive inhibitors. Since SAM serves as the universal methyl donor for methyltransferases, such analogs could bind multiple enzyme classes [34, 60, 138, 139](**Figure 4**). However, this approach poses specific challenges, as it may disrupt a broad range of methylation reactions beyond

Figure 4: Deposition of Nm by writers. The chemical structures of the methyl donor SAM will, in turn, be converted into SAH mediated by the Nm modification writer. Created on the platform BioGDP.com.



Nm modification.

5 Conclusion and Perspective

This review provides a comprehensive overview of the emerging regulatory functions of Nm modification in PCa. Large-scale transcriptomic datasets consistently reveal dysregulation of enzymes and cofactors involved in Nm modification across PCa cohorts, suggesting that aberrant Nm signaling may contribute to tumor initiation, progression, and therapeutic resistance [83]. Despite these correlative observations, functional studies directly dissecting the roles of Nm in PCa remain limited. Moreover, no small-molecule inhibitors targeting Nm-related

enzymes have entered clinical development, reflecting both the technical and conceptual challenges in this nascent field.

A major obstacle in studying Nm biology lies in the multifunctionality of the enzymes that catalyze or read these modifications. For example, FBL not only serve as "writers" of Nm but also function as canonical RNA-binding proteins (RBPs) that influence RNA splicing, stability, and translation independently of their catalytic activities [140, 141]. As another example, IGF2BP1 is well known as an m^6A reader [142], but recent evidence suggests it may also recognize Nm-modified transcripts [78], potentially linking multiple epitranscriptomic pathways in PCa [143, 144]. The redundancy and pleiotropy of these proteins obscure mechanistic interpretation and complicate the development of selective inhibitors.

Adding further complexity, Nm modifications can occur across diverse RNA classes-including mRNA, tRNA, rRNA, and snRNA-with enzyme specificity influenced by subcellular localization, snoRNA scaffolds, and cell-type context [30]. In some cases, the same nucleotide position may be modified by distinct enzymes under different physiological conditions [145]. This raises the possibility that Nm may serve as a dynamic regulatory code, akin to the "histone code" in chromatin biology, rather than a static post-transcriptional mark. Elucidating this context dependency remains a key unmet need.

Technological barriers have also hindered progress. Although methods such as RiboMeth-seq, Nm-seq, nanopore direct RNA sequencing, and LC-MS/MS have enabled transcriptome-wide profiling of Nm sites, each suffers from trade-offs between sensitivity, resolution, and input requirements [146-150]. Reverse transcriptase (RT) stalling at Nm-modified nucleotides, once considered a major limitation, is now being leveraged as a feature for detection. However, distinguishing genuine RT stops caused by Nm from those due to RNA secondary structures or sequencing artifacts remains challenging [151, 152]. Further innovation in high-resolution, single-base detection, potentially through direct chemical labeling or structure-sensitive enzymatic assays, will be essential for confidently mapping Nm across low-abundance transcripts, especially in clinical samples [153].

To further advance the field, integrated multi-omics strategies, combined with improvements in sequencing resolution and modification specificity, will be critical. These approaches will facilitate the identification of novel Nm-modified targets, support the rational design of selective inhibitors, and ultimately enable the translation of molecular insights into therapeutic applications, thereby improving the diagnosis and treatment of PCa.

Author Contributions

S.W. wrote the review with the help of R.W.. Y.Y. supervised and revised the paper; All authors discussed the results and commented on the manuscript.

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Competing interests

The authors declare that there is no potential conflict of interest.

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Table 1: Nm-related writers

Methyltransferase	Superfamily	Catalytic dependency	Primary RNA substrate	Key modification site	Expression	Biological function	Ref
FBL	RFM/RrmJ	SnoRNA-dependent	rRNA, snRNA, mRNA	Numerous sites	Upregulate	Ribosome biogenesis, snoRNA-guided bulk methylation	[31, 83, 88, 154]
TARBP1	SPOUT	SnoRNA-independent	tRNA	Gm18	Upregulate	tRNA stability, translation regulation	[41, 83]
FTSJ1	RFM/RrmJ	SnoRNA-independent	tRNA	Cm32/Gm32, Am34	Upregulate	Translational fidelity and efficiency	[83, 155-157]
TRMT44	SPOUT	SnoRNA-independent	tRNA	Um44	Downregulate in mCRPC	tRNA stability	[42]
TRMT13	SPOUT	SnoRNA-independent	tRNA	Nm4	Upregulate in mCRPC	tRNA biogenesis and function	[40, 83]
FTSJ3	RFM/RrmJ	SnoRNA-independent	mRNA	Numerous sites	Upregulate in Primary prostate cancer; Downregulate in mCRPC	Innate immune evasion, mRNA stability and export	[69-71, 83]
CMTR1	RFM/RrmJ	SnoRNA-independent	RNA polymerase II transcripts	Cap1	/	Innate immune evasion, RNA stability, translational efficiency	[83, 158, 159]
CMTR2	RFM/RrmJ	SnoRNA-independent	RNA polymerase II transcripts	Cap2	Downregulate in primary prostate cancer	Innate immune evasion, RNA stability, translational efficiency	[29, 83, 160]
HENMT1	HEN	SnoRNA-independent	miRNA, piRNA,3'-tRFs	3'-terminal 2'-O-Me	Upregulate	Stabilizes small RNAs, prevents exonucleolytic degradation	[46, 47, 83]
MRM1	RFM/RrmJ	SnoRNA-independent	Mitochondrial 16S rRNA	Gm1145	Upregulate in primary PCa; Downregulate in mCRPC	Stabilizes catalytic core, ensure proper ribosome assembly and translation efficiency	[73, 83]
MRM2(FTSJ2)	RFM/RrmJ	SnoRNA-independent	Mitochondrial 16S rRNA	Um1369	Upregulate in mCRPC	Ensure accurate codon-anticodon recognition and proper SSU-LSU functional coupling	[73, 83]
MRM3	RFM/RrmJ	SnoRNA-independent	Mitochondrial 16S rRNA	Gm1370	Upregulate in primary PCa	Stabilizes A-site Trna binding and maintains translation speed and fidelity	[73, 83]