

Role of RNA 5-methylcytosine modification in cancer: Insights from coding and non-coding RNAs (Review)

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Received April 16, 2025; Accepted December 17, 2025

DOI: 10.3892/mmr.2026.13909

Abstract. RNA modifications serve notable roles in various biological processes, with >170 identified modifications. These modifications increase the complexity of RNA species by influencing their tertiary structure, biogenesis, localization and function. The combination of high-throughput detection technologies and corresponding analytical workflows provides a precise 5-methylcytosine (m⁵C) landscape, helping to elucidate its biological functions. The m⁵C methylation occurs in coding and non-coding RNAs and is dynamically regulated by related enzymes, including methyltransferases (writers), demethylases (erasers) and binding proteins (readers). m⁵C is involved in various physiological functions and regulates the progression of numerous types of tumors. Aberrant m⁵C RNA modifications contribute to the proliferation, migration and drug resistance of cancer cells, suggesting that targeting aberrant posttranscriptional modifications in cancer cells may hold promise as an efficient therapy for tumors. The present review systematically outlines the regulatory components of m⁵C modification, emphasizing their dynamic regulatory roles in RNA metabolism and function. The mechanisms by which m⁵C modification promotes tumor progression through the regulation of cancer cell proliferation, migration and drug resistance are summarized. The present review proposes that targeting abnormal m⁵C modifications could serve as a novel strategy for cancer treatment, offering new research directions in oncology.

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1. Introduction

Aberrant epigenetic modifications serve pivotal roles in the pathogenesis of a wide range of diseases. A key player in this process is methylation, a biochemical modification catalyzed by methyltransferases that add methyl groups to various targets, including DNA, RNA, histones and other proteins. The specificity and outcome of these methylation events are finely regulated by a complex network of proteins known as methyltransferases (writers), demethylases (erasers) and specific binding proteins (readers) (1). Recent multiomics studies have clarified that DNA and RNA methylation should not be viewed as isolated layers of regulation; integrated analyses have shown that compared with DNA methylation, RNA methylation often explains a comparable or even larger fraction of transcriptional variance (2,3). RNA modifications, such as N⁶-methyladenosine (m⁶A), 5-methylcytosine (m⁵C) and 7-methylguanylate (m⁷G), are crucial for the epigenetic regulation of gene expression and cellular functions, and markedly impact the development of numerous human diseases (4,5). Advances in single-nucleotide resolution sequencing and m⁵C antibody-based sequencing technologies have reignited interest in the role of m⁵C modifications in human diseases (6).

m⁵C is a widespread, evolutionarily conserved modification present in both coding and non-coding RNAs (ncRNAs) and is essential for regulating RNA metabolism and function. It influences several aspects of RNA processing, including transfer RNA (tRNA) stability, ribosomal RNA (rRNA) assembly, mRNA translation and the regulation of ncRNA (7-9). Dysregulation of m⁵C modification, often due to abnormal expression of related enzymes, alters RNA methylation and disrupts multiple signaling pathways (10). This disruption contributes to the onset and progression of

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Key words: RNA modification, 5-methylcytosine, mRNA, non-coding RNA, cancer

neurological disorders, metabolic diseases and cancers (11). In particular, the study of RNA m⁵C modifications has become a central focus in cancer research. These modifications are known to impact critical cancer-related processes such as cell proliferation, metastasis, therapy resistance and the modulation of the tumor microenvironment (12). Due to the marked role of m⁵C modifications in tumor immunity, therapeutic strategies targeting these modifications are being explored as potential cancer immunotherapies (13,14).

The present review aimed to provide an in-depth overview of the distribution and regulation of m⁵C modifications in both coding and ncRNAs and to explore their roles in cancer progression. By examining how m⁵C modification influences RNA metabolism and function, the present study aimed to deepen the understanding of the critical role of RNA methylation in epigenetic regulation and offer new insights into the regulatory networks contributing to cancer.

2. Writers, erasers and readers for m⁵C modification

RNA methyltransferases use S-adenosylmethionine (SAM) as a methyl donor to transfer a methyl group to cytosine, forming m⁵C, which occurs at position 5 of cytidine residues in RNA (15,16). Key enzymes involved in this process include members of the NOL1/NOP2/SUN domain (NSUN) family, the DNA methyltransferase (DNMT) homolog DNMT2 and members of the tRNA-specific methyltransferase family. NSUN proteins contain an RNA recognition motif and a Rossmann fold catalytic core that accommodates the SAM cofactor; NSUN proteins use two catalytic cysteines in the active site, whereas DNMT2 uses a single active site cysteine (17,18). A covalent intermediate is formed between the protein cysteine and the cytosine in RNA, which activates the electron-deficient pyrimidine ring, allowing the nucleophilic attack of carbon 5 on the methyl group of SAM. NSUN family proteins use the cysteine in motif VI to perform a nucleophilic attack on carbon 6 of the target cytosine in RNA, whereas DNMT family proteins use the cysteine in motif IV (19,20). The NSUN family, which spans NSUN1 to NSUN7, serves extensive and potentially overlapping roles in regulating early embryogenesis, tumorigenesis and cell proliferation (21). DNMT2, traditionally known as a cytosine DNMT, also functions as an RNA methyltransferase because of its unique sequence and structure in the catalytic motif, notably impacting cell proliferation and migration (22-24).

Demethylases involved in m⁵C modification include members of the ten-eleven translocation (TET) protein family (TET1, TET2 and TET3), which can oxidize 5-formylcytosine (f⁵C) in RNA or contribute to RNA degradation (25-27). TET family proteins are more active in DNA than in RNA and are typically responsible for DNA demethylation (28,29). In RNA samples from various tissues and cells, ~0.02% of m⁵C is modified to f⁵C (30). TET-mediated m⁵C oxidation is involved in several physiological functions, this m⁵C oxidation activity dictates the global chromatin regulation in mouse embryonic stem (mES) cells, human hematopoietic stem cells (HPSCs) and leukaemia cells (28,31).

Additionally, the α -ketoglutarate-dependent dioxygenase ABH1 (ALKBH1) functions as a demethylase and serves a well-documented role in the mitochondria (32). ALKBH1 is

involved in the biogenesis of 5-hydroxymethyl-2'-O-methylcytidine (hm⁵Cm) and 5-formyl-2'-O-methylcytidine (f⁵Cm) at position 34 of cytoplasmic tRNA-leucine (ct-tRNA^{L^{eu}}) and f⁵C in mitochondrial (mt)-tRNA^{Met}, which are essential for efficient translation (33). ALKBH1 exhibits enzymatic activity against several substrates, including m⁶A, N1-methyladenosine, N3-methylcytidine, m⁵C and histone H2A (34); this substrate diversity and specificity are key to its association with tumors.

Proteins that specifically bind to methylation sites, such as RNA export factor binding protein 2 [Aly/REF export factor (ALYREF)] and Y-box binding protein 1 (YBX1), are crucial for determining the biological functions of RNA modifications (35,36). ALYREF is responsible for exporting mRNA from the nucleus, specifically binding to m⁵C-modified mRNA to form mRNA ribonucleoprotein complexes. YBX1 can recognize and bind to m⁵C-modified mRNA through the Trp45 residue in its cold shock domain, thereby maintaining its stability; however, a mutation in the indole ring of Trp45 disrupts this interaction and abolishes its binding capability (37-40). Another notable binding protein for m⁵C is methyl-CpG binding protein 2 (MeCP2), which interacts with the m⁵C-modified long non-coding RNA (lncRNA) lncRnc3 to maintain the neural progenitor pool, thereby limiting neuronal differentiation. Bioinformatic analyses also suggest that MeCP2 may bind various cytosine-methylated RNAs in the brain (41). Moreover, while the YTH domain family protein 2 (YTHDF2) is a reader for RNA m⁶A modification, studies have indicated that it can also directly bind to m⁵C in RNA, although with a lower affinity compared with m⁶A (42).

3. Methods for detecting m⁵C modification

The detection of target genes with RNA methylation modifications is essential for studying m⁵C modifications. Current technologies and methods enable both qualitative and quantitative analysis at the single-nucleotide level, offering new approaches for research. The primary methods for analyzing m⁵C methylation are discussed in the present section.

Mass spectrometry (MS) and liquid chromatography-tandem mass spectrometry (LC-MS/MS). MS uses nucleases to catalytically degrade RNA sequences into nucleosides, which are then separated and identified based on their mass-to-charge ratio; this approach enables the detection of modified nucleosides. MS is widely used for detecting various RNA modifications; however, it requires markedly purified and concentrated target RNA and has relatively low sensitivity (43). LC-MS/MS partially addresses these limitations, as it can determine the overall abundance of RNA m⁵C modifications in a sample; however, its resolution does not reach the single-nucleotide level (44,45).

RNA bisulphite sequencing (RNA-BisSeq) and TET-assisted peroxotungstate oxidation (TAWO) sequencing. RNA-BisSeq is a widely applicable and cost-effective method for the specific analysis of m⁵C RNA. Bisulfite treatment converts unmethylated cytosines in single-stranded RNA to uracil, whereas methylated cytosines remain unchanged. When combined with high-throughput sequencing, this technique can generate a transcriptome-wide m⁵C map with single-nucleotide

resolution. However, this method can be influenced by factors such as RNA structure, experimental temperature and other variables, which may lead to false-positive results (16,46).

TAWO sequencing combines peroxotungstate oxidation with TET enzyme oxidation, using peroxotungstate to oxidize 5^mC to trihydroxylated thymine (thT), and thermostable group II intron reverse transcriptase to convert thT to T during cDNA synthesis. TAWO sequencing allows for the direct detection of modified cytosines without affecting unmodified cytosines, thus overcoming the false-positive issue associated with BisSeq (47). However, because TAWO sequencing relies on the conversion of m⁵C, the conversion efficiency in mRNA samples requires further optimization.

Immunoprecipitation. Methylated RNA immunoprecipitation sequencing (MeRIP-seq) identifies markedly methylated RNA fragments by using an RNA m⁵C antibody to bind m⁵C-modified RNA, combined with high-throughput sequencing. This method can detect low-abundance m⁵C modification sites and avoids interference from other RNA modifications, although it is notably dependent on the specificity of the m⁵C antibody (48,49). Methylation individual-nucleotide resolution crosslinking and immunoprecipitation (miCLIP) uses an NSUN2 antibody instead of an m⁵C antibody to target the NSUN2 m⁵C transcriptome landscape (50,51). Moreover, 5-azacytidine-mediated RNA immunoprecipitation (5-azaIP) utilizes 5-azacytidine, a cytidine analogue that forms a reversible covalent bond with RNA m⁵C methyltransferases; methyltransferase antibodies are then used to pull down the target RNA. 5-azaIP reduces the non-specific RNA background and can identify enzyme-specific methylation sites with single-nucleotide resolution; however, 5-azacytidine is markedly toxic, and its incorporation efficiency into RNA can influence the results (52,53).

Nanopore sequencing. Nanopore direct RNA sequencing (DRS) enables direct interrogation of RNA molecules without the need for cDNA conversion, allowing for the capture of transcript isoforms and the preservation of epitranscriptomic modifications (54,55). As RNA passes through the nanopore, the bases cause characteristic changes in the electrical current, which are then used to infer the corresponding bases from the signal data (56). DRS provides a powerful platform for parallel profiling of various RNA modifications, revealing the complexity of the epitranscriptome (57). However, its implementation poses challenges due to high costs and a reliance on machine learning-based analysis, where methodological variability can limit reproducibility (55); therefore, the development of more robust and integrative analytical tools remains a critical hurdle.

Predictive models. Experimental detection methods provide precise information about RNA modifications; however, they are not without limitations, including operational complexity and high costs. As a result, several models have been developed to predict RNA m⁵C modifications. These include PEA-m⁵C (58), m⁵C-PseDNC (59) and m⁵C-HPCR (60). Additionally, some models, such as m⁵C-Pred-SVM (61) and DeepMRMP (62), are designed to predict specific m⁵C sites. However, the predictive accuracies of these models vary

notably across different species, highlighting the need to improve the accuracy and specificity of traditional prediction models. The integration of experimental detection tools with predictive models can lead to more accurate experimental data, ultimately enhancing the precision of research outcomes.

4. Modification of m⁵C in mRNA

Advances in high-throughput sequencing, such as m⁵C-RIP-seq (49), miCLIP-seq (63,64), AZA-IP-seq (52) and RNA-BisSeq (16), have revealed that m⁵C is present on mRNA. The distribution of m⁵C is species-specific, and is predominantly located in the 5'untranslated regions (UTRs) of *Drosophila*, coding sequence regions (CDSs) and 3'UTRs in mouse and human HeLa cells (65), whereas in zebrafish embryos, m⁵C sites are enriched primarily in only CDS regions (38).

Studies have shown that the m⁵C modification in mRNA is catalyzed mainly by NSUN2 and NSUN6; as a primary m⁵C methyltransferase, NSUN2 regulates the cell cycle, cell differentiation and proliferation (22,66). In endometrial cancer, NSUN2 has been demonstrated to promote tumor cell proliferation both *in vivo* and *in vitro*. The m⁵C modification ability of NSUN2 depends mainly on the release site (cysteine 271) and the catalytic site (cysteine 321); when these two sites are mutated, NSUN2 is unable to promote tumorigenesis through methylation modification (Table I) (67). Methylation of oncogenes by the m⁵C methyltransferase promotes mRNA stability, leading to tumor development.

NSUN6 also modifies mRNA, primarily by targeting the 3'UTR within the consensus sequence motif CTCCA present in hairpin loop structures, which marks the translation termination site. NSUN6 expression varies across human tissues, being most prevalent in the testes and least prevalent in the blood (68). The role of NSUN6 in tumorigenesis remains a subject of ongoing scientific debate; in cancers such as pancreatic cancer, glioblastoma and esophageal squamous cell carcinoma, NSUN6 acts as a tumor suppressor, potentially inhibiting malignant progression or modulating therapeutic responsiveness through m⁵C-mediated mRNA methylation (Table I) (69-71). Conversely, NSUN6 is frequently upregulated in cervical cancer, gastric cancer and triple-negative breast cancer, where it facilitates tumor initiation and aggressiveness (Table I) (72-74). This functional dichotomy may be linked to the complexity of the tumor microenvironment (TME), where extracellular matrix interactions and metabolic heterogeneity collectively influence oncogenesis and metastatic behavior (75,76). Immune infiltration patterns also differ markedly between NSUN2 and NSUN6: NSUN2 expression predominates in monocytes/macrophages and proliferative T cells, whereas NSUN6 is most abundant in regulatory T cells (74,77). Such distinctions may help explain the context-dependent roles of NSUN6 across cancer types and disease stages. Future studies should aim to elucidate the integrated expression dynamics of NSUN6 within both the tumor parenchyma and stromal compartments to advance personalized oncology strategies.

TET2, a recognized tumor suppressor, inhibits myeloid tumors in an enzyme activity-dependent manner and serves a marked role in inflammatory responses (78); however,

Table I. m⁵C-modifying enzymes regulate the role of different RNAs in cancer.

A, mRNA			
m ⁵ C regulators	Cancer	Function	(Refs.)
ALYREF	Non-small cell lung cancer	LINC02159 binds to ALYREF and enhances m ⁵ C modification of YAP1 mRNA, activating the Hippo and β -catenin pathways.	(82)
NSUN2/YBX1	Endometrial cancer	NSUN2/SLC7A11/YBX1 axis can inhibit tumor growth by increasing lipid peroxidation and ferroptosis in endometrial cancer cells.	(67)
	Bladder cancer	NSUN2/heparin-binding growth factor/YBX1 axis activates oncogenic pathways in bladder urothelial carcinoma.	(83)
NSUN5	Glioma	NSUN5 downregulates β -catenin expression by promoting the degradation of β -catenin mRNA, thereby enhancing the phagocytic activity of tumor-associated macrophages.	(80)
NSUN6	Pancreatic cancer	NSUN6 inhibits the proliferation of pancreatic cancer cells and is associated with recurrence and prognosis.	(70)
	Cervical cancer	NSUN6/N-myc downstream regulated 1/ALYREF pathway activates radiosensitivity.	(73)
	Gastric cancer	NSUN6/CEBPZ m ⁵ C methylation activates the p53/mTOR pathway and inhibits autophagy.	(72)
	Glioblastoma	NSUN6 controls the response to temozolomide treatment through m ⁵ C-mediated regulation of NELFB and RPS6BK2, and high NSUN6 expression prolongs the survival of patients with glioblastoma.	(69, 140)
	Esophageal squamous cell carcinoma TNBC	NSUN6 inhibits the progression of esophageal squamous cell carcinoma by regulating CDH1 mRNA translation. NSUN6 expression is higher in TNBC than in normal tissues, and may be involved in extracellular matrix receptor interactions, metabolism and cell adhesion.	(71) (74)
TET2	Leukemia	TET2 deficiency leads to the accumulation of TSPAN13 mRNA m ⁵ C modification, which plays a marked functional role in leukemia development, leukemia blast migration/homing and leukemia stem cell self-renewal.	(79)
B, tRNA			
m ⁵ C regulators	Cancer	Function	(Refs.)
NSUN2	ATC	NSUN2 plays a role in stabilizing tRNA, promoting the transport of amino acids such as leucine, and improving translation efficiency in ATC cells, and is involved in the formation, proliferation, and drug resistance of ATC cells.	(92)
	TNBC	NSUN2 mediates m ⁵ C modification of tRNA Val-CAC, enhancing codon frequency-dependent translation of key glycolysis-related genes (including ALDH3A2, ALDH7A1, HK1 and PFKM), thus conferring docetaxel resistance in TNBC cells.	(141)
	Colon cancer	Under hypoxic conditions, NSUN2 modulates the tRNA-Arg C34 site via m ⁵ C modification, regulating the selective expression of tRNA-derived fragment and promoting tumor metastasis.	(93)
C, Ribosomal RNA			
m ⁵ C regulators	Cancer	Function	(Refs.)
NSUN5	HCC	NSUN5 can promote the proliferation and migration of HCC cells and is associated with poor patient prognosis.	(109)

Table I. Continued.

D, lncRNA			
m ⁵ C regulators	Cancer	Function	(Refs.)
NSUN2	Gastric cancer	NSUN2-methylated NR_033928 promotes gastric cancer progression through GLS-mediated glutamine metabolism.	(142)
	Cholangio-carcinoma	NSUN2 interacts with lncRNA NKILA, increasing its m ⁵ C level and promoting its interaction with YBX1, thus accelerating cholangiocarcinoma progression via the miR-582-3p-YAP1 axis.	(128)
	HCC	m ⁵ C-modified H19 lncRNA may promote tumorigenesis and development by recruiting G3BP1 oncoprotein.	(143)
E, circRNA			
m ⁵ C regulators	Cancer	Function	(Refs.)
NSUN2	Breast cancer brain metastasis	NSUN2-mediated m ⁵ C methylation enhances the stability of hsa_circ_0004516 and activates the AKT signaling pathway.	(136)
NSUN2	Lung cancer	NSUN2-mediated m ⁵ C modification of circFAM190B enhances its expression, thereby inhibiting autophagy through the novel SFN/mTOR/ULK1 signaling pathway.	(133)
NSUN2/ ALYREF	Lung cancer	NSUN2/circRREB1/ALYREF mediates m ⁵ C modification, initiates mitophagy, and promotes lung cancer progression.	(134)
NSUN4	Lung cancer	NSUN4-mediated m ⁵ C modification enhances the nuclear export of circERI3, which regulates mitochondrial energy metabolism through the DDB1/PGC-1 α signaling pathway.	(135)

m⁵C, 5-methylcytosine; TNBC, triple negative breast cancer; HCC, hepatocellular carcinoma; ATC, anaplastic thyroid cancer; lncRNA, long non-coding RNA; tRNA, transfer RNA; NSUN, NOL1/NOP2/SUN family; YBX1, Y-box binding protein 1; ALYREF, Aly/REF export factor; TET, ten-eleven translocation; GLS, glutaminase; circRNA, circular RNA; SLC7A11, solute carrier family 7 member 11; YAP1, Yes1 associated transcriptional regulator; NELFB, negative elongation factor complex member B; RPS6BK2, NOP2/Sun RNA methyltransferase 6; CDH1, cadherin-1; TSPAN13, tetraspanins-13; ALDH, aldehyde dehydrogenase; HK1, hexokinase-1; PFKM, phosphofructokinase, muscle; SFN, stratifin; ULK1, unc-51 like autophagy activating kinase 1; DDB1, DNA damage-binding protein 1; PGC-1 α , Pparg coactivator 1 α .

the involvement of TET2 in m⁵C methylation modification remains controversial. A study by Li *et al* (79) demonstrated that TET2 functions as an m⁵C demethylase and its deficiency leads to the accumulation of TSPAN13 mRNA, promoting leukemogenesis and enhancing the self-renewal capacity of leukemia stem cells. Zou *et al* (31) proposed that TET2 acts as a DNA 5mC (abbreviated as ‘5mC’ in DNA) oxidase in leukemia; additionally, TET2-mediated oxidation also serves a role in glioma (80). However, TET2 promotes M2 macrophage polarization in allergic rhinitis by regulating m⁵C oxidation in mRNA (81). In patients with diabetic nephropathy (DN), TET2 expression is associated with renal impairment, and TET2-mediated m⁵C modification regulates mitophagy, suggesting its potential as a therapeutic strategy for DN (82). Due to the multifaceted biological functions of TET2, the specific molecular mechanisms by which it acts as an RNA m⁵C demethylase in tumors have not yet been fully elucidated. This lack of clarity may arise from its broad range of actions and involvement in multiple signaling pathways, which could contribute to a non-specific role in RNA methylation modification. Future research should integrate both

the oxidative and demethylating functions of TET2 to further explore its impact on the TME, thus providing directions for the development of novel targeted or immunotherapeutic strategies.

In addition, Chen *et al* (83) found that YBX1 can recognize m⁵C-modified mRNA through the indole ring of W65 in its cold shock domain; high levels of m⁵C modification promote bladder cancer progression and are associated with the role of YBX1 in stabilizing mRNA in the cytoplasm. ALYREF, an mRNA export factor, serves as the principal nuclear m⁵C reader that promotes mRNA export (22). In non-small cell lung cancer, ALYREF recognizes m⁵C-methylated mRNA, which increases its stability and leads to its translation, thus activating the oncogenic pathway (Table I) (84). The oncogenic role of the downstream reader proteins in m⁵C modification is also critical. In addition to affecting mRNA stability and export, studies using bisulfite conversion and RNA sequencing have shown that m⁵C levels are negatively associated with mRNA translation, particularly within the CDS (85); however, the precise mechanism by which m⁵C affects mRNA translation still requires further investigation.

5. Modification of m⁵C in ncRNAs

The m⁵C modification in ncRNAs, including tRNA, lncRNAs, small ncRNAs (sncRNAs) and rRNA, is also involved in carcinogenesis. These ncRNAs regulate gene expression, maintain genome stability and mediate key cellular processes.

tRNA. The m⁵C modification in tRNA is among the earliest and most extensively studied aspects of RNA biology. Methylation typically occurs at the junction of the variable loop and the T stem, often involving positions 47-50, with 1-3 cytosine residues (51). In mice, cytosine at position 38 within the anticodon loop of tRNA is another frequent modification site (86). The m⁵C sites in cytoplasmic tRNA are relatively conserved and play a vital role in maintaining tRNA secondary structure stability and regulating translation efficiency. Moreover, the m⁵C modification regulates codon/anticodon pairing, which is essential for the correct loading of amino acids and the prevention of misloading (25).

Among the tRNA methyltransferases, DNMT2 and NSUN enzymes have been extensively studied (23). The deletion of DNMT2 in mouse cells increases the proportion of uncharged tRNA^{Asp}, resulting in decreased translation efficiency of proteins containing polyaspartic acid (86); this deletion also triggers tRNA fragmentation and decreases steady-state levels (87,88). Additionally, the m⁵C modification safeguards tRNAs against stress-induced endonuclease-mediated fragmentation, thereby ensuring the accurate translation of near-cognate codons. For example, DNMT2-mediated m⁵C modification in tRNA^{Asp} helps to distinguish it from tRNA^{Glu}, preventing amino acid misincorporation (89). Studies have also shown that a reduction in DNMT2 methyltransferase activity due to somatic cancer mutations strongly associated with decreased tRNA levels (90,91). Furthermore, the TET2-mediated oxidation of m⁵C in tRNA disrupts the binding of readers to RNA and produces 5-hydroxymethylcytosine (hm⁵C), altering tRNA methylation and thus impacting translation (25). In anaplastic thyroid cancer (ATC), NSUN2 knockdown markedly reduces tRNA m⁵C modification, suggesting it has a role in stabilizing tRNA in ATC, facilitating the transport of amino acids such as leucine and enhancing translation efficiency. Moreover, NSUN2 also contributes to the formation, proliferation and drug resistance of ATC cells (Table I) (92). In addition to influencing codon translation, NSUN2 also regulates the cleavage site of tRNA^{Arg} in colorectal cancer in an m⁵C-dependent manner under hypoxic conditions and serves a critical role in tumor metastasis (Table I) (93).

Mt-tRNA^{Met} also undergoes m⁵C modification, which is essential for the expression of the mitochondrial genome. This modification supports cellular energy metabolism and various metabolic pathways and plays notable roles in tumorigenesis and metastasis (94). The mt-tRNA^{Met} not only decodes the conventional AUG codon but also mediates the incorporation of methionine into the AUA codon during translation initiation (32,95,96). These modifications of mt-tRNA^{Met} predominantly occur in the anticodon and its surrounding regions, especially at the wobble position. Additionally, f⁵C, an oxidation product of m⁵C, plays a critical role in enabling mt-tRNA^{Met} to decode the AUA methionine codon during mitochondrial translation (32,97,98). A previous study highlighted

that the α -ketoglutarate-dependent dioxygenase ALKBH1 is involved in the biosynthesis of f⁵C at the first position of the anticodon (position 34 of tRNA) in mt-tRNA^{Met}, thus facilitating this decoding process. ALKBH1 also promotes the formation of hm⁵Cm and f⁵Cm at the same position in cytoplasmic tRNA^{Leu} (99). The ALKBH1-mediated oxidation of m⁵C has been shown to be vital for translation and mitochondrial function, underscoring its notable role in cellular processes (97). Furthermore, m⁵C modification of mt-tRNA^{Met} dynamically regulates tumor cell metastasis and invasion by modulating the initiation and maintenance of mt-mRNA translation (100). Depletion of NSUN3 results in marked reductions in the levels of m⁵C and f⁵C at C34 in mt-tRNA^{Met}, altering mitochondrial morphology and reducing the number of mitochondrial cristae, thereby regulating cellular energy metabolism (101).

In conclusion, the m⁵C modification of tRNA enhances translation efficiency and helps prevent amino acid mismatches. Studies have also revealed that m⁵C modification contributes to the site-specific cleavage of tRNA. Targeting tRNA methylation modifications may serve as a therapeutic target for certain malignancies with poor prognosis, such as ATC. Moreover, due to the aberrant energy metabolism in malignant tumors, especially the increased metabolic plasticity in aggressive and metastatic cancers, inhibiting the m⁵C modification of mt-tRNA^{Met} presents a promising therapeutic strategy to effectively curb the spread of malignant tumors (94).

rRNA. A growing body of evidence links alterations in rRNA modification levels and defects in components of the rRNA modification machinery to tumors. rRNAs are extensively modified during their transcription and subsequent maturation in the nucleolus, nucleus and cytoplasm (102,103). The m⁵C modification in rRNA is associated with ribosome synthesis and protein translation; the m⁵C modification stabilizes the RNA structure by promoting base stacking and increasing the thermal stability of hydrogen bonding with guanine. In functionally crucial areas of the ribosome, m⁵C modification helps stabilize rRNA folding, which is essential for efficient ribosome function (42,104,105). Studies have shown that NSUN4 is crucial for the biosynthesis of mitochondrial rRNA; the knockout of NSUN4 results in the loss of methylation at position C911 of mitochondrial rRNA in mouse hearts, suggesting its key role in coordinating mitochondrial ribosome biosynthesis (106,107). Similarly, NSUN5 is necessary for the specific methylation of 28S rRNA in humans and mice, and its absence reduces protein synthesis, underscoring the importance of NSUN5 in ribosome function and translation regulation (108). In hepatocellular carcinoma, overexpression of NSUN5 upregulates the m⁵C level of 28S rRNA, which in turn promotes growth and metastasis (Table I) (109). Additionally, the depletion of YTHDF2, a multifunctional reader, results in a marked increase in m⁵C levels at various sites within rRNA. This alteration may influence translation fidelity, suggesting a potential link between the role of YTHDF2 in recognizing m⁵C and its impact on translation accuracy (42). A number of studies have shown that YTHDF2 is dysregulated in malignant tumors such as bladder cancer, liver cancer, gastric cancer, osteosarcoma and blood system by m⁶A methylation modification (110-115), while, to the best

of our knowledge, no studies have investigated the specific mechanism of m⁵C methylation modification in malignant tumors. Further research is expected to uncover the potential biological functions of YTHDF2.

sncRNAs. The sncRNAs, which include microRNAs (miRNAs), PIWI-interacting RNAs, small interfering RNAs and tRNA-derived small RNAs (tsRNAs), play vital roles within cells by binding to proteins, regulating transcription factors and participating in genome stability (24). High-throughput next-generation sequencing-based methods, such as bisulphite miRNA sequencing, and an analysis pipeline such as methylation assessment of miRNAs after bisulphite analysis, have revealed widespread m⁵C modifications in miRNAs (116). The miRNAs regulate gene transcription and posttranscriptional regulation through partial base pairing with sequences mainly in the 3'UTRs of target mRNAs, guiding the RNA-induced silencing complex to suppress mRNA translation. The m⁵C modification in miRNA affects its pairing with mRNA, potentially disrupting its gene-silencing activity (117). To the best of our knowledge, few studies have investigated the role of RNA m⁵C methylation in miRNAs in cancer, although some studies have explored DNA methylation. In glioblastoma, miRNA is methylated at cytosine residues through complexes such as DNMT3A/AGO4, which abrogates its inhibitory effect on gene expression and is associated with poor prognosis (118). Additionally, a pivotal study has successfully developed a serum diagnostic signature using m⁵C-modified miRNAs (119). Due to the potential crosstalk between DNA methylation and RNA methylation, studying the role of RNA methylation in miRNAs may lead to notable breakthroughs in tumor treatment and diagnosis (119).

tsRNAs, derived from tRNA, interact with cytoplasmic ribonucleoproteins (RNPs) to form tsRNA-RNP complexes. These complexes can bind to Argonaute family proteins, effectively mediating posttranscriptional gene silencing through mechanisms such as RNA interference or direct inhibition of mRNA translation (120). DNMT2-mediated m⁵C modifications in mice markedly affect sperm tsRNA levels, with the loss of DNMT2 disrupting the transmission of high-fat diet-induced metabolic disorders mediated by sperm sncRNA to offspring (24). It has been demonstrated that tsRNAs can influence translation rates through YBX1. In breast cancer cells, tsRNAs can induce tumor-suppressive effects by replacing YBX1 with oncogenic mRNAs, thereby promoting the degradation of such transcripts (121-123). Thus, the m⁵C modifications in tRNA notably influence tsRNA functionality, and the regulation of protein translation mechanisms by tsRNA represents a promising new avenue of investigation in cancer studies.

The utilization of innovative detection technologies has notably advanced research into m⁵C modifications of sncRNAs. These advancements enhance the understanding of intracellular signaling mechanisms associated with various diseases. These findings open new avenues for targeted sncRNA therapies, offering more precise and effective treatment options.

lncRNAs. lncRNAs, a category of ncRNAs >200 nucleotides in length, constitute a substantial part of the mammalian

transcriptome (124). lncRNAs participate in various physiological and pathological processes in normal tissues and tumors by interacting with RNA-binding proteins. These processes include chromatin modification, transcriptional activation, transcriptional interference and nuclear transport (125,126). One of the enzymes involved in modifying lncRNAs is NSUN2, which has been linked to the development of various cancers. In gastric cancer, NSUN2 modifies the lncRNA NR_033928, which in turn affects the stability of glutaminase mRNA and participates in metabolic reprogramming (127). Similarly, in cholangiocarcinoma, NSUN2 interacts with the lncRNA NKILA, promoting disease progression (Table I) (128). In the nervous system, lncRNAs are involved in ischemic stroke through mechanisms such as calcium overload, oxidative stress, hypoxia and inflammatory responses (129). Notably, MeRIP-Seq analysis revealed increased methylation levels and frequency in lncRNAs in the middle cerebral artery occlusion model, which simulates human ischemic stroke. These differentially methylated lncRNAs are associated with several notable pathways, including mTOR signaling, Rap1 signal transduction, pyrimidine metabolism, dopamine receptor binding and phosphatidylinositol phosphate kinase activity (130).

The study of m⁵C modifications in lncRNAs within tumors is still in its early stages, with NSUN2 currently being the only gene known to function as a methyltransferase for lncRNAs. As bioinformatics analyses continue to accumulate, a high m⁵C score has been closely associated with the activation of malignant tumor-related pathways and the impairment of immune microenvironment functions. Furthermore, m⁵C-related lncRNAs show promise as biomarkers for malignant tumors and potential therapeutic targets, making this a notable prospective research direction (131).

Circular RNAs (circRNAs). circRNAs are a class of covalently closed RNA molecules lacking free 5' and 3' ends, and most circRNAs are non-coding; however, growing evidence indicates that some can encode functional proteins (132). Although circRNAs play marked roles in the pathogenesis of various diseases, the functional implications of m⁵C methylation on circRNAs remain largely unexplored. The emerging role of m⁵C-modified circRNAs in lung cancer pathogenesis has attracted increasing attention in recent studies. Chen *et al* (133) reported that NSUN2-mediated m⁵C modification of circFAM190B enhances its stability, suppresses autophagy and ultimately drives tumorigenesis. The study by Wu *et al* (134) revealed that NSUN4-catalyzed m⁵C modification of circERI3 facilitates its nuclear export, which in turn promotes the development and progression of lung cancer by enhancing mitochondrial energy metabolism. Cai *et al* (135) identified circRREB1 as an m⁵C-modified circRNA in lung cancer. This modification, mediated by the methyltransferase NSUN2 and recognized by the reader protein ALYREF, activates mitophagy to promote tumor progression. The functional relevance of circRNA m⁵C modification has also been implicated in other malignancies, including esophageal squamous cell carcinoma and breast cancer (Table I) (136,137). These findings demonstrate that m⁵C methylation can regulate broader processes such as autophagy and mitochondrial metabolism through

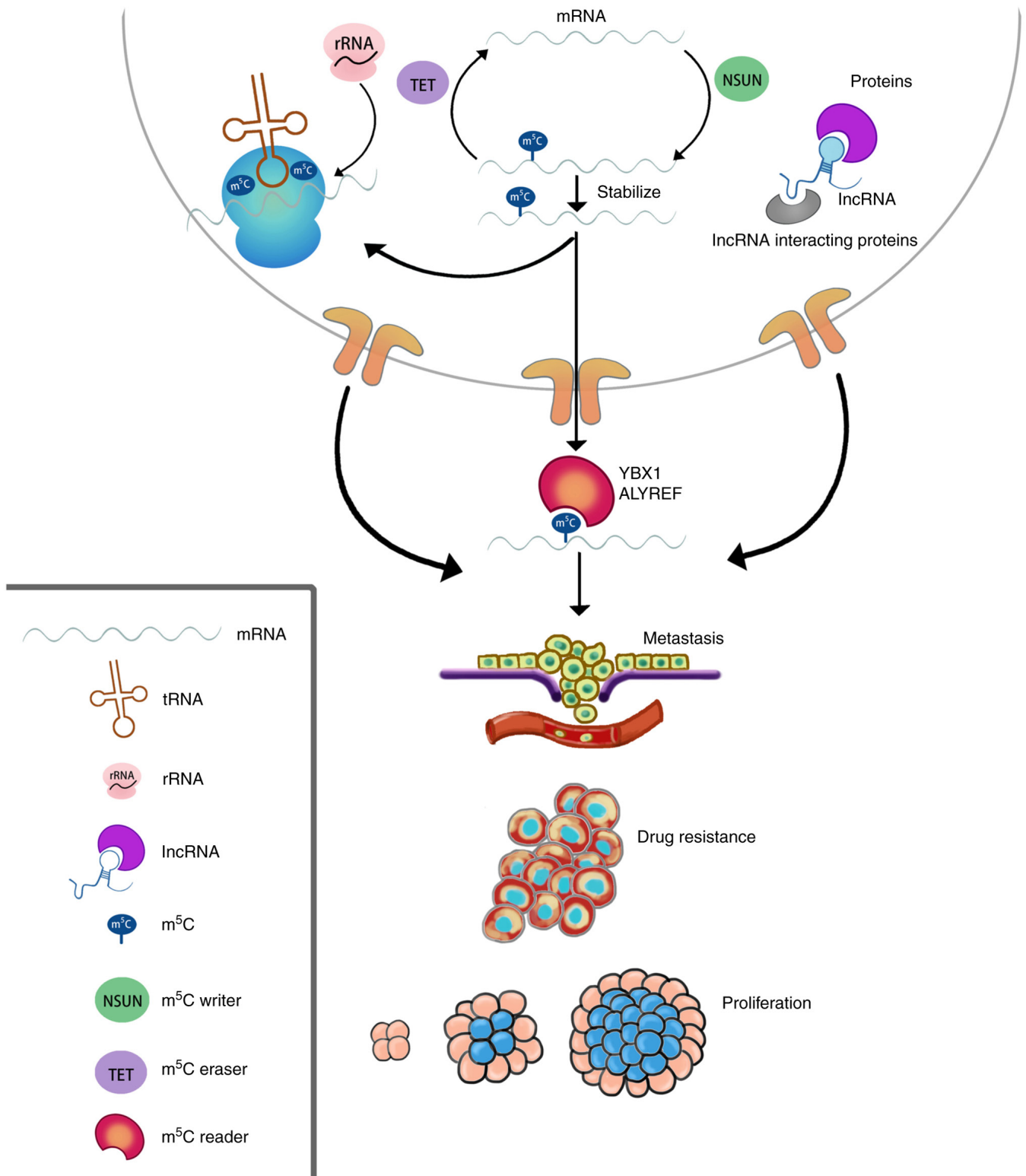


Figure 1. m⁵C modification in coding and non-coding RNAs. NSUN family proteins, as ‘writers’ promote the stability of downstream target mRNAs through m⁵C methylation and facilitate mRNA nuclear export. The TET family, which acts as ‘erasers’ removes methylation modifications, whereas YBX1 or ALYREF, which act as ‘readers’ recognize methylated mRNA. These processes mediate tumor cell development, metastasis and drug resistance. The m⁵C modification in rRNA is linked to ribosome synthesis and protein translation. The m⁵C modification in lncRNAs affects interactions with RNA-binding proteins. m⁵C, 5-methylcytosine; tRNA, transfer RNA; rRNA, ribosomal RNA; lncRNA, long non-coding RNA; NSUN, NOL1/NOP2/SUN family; YBX1, Y-box binding protein 1; ALYREF, Aly/REF export factor; TET, ten-eleven translocation.

the modification of circRNAs. Given the fundamental role of RNA modifications in physiology, research on m⁵C methylation in ncRNAs has been increasing, extending

beyond mRNA, tRNA and rRNA to include insights into the mechanisms of lncRNA and circRNA modification. Future studies should further expand the scope from cancer

to other pathological and functional fields, providing new perspectives for diagnosing diseases through non-invasive examinations.

6. Conclusions

RNA modifications, particularly RNA methylation, represent a rapidly evolving field and play an indispensable role in regulating gene transcription, expression, editing, stability and degradation. A notable number of tumors exploit RNA modifications as a mechanism to enhance or suppress gene expression, ultimately leading to tumor development (Fig. 1). Methylation is critically involved in diverse physiological and pathological processes; notably, its reversible nature underscores the considerable therapeutic potential of targeting specific methylation sites in cancer, positioning it as a promising strategy for future early-stage interventions. Pioneering this approach, the DNA methyltransferase inhibitor 5-azacytidine was developed to reverse aberrant methylation and is now a standard treatment for high-risk myelodysplastic syndromes (138). This success has spurred interest in targeting RNA modifications as a novel strategy for treating various diseases. Key research directions in precision medicine now include developing small-molecule inhibitors against specific modifying enzymes and utilizing serum methylation patterns to predict disease progression. However, both DNA- and RNA-methylation-targeted therapies still face notable challenges, such as selectively targeting the oncogenic functions of these proteins without inducing widespread epigenetic disruption. Inhibiting methylation or demethylation at specific loci often results in limited efficacy, likely due to functional redundancy and crosstalk among different methyltransferases. Therefore, future efforts must prioritize understanding these interactions within specific tumor contexts and developmental stages, while rigorously evaluating the safety profiles of these emerging therapies (139).

Although some clues regarding the potential role of m⁵C methylation in tumors have been uncovered, the specific molecular mechanisms of m⁵C ‘writers’, ‘erasers’ and ‘readers’ still require further elucidation. Regarding molecular mechanisms, future research on m⁵C methylation in tumors should extend beyond its effects on proliferation and migration to elucidate its role in the TME. By integrating multiomics approaches, including proteomics and metabolomics, it will be possible to investigate how m⁵C methylation influences immune cells, an endeavor expected to yield novel therapeutic strategies. Furthermore, translating the functional relevance of m⁵C methylation into clinical practice is crucial. This includes developing small-molecule inhibitors that target either m⁵C modifications or their associated writers/erasers and delivering them precisely to primary tumors using nanocarriers to effectively halt tumor progression. Notably, future studies should move beyond analyzing m⁵C in isolation and instead dissect how it cooperates with DNA methylation and the methylation of other RNAs, particularly m⁶A, at defined genomic loci and on specific transcripts. Advances in single-molecule and multiomics technologies will be essential to resolve this hierarchy in tumors and inform the rational design of more effective combination epigenetic therapies. Taken together, m⁵C methylation represents a promising target for future research and tumor treatment.

Acknowledgements

Not applicable.

Funding

The present study was supported by Provincial Natural Science Foundation of Hunan (grant no. 2023JJ30769, 2022JJ30806), Health Research Project of Hunan Provincial Health Commission (grant no. W20243103) and Natural Science Foundation of Changsha (grant no. kq2208345).

Availability of data and materials

Not applicable.

Authors' contributions

The present review was designed by QJ and YG. QJ and YG wrote the manuscript, and SL contributed to the drafting, editing, and critical revision of the manuscript. MZ and TT participated in revising the manuscript for important intellectual content. All authors read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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