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5-Methylcytosine RNA modification and its roles in cancer and cancer chemotherapy resistance

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Abstract

Recent advancements in cancer therapies have improved clinical outcomes, yet therapeutic resistance remains a significant challenge because of its complex mechanisms. Among epigenetic factors, m5C RNA modification is emerging as a key player in cancer drug resistance, similar to the well-known m6A modification. m5C affects RNA metabolism processes, including splicing, export, translation, and stability, thereby influencing drug efficacy. This review highlights the critical roles of m5C in modulating resistance to chemotherapy, targeted therapy, radiotherapy, and immunotherapy. This review also discusses the functions of key regulators, including methyltransferases, demethylases, and m5C-binding proteins, as essential modulators of the m5C epigenetic landscape that contribute to its dynamic and complex regulatory network. Targeting these regulatory components offers a promising strategy to overcome resistance. We highlight the need for further research to elucidate the specific mechanisms by which m5C contributes to resistance and to develop precise m5C-targeted therapies, presenting m5C-focused strategies as potential novel anticancer treatments.

Keywords Cancer drug resistance, m5C, RNA modification, Chemotherapy

Background

RNA methylation, an epigenetic mark that maintains the gene sequence intact, is implicated in a multitude of biological functions. The machinery governing RNA methylation comprises "writers" that add methyl groups, "erasers" that eliminate them, and "readers" that identify methylated RNA. Specific forms of RNA methylation, including m6A, m5C, m3C, m1A, and m7G, have emerged as potential therapeutic targets for disease intervention [1–3]. Abnormalities in RNA modification are linked to diseases like cancer and neurodegenerative disorders. Therefore, better understanding of the mechanisms of RNA modification may provide insights into gene expression complexity and developing new cancer treatments.

RNA methylations are present in various RNA species, including messenger RNA, transfer RNA, ribosomal

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RNA, microRNA, long noncoding RNA, small nuclear RNA, and small nucleolar RNA. The modification of RNA with N6-methyladenosine (m6A) plays a critical function in biological processes such as cell differentiation, development, and stress response [4, 5]. Same as m6A modification, 5-methylcytosine (m5C), plays a crucial role in mRNA stability, nuclear export, and translation. Research has shown that RNA methylation regulatory factors are frequently disordered in several tumors. Aberrant mRNA m5C modification has been reported to be involved in the etiology of multiple diseases, including arteriosclerosis, autoimmune diseases, and cancer.

Despite the rapid development of modern medicine, cancer is a complex disease that remains a significant cause of death worldwide. Drug resistance is the main obstacle in cancer treatment, and a variety of drug resistance mechanisms have been identified, such as drug efflux, metabolic changes, target mutations, and cell death mechanism escape [6]. Hypoxia, acidic conditions, and immune escape in the tumor microenvironment also adversely affect the therapeutic effect of drug treatments. These complex drug resistance phenomena make it difficult to achieve treatment efficacy. Therefore, there is an urgent need for the exploration of innovative therapies.

Epigenetic regulation plays a critical function in mediating drug resistance, and RNA methylation contributes to drug resistance through various mechanisms. In hepatocellular carcinoma (HCC), upregulation of METTL1 and WDR4, components of the tRNA m7G methyltransferase complex, is associated with lenvatinib resistance [7]. m6A plays a regulatory role in various aspects of cancer resistance, influencing processes such as drug transportation and metabolism, the activity of target receptors, the characteristics of cancer stem cells, DNA repair mechanisms, and cellular apoptosis [8, 9]. For example, METTL3 facilitates m6A methylation of DCP2 mRNA, promoting its degradation and increasing mitochondrial autophagy via the Pink1-Parkin pathway, thus aiding chemotherapy resistance. Moreover, depleting METTL3 leads to sorafenib resistance in HCC by affecting FOXO3 mRNA stability, while overexpressing FOXO3 restores sensitivity to sorafenib [10]. Better understanding of these resistance mechanisms is crucial for the development of novel therapeutic strategies targeting these modifications, thereby enhancing the efficacy of cancer treatments.

Studies have shown that m5C modifications are closely related to drug resistance in tumors. In ovarian cancer, the m5C modification stabilizes CHD3 mRNA via the recruitment of PABPC1, thereby augmenting nuclear accessibility and the efficacy of homologous recombination repair, which contributes to the resistance of tumor cells against cisplatin-induced apoptotic stress. This review discusses the clinical potential of targeting m5C modifications to overcome drug resistance and optimize cancer therapy. The regulatory factors and mechanisms that modulate m5C modification and the m5C detection methodologies are first presented. The review primarily highlights the significant role of m5C modifications in tumor drug resistance by exploring the mechanisms through which RNA m5C modifications contribute to drug resistance. Furthermore, the review discusses strategies for targeting m5C alterations to predict and treat cancer resistance. The aim is to inspire the development of novel therapeutic approaches targeting m5C modifications.

Dynamic regulation of m5C

The m5C modification is a widespread RNA modification detected in mRNA and non-coding RNAs including transfer RNA, ribosomal RNA, long non-coding RNA, small non-coding RNA, microRNA, and enhancer RNA [11–14]. In m5C modification, an active methyl group from the donor, usually S-adenosyl-methionine, is added to the carbon-5 position of the cytosine base in RNA. m5C modification is mediated by methyltransferases (writers), demethylases (erasers), and binding proteins (readers) as shown in Fig. 1 [15–17]. The interplay between these proteins enables the dynamic and reversible regulation of m5C methylation. m5C modification impacts various RNA processes, including splicing, translation, maturation, transport, localization, and protein interactions (Table 1) [18].

m5C writers

The m5C methylation process is catalyzed by metRNA cytosine methyltransferases, including NOL1/NOP2/sun (NSUN) family proteins (NSUN1–7) and DNA methyltransferase 2 (DNMT2). The localization of methyltransferases in cells is highly specific. Within the nucleus, NSUN2, NSUN5, NSUN6, NSUN7, and NOP2 primarily mediate m5C modification on mRNA, tRNA, 28 S rRNA, and non-coding RNA [19]. In mitochondria, NSUN2 and NSUN3 methylate tRNA, while NSUN4 facilitates mitochondrial ribosome assembly by methylating 12 S rRNA [20]. NSUN2 is a major RNA cytosine methyltransferase that is distributed in both the nucleus and the cytoplasm, including ribosomes and mitochondria.

NSUN2 is highly expressed in multiple tumor types and is involved in multiple malignant cellular functions, such as tumorigenesis, cell proliferation, tumor invasion, and cell differentiation [21, 22]. Studies in hepatitis B virus (HBV) showed that the host NSUN2 methyltransferase mediates m5C modification on HBV RNA, thereby increasing the stability of viral RNA and promoting HBV replication and expression [23]. Recent reports have shown that m5C modification of mitochondrial tRNA mediated by the NSUN3 methyltransferase enhances

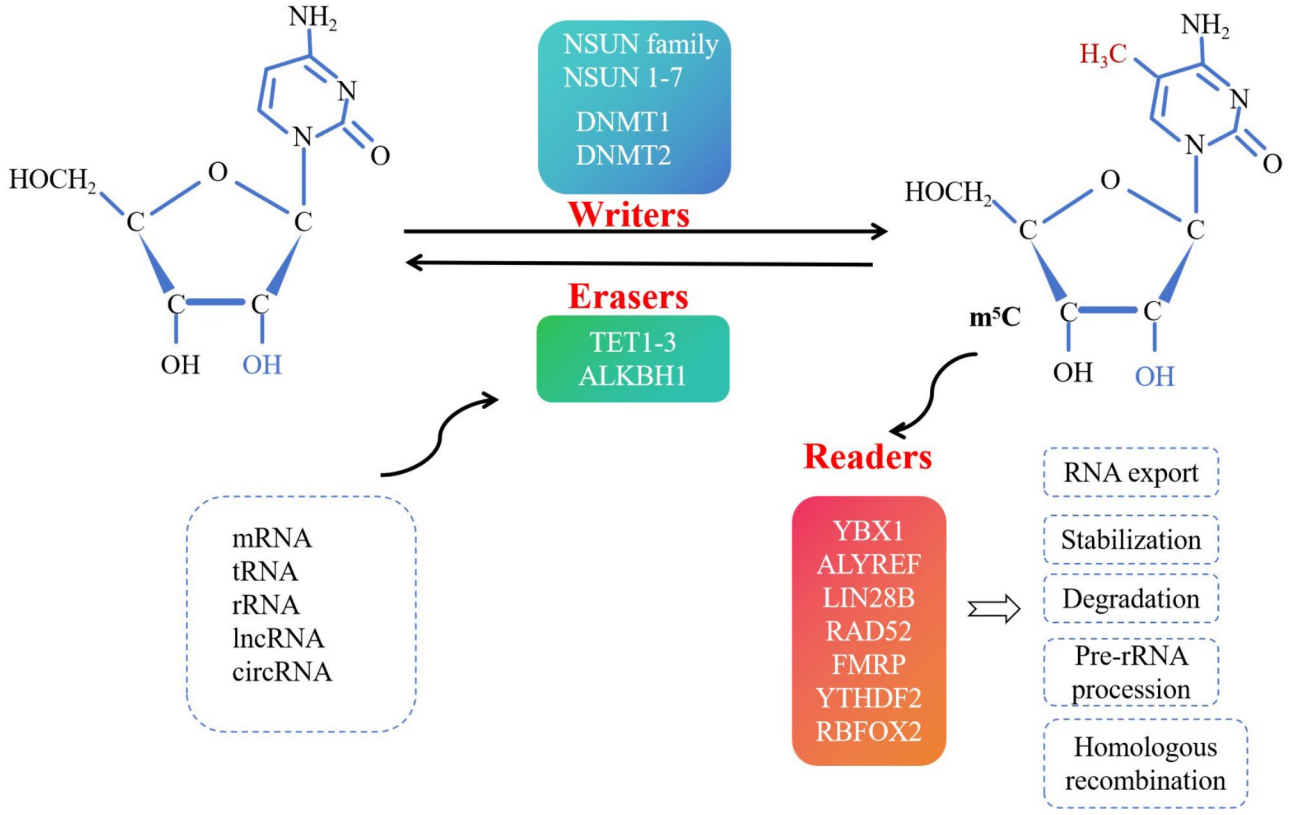

Fig. 1 Chemical structures and regulatory enzymes for m5C modifications

Table 1 Regulator of m5C

RNA	Writer	Eraser	Reader	Function
mRNA	DNMT2, NSUN2/4/5/6	TET1 TET2	YBX1, LIN28B ALYREF, RAD52, FMRP RBFOX2	Stabilization RNA export Homologous recombination Degradation
tRNA	DNMT2, NSUN2/3/6	ALKBH1	YBX1, YBX2	Stabilization
rRNA	NSUN1/4/5	TET2, ALKBH1	YTHDF2	Pre-rRNA procession
lncRNA	NSUN2/3/6	TETs	YBX1, ALYREF	Stabilization
CircRNA	NSUN4	NA	NA	Nuclear export

energy supply by promoting the protein synthesis of the mitochondrial respiratory chain, thereby promoting cancer cell invasion and metastasis [24]. A combined m5C microarray analysis showed that circERI3 is modified by m5C, and NSUN4-mediated m5C modification enhances circERI3 nuclear export.

TRDMT1 (DNMT2), a member of the 5-DNA methyltransferase protein family, is a conserved eukaryotic cytokine that is present in the nucleus or cytoplasm. While DNMT2 was found to mediate m5C methylation modification of DNA, DNMT2 also mediates m5C modification of tRNA [25]. In eukaryotic cells, DNMT2 mainly mediates the methylation of tRNA at position C38 [26]. Researchers showed that DNMT2-mediated RNA methylation plays an important role in organ differentiation and environmental tolerance in zebrafish and Drosophila [27].

m5C erasers

Demethyltransferases, or "erasers," mediate the demethylation of RNA, making m5C a reversible process. In recent years, liquid chromatography tandem mass spectrometry quantitative analysis showed that enzyme family has the potential to act as RNA demethylases, and its overexpression significantly increases the level of RNA 5hmC in HEK293T cells.

ALKBH1, a mitochondrial DNA and RNA dioxygenase, is also involved in the demethylation of cytoplasmic tRNAs [28]. Loss of ALKBH1 leads to severe deficiencies in mitochondrial translation and oxygen consumption, indicating that ALKBH1 may regulate mitochondrial activity [29]. In acute myeloid leukemia, TET2 deficiency leads to an accumulation of m5C modifications in TSPAN13 mRNA, which is recognized by YBX1, resulting in transcript stabilization and enhanced TSPAN13 expression. This process contributes to rapid leukemogenesis, not only through cell proliferation advantages but also through increased leukemic stem cell homing and self-renewal [30]. Studies have shown that the

TET-mediated oxidation product 5-hydroxylmethylcytosine (hm5C) is specifically enriched in tRNA, and TET2 may influence translation through impacting tRNA methylation [31]. In allergic rhinitis, TET2 plays a protective role by negatively regulating M2-related factors through mRNA m5C demethylation.

m5C readers

m5C modification of mRNA plays a crucial role in various biological processes and diseases through its recognition by specific reader proteins. Identifying these m5C reader proteins is essential for elucidating the mechanisms that influence the fate and function of m5C-modified RNA. The currently known m5C reader proteins include ALYREF, YBX1, YBX2, YTHDF2, RAD52, FMRP and SRSF2 [32–36]. The m5C reader ALYREF binds to lysine 171 and promotes mRNA extravasation in bladder cancer. ALYREF acts as an oncogenic factor by binding to m5C in EGFR mRNA to stabilize EGFR expression, thereby activating the STAT3 pathway and promoting liver hepatocellular carcinoma progression [17]. The RNA-binding protein YBX1 functions as an m5C reader and participates in NSUN2-mediated E2F1 regulation to drive ovarian cancer progression [37]. YBX2, a homologous protein of YBX1, is another m5C binding protein and interacts with m5C-modified RNAs [38]. In a study using an unbiased quantitative proteomic approach, YTHDF2 was identified as a reader of m5C in RNA [39]. FMRP, a m5C reader, is a coordinator between m5C writers and erasers to promote mRNA-dependent repair and cell survival in cancer [40]. SRSF2 is a novel reader of m5C. The SRSF2P95H common gene mutation in leukemia impairs the ability of SRSF2 to read m5C-labeled mRNA and significantly reduces its binding to key leukemia-associated transcripts in leukemia cells, thus participating in leukemogenesis [41].

Methods for detecting m5C methylation modifications

Liquid chromatography-mass spectrometry was initially used to identify m5C, but sensitivity and reliability issues were challenges for detection of low abundance m5C. Recent advancements in high-throughput sequencing and derived techniques now allow for precise identification and quantification of nucleotide methylation modifications, including m5C, within the transcriptome [42–44]. The detection of m5C methylation modifications has evolved from liquid chromatography-mass spectrometry, which faced sensitivity and reliability challenges, to high-throughput sequencing techniques. The common methods for m5c detection are shown in Fig. 2. These technological advancements have significantly advanced research in transcriptome epigenetics and have become valuable tools for investigating the roles of m5C in tumor immunity, metastasis, and other biological functions. Additionally, these techniques hold potential clinical applications in cancer diagnosis and surgical support.

m5C RNA immunoprecipitation sequencing (m5C-RIP-seq)

In m5C-RIP-seq, antibodies that specifically recognize m5C modification sites are used to enrich RNA fragments containing m5C by antibody affinity chromatography for library construction and sequencing. However, because of certain limitations, m5C-RIP-seq is unable to identify methylation on low abundance mRNAs [45]. Similarly, miCLIP-seq using NSUN2-specific antibody has been performed to selectively capture RNA fragments targeted by NSUN2. The drawbacks of miCLIP-seq detection of NSUN2-mediated m5C modification in RNA is the inability to obtain a comprehensive transcriptomic profile because of the use of antibodies against a single RNA m5C methyltransferase and being limited to in vitro analyses in cells [36].

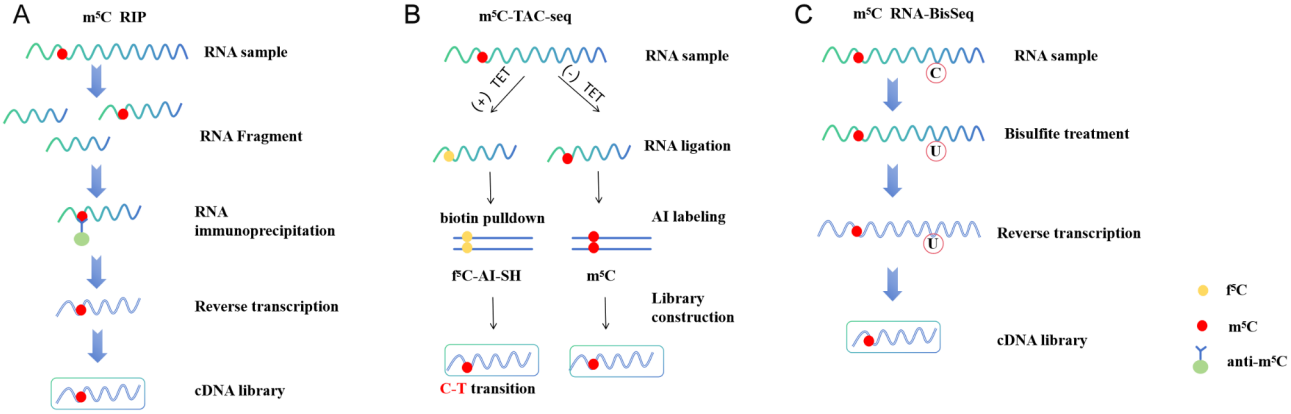

Fig. 2 Methods for detecting m5C methylation modifications

RNA bisulfite sequencing (RNA-BisSeq)

RNA-BisSeq is the most widely used method for detecting RNA m5C methylation modification. During RNA-BisSeq, the heavy sulfate treatment of the sample converts unmethylated cytosine in mRNA to uracil, and subsequent reverse transcription generates cDNA, enabling identification of m5C modification sites [46]. This technique offers significant advantages in terms of single nucleotide resolution and accurate prediction of methylation levels. However, a limitation lies in its inability to effectively distinguish between unconverted cytosine derived from m5C or hm5C [47]. Bisulfite can only convert single-stranded cytosines; thus, incomplete conversion as a result of partial double-stranded RNA formation may lead to misidentification as methylated cytosines.

Ultrafast bisulfite sequencing (UBS-seq)

Compared with m5C in DNA, m5C has fewer modification sites in mRNA and lower levels, presenting challenges in RNA bisulfite sequencing such as avoiding false positives, reducing RNA degradation, and accurately detecting m5C sites. UBS-seq, developed by Chuan He's team at the University of Chicago, addresses these challenges by using ammonium bisulfite, which enhances bisulfite treatment efficiency [48]. This method allows for rapid and complete conversion of cytosine to uracil while preserving 5mC, minimizing degradation. UBS-seq enables efficient and accurate sequencing of both DNA and RNA, including single-cell samples, with reduced background noise and false positives. This method offers a powerful tool for studying the biological functions mediated by m5C.

m5C-TAC-seq

Li Xiao yu et al. developed a novel detection technology called m5C-TAC-seq (m5C detection strategy enabled by TET-assisted chemical labeling), which eliminates the need for bisulfite treatment [49]. This method enables accurate and sensitive detection of m5C at single-base resolution across the transcriptome. The technology combines an optimized TET enzymatic reaction to oxidize RNA m5C to f5C, followed by chemical labeling with azindone for direct detection. Using m5C-TAC-seq, researchers observed down-regulation of m5C methylation during mouse embryonic stem cell differentiation, particularly in transcripts related to the cell cycle and division, suggesting a role for m5C in differentiation. This method allows for the detection of low abundance RNA with low sequence complexity and low modification ratios and facilitates dynamic monitoring of m5C modifications in various biological processes, enabling insights into the biological function of m5C modifications.

The applicability of m5C detection methods varies depending on the research context. miCLIP-seq, while specific for NSUN2-mediated m5C, offers a restricted view of the transcriptome and is primarily applicable in vitro. Technological bottlenecks include the need for high-quality antibodies, the complexity of data analysis, and the challenge of profiling low-abundance m5C modifications. Advances in sequencing depth and bioinformatics are ongoing to mitigate these limitations and enhance the utility of these techniques in both research and clinical settings.

m5C and cancer progression

Research has shown that the RNA modification m5C is intimately associated with cancer progression. m5C modification and its regulatory factors, including writers, erasers, and readers, are abnormally expressed across various types of cancer, leading to the initiation and development of tumors. Studies have demonstrated that the role of m5C is correlated with DNA methylation and the regulation of gene expression, and alterations in m5C patterns may influence the proliferation, migration, and drug resistance of cancer cells. In multiple myeloma, m5C modifications by NSUN2 and YBX1 upregulate lncRNA MALAT1, which is then transported to osteoclasts via exosomes, promoting bone lesions [50]. The modulation of cancer cell behavior by m5C alterations is critical and may influence the development of therapeutic resistance in tumors.

Most m5C RNA methylation regulators exhibit differential expression and prognostic values in cancer. The great significance of the m5C-related signature in predicting the survival of patients with glioma was confirmed in validation sets and a CGGA cohort [51].

m5C and chemotherapy resistance

Chemotherapy is one of the most effective methods for the treatment of cancer, and it is a particularly effective clinical treatment strategy for patients with advanced malignant tumors [52–54]. However, resistance to chemotherapeutic agents greatly limits their overall therapeutic effect. Cancer resistance is caused by a variety of factors, such as individual differences in drug sensitivity, tumor location, tissue spectrum, tumor aggressiveness, and alterations in intracellular molecules [55, 56]. An increasing number of studies have implicated m5C modification as a potential determinant of tumor response to chemotherapy [57–59]. This modification appears to play a crucial role in how cancer cells react to treatment. Studies have also shown that m5C functions in drug resistance by influencing DNA repair, adaptive responses like apoptosis and autophagy, cell stemness, the tumor immune environment, and non-coding RNAs (Table 2).

Table 2 Role of m5C regulator in chemotherapy

Drug	Regulator	Role	Level	Cancer	Target	Mechanism	References
Cisplatin	YBX1	oncogene	high	OC	CHD3	maintained CHD3 mRNA stability by recruiting PABPC1 protein	[65]
Cisplatin	YBX1	oncogene	high	EOC	SIAH1	SIAH1 as a novel E3 ligases to trigger degradation of YBX-1	[66]
Cisplatin	DNMT1	suppressor	low	Osteosarcoma	AXL, NOTCH2, YAP1	Increased DNMT1 reduces anti-apoptotic genes' expression	[68]
Cisplatin	NSUN2	oncogene	high	ESCC	NMR	bind to chromatin regulator BPTF and promote MMP3 and MMP10 expression by ERK1/2 pathway	[67]
Cisplatin 5-FU	NSUN2	oncogene	high	GC	Bcl-2, Bax	NSUN2 downregulation declines in ERK1/2-induced proliferation	[69]
Cisplatin Olaparib	NSUN6	oncogene	high	CC	NDRG1	NSUN6 promoted the m5C modification of NDRG1 mRNA, enhanced NDRG1 mRNA stability	[97]
Temozolomide	NSUN6	Suppressor	low	Glioma	NELFB, RPS6BK2	regulates mRNA stability in time-dependent manner through NELFB coordinated transcriptional pausing	[74]
5-FU	NSUN2	oncogene	high	CCA		stabilize mature tRNA molecules and prevent rapid tRNA decay	[82]
Doxorubicin HCl, Cisplatin	NSUN2	oncogene	high	ATC	c-Myc, BCL2, RAB31, JUNB	stabilizing tRNA, increased efficiency necessary to support a pro-cancer translation program	[70]
5-Azacytidine	DNMT2	oncogene	high	myeloid leukemias; CRC	DNMT2	Azacytidine inhibits RNA methylation at DNMT2 target sites	[83]
5-Azacytidine	DNMT2	oncogene	high	leukemias	hnRNPK	Disrupt the active chromatin structure associated with hnRNPK.	[59]
AZD9291	NSUN2	oncogene	high	LUAD	YAP	increased the stability of YAP mRNA	[90]
Gefitinib	NSUN2, YBX1	oncogene	high	NSCLC	QSOX1	methylated QSOX1 coding sequence region, enhanced QSOX1 translation	[91]
Osimertinib	TET2	Suppressor	low	NSCLC	IL6, TNF	Loss of TET2 resulted in the upregulation of TNF/NF-κB signaling	[92]
Erlotinib	ALYREF	oncogene	high	LIHC	EGFR	stabilization of EGFR mRNA and subsequent activation of the STAT3 signaling pathway	[93]
Sorafenib	NSUN2	oncogene	high	HCC	GRB2	modulating the Ras signaling pathway	[94]

Platinum drugs

The platinum compound cisplatin was approved by the Food and Drug Administration for the treatment of metastatic testicular, ovarian, and bladder cancer in 1978 [60–62]. The second-generation platinum drug carboplatin and third-generation oxaliplatin are a major component of chemotherapy regimens for many other cancers [63, 64]. While platinum has become the first-line chemotherapeutic drug in the clinical treatment of cancer, drug resistance greatly limits its clinical application. Recent studies have revealed that m5C modification and its regulatory factors modulates cancer resistance to cisplatin.

Inhibiting the m5C reader YBX1 significantly enhances the sensitivity of ovarian cancer to platinum-induced stress, indicating the potential of targeting YBX1 to overcome platinum-resistant therapy in ovarian cancer [65]. The overexpression of SIAH1 has been shown to enhance the antitumor efficacy of cisplatin in both in vitro and in vivo. This enhancement is partially compromised by either the ectopic expression of YBX1 or the depletion of YBX1 ubiquitination. The interaction between SIAH1 and YBX1 may be a potential therapeutic target for overcoming chemoresistance in epithelial ovarian cancer [66]. A novel NSUN2 methylated lncRNA (NMR), which was significantly upregulated in esophageal squamous cell carcinoma, functions as a key regulator of tumor metastasis and drug resistance in esophageal squamous cell carcinoma [67]. During the induction of apoptosis in osteosarcoma cells by cisplatin and doxorubicin, DNMT1 and NSun2 exhibit inversely levels. DNMT1 can silence the of NSUN2, thereby reducing its methylation on NOTCH2, AXL, and YAP1 mRNA, which impacts the apoptosis process. Modulating the of DNMT1 or NSUN2 can regulate the in osteosarcoma [68]. Downregulation of NSUN2 promotes gastric cancer chemosensitivity to inverse modulation by chemotherapeutic agents of Bcl-2 and Bax expression levels and declines

in ERK1/2-induced proliferation [69]. The expression of the tRNA m5C methyltransferase NSUN2 is upregulated in undifferentiated thyroid carcinoma, promoting cancer cell dedifferentiation, and its inhibition enhances the sensitivity of tumors to genotoxic drugs by catalyzing the m5C modification of tRNA, stabilizing tRNA, and accelerating amino acid transport, particularly leucine [70].

Temozolomide (TMZ)

TMZ is an alkylating agent distinct from platinum-based drugs and serves as the primary first-line chemotherapeutic agent in the standard treatment regimen for advanced gliomas, in combination with radiotherapy. The overall clinical efficacy of this regimen in treating glioblastoma is suboptimal because of both inherent and acquired resistance to TMZ therapy [71–73].

In glioma, NSUN6 interacts with NELFB and RPS6KB2, which together modulate the adaptability of glioblastoma cells to TMZ treatment. Elevated levels of NSUN6 promote tumor cell survival in the presence of chemotherapy, suggesting that targeting NSUN6 may enhance therapeutic efficacy in glioblastoma management [74].

Anthracyclines

Anthracyclines are some of the most commonly used antitumor drugs for treating various hematologic and solid tumors; these drugs are used alone or in combination with targeted therapies or cytotoxic agents [75–77]. Doxorubicin and its derivative epirubicin are widely used in the treatment of breast cancer, endometrial cancer, gastric cancer, pediatric solid tumors, soft tissue sarcomas, and aggressive lymphocytic or myelocytic leukemia [78–81].

The simultaneous overexpression of NSUN2 and METTL1 is common in human cancers, suggesting that targeting both proteins may be an effective cancer chemotherapy strategy. Overexpression of the dephosphorylated forms of these methyltransferases suppresses sensitivity to 5-FU [82].

5-Azacytidine

The cytosine analogues azacytidine and decitabine, which exhibit demethylating effects, are being developed as drugs for epigenetic cancer therapy. Studies have shown that changes in RNA methylation patterns can lead to alterations in gene expression and chromatin structure, thereby influencing the efficacy of 5-azacytidine [83]. Resistant leukemia cells exhibit distinct methylation profiles compared with sensitive cells, pointing to methylation status as a critical factor in the development of drug resistance. Such insights underscore the importance of understanding the mechanisms of RNA methylation and its implications for therapeutic strategies in leukemia [59].

In myeloid leukemias, azacytidine inhibits the RNA methyltransferase DNMT2, which in turn inhibits the methylation of cytosine 38 in tRNA (Asp). This raises the possibility that tRNA hypomethylation might contribute to drug susceptibility [83]. These findings suggest that targeting RNA methylation pathways may provide a novel approach to overcoming resistance to 5-azacytidine and improving treatment outcomes for leukemia patients.

m5C and resistance to targeted therapy

Targeted therapy represents a revolutionary approach in cancer treatment by disrupting specific molecules involved in cancer growth, spread, and progression [84–87]. Numerous targeted drugs have received FDA approval and have shown significant clinical benefits for various cancers, such as liver, colorectal, lung, breast, and ovarian cancers. However, resistance to these therapies often develops, mainly as a result of mutations in the target, activation of alternative signaling pathways, and cellular adaptability.

EGFR tyrosine kinase inhibitors (EGFR-TKIs)

EGFR-TKIs are small molecule drugs that target the tyrosine kinase domain of EGFR, effectively inhibiting tumor growth by blocking the EGFR signaling pathway. Three generations of EGFR-TKIs are currently available [88, 89].

Recent findings suggest that YAP m5C modification, along with its function in promoting exosome secretion, contributes to the malignant phenotype and resistance to AZD9291 (a third-generation EGFR-TKI) in lung adenocarcinoma cells, indicating that blocking YAP m5C modification may be beneficial for lung adenocarcinoma treatment [90]. In non-small cell lung cancer, aberrant m5C hypermethylation plays a critical role in mediating resistance to gefitinib, an EGFR inhibitor. The NSUN2 methyltransferase enhances the m5C methylation of specific mRNAs, leading to altered stability and translation of these targets, including YBX1. This alteration in mRNA processing contributes to the resistance phenotype, as elevated levels of YBX1 subsequently regulate QSOX1 expression, reinforcing cellular pathways that diminish gefitinib efficacy [91]. TET2 undergoes polyubiquitination by the E3 ligase complex $CUL7^{FBXW11}$, leading to TET2 degradation in EGFR-TKI-resistant non-small cell lung cancer cells [92].

In HCC, ALYREF-mediated m5C modification stabilizes EGFR mRNA, leading to enhanced activation of the STAT3 signaling pathway. This stabilization promotes HCC progression by facilitating the expression of oncogenic factors associated with tumor growth and survival,

and contributes to the development of resistance to erlotinib [93]. NSUN2 also enhances the stability of specific mRNAs involved in tumorigenesis, and is closely associated with sorafenib resistance, further supporting the notion that m5C modifications are integral to the molecular mechanisms driving HCC [94].

m5C and radiosensitivity

Radiotherapy has long been a crucial pillar in cancer treatment, especially for solid tumors such as lung cancer, breast cancer, esophageal cancer, colorectal cancer, and glioblastoma [95, 96]. However, resistance to radiotherapy remains a significant challenge in clinical cancer treatment.

In cervical cancer, aberrant m5C hypermethylation and overexpression of NSUN6 drive resistance to radiotherapy. Elevated NSUN6 expression promotes radioresistance by activating the NSUN6/ALYREF-m5C-NDRG1 pathway [97]. While targeting R-loop modifiers and RNA modification enzymes is an attractive strategy in cancer therapy, further investigation is warranted to understand the molecular mechanisms by which RNA modifications regulate DNA repair. The RNA methyltransferase TRDMT1 generates m5C on mRNA at transcription-associated DNA double-strand breaks (DSBs), promoting homologous recombination (HR). The fragile X mental retardation protein (FMRP) aids HR by interacting with TRDMT1 and TET1, functioning at active DSB sites. FMRP binds m5C-modified RNA in DNA: RNA hybrids and promotes TET1-mediated m5C demethylation in vitro. Loss of FMRP impairs m5C demethylation, hindering DSB repair and increasing radiosensitivity in cancer cells via BRCA-independent pathways [40]. TRDMT1 deficiency compromises HR, preventing RAD51 and RAD52 from localizing to DNA damage sites, and increases sensitivity to PARP inhibitors [34]. These findings highlight the TRDMT1-m5C axis in HR, suggesting that post-transcriptional RNA modifications regulate DNA repair and radiosensitivity.

The potential of m5C in clinical applications

Research has confirmed m5C as a significant epigenetic modification in RNA. The potential of m5C in clinical applications is substantial, particularly in the context of cancer diagnosis, prognosis, and therapy.

m5C in immunotherapy and the tumor microenvironment

Immunotherapy is a powerful approach to cancer treatment that leverages the patient's own immune system to combat cancer cells [4, 98]. Recent studies have illuminated the role of RNA m5C modifications in modulating immune responses, thereby influencing the efficacy of immunotherapy. m5C modifications are implicated in the regulation of gene expression and mRNA stability, which can affect the immune landscape within the tumor microenvironment.

Recent studies have highlighted how m5C modifications, particularly modifications mediated by NSUN2, influence the efficacy of immunotherapy in cancer treatment. Understanding the mechanisms by which m5C modifications influence cancer and immune responses may provide new avenues for enhancing the effectiveness of immunotherapeutic strategies. The deletion of genes in the glucose/NSUN2/TREX2 pathway promotes apoptosis and CD8 + T cell infiltration by activating the cGAS/STING pathway, thereby inhibiting tumorigenesis and overcoming tumor resistance to PD-L1 immunotherapy [99]. In head and neck squamous cell carcinoma, NSUN3 knockdown reduces M2 macrophage infiltration and increases M1 macrophage infiltration. This highlights the potential of NSUN3 as a therapeutic target for improving treatment outcomes in cancer [100]. NSUN6 promotes HDAC10 expression, inhibiting macrophage-associated chemokines and reducing M2 macrophage recruitment, thereby improving prognosis in bladder cancer patients. High YBX1 expression is linked to M2 macrophage infiltration and T cell depletion and may be targeted with M1 polarization agents alongside immunotherapy [101]. m5C modifications appear to play a leading role in the intricate interplay with m6A, finely tuning gene expression and cellular functions. This interaction underscores the complexity of the epigenetic regulatory network, which may significantly influence cancer progression and the development of therapeutic resistance [102]. YTHDF2 is overexpressed in B-cell malignancies and contributes to cancer cell survival and immune evasion via two distinct pathways. It stabilizes m5C-modified ATP synthase subunit mRNAs, enhancing ATP synthesis and supporting cancer cell proliferation. Additionally, YTHDF2 induces the degradation of m6A-modified CD19 and MHC-II mRNAs, reducing cancer cell detection by the immune system and aiding in evading immunotherapy. Understanding the mechanisms by which m5C modifications influence cancer and immune responses may provide new avenues for enhancing the effectiveness of immunotherapeutic strategies.

The pivotal role of m5C in modulating interactions between tumor cells and the immune microenvironment significantly influences tumorigenesis. Studies have shown that NSUN3 and NSUN4 predict lung squamous cell carcinoma prognosis and modulate the immune microenvironment. In lung adenocarcinoma, distinct m5C patterns correlate with immune cell infiltration in the tumor microenvironment, where higher m5C scores are linked to better prognosis [103]. Additionally, m5C-regulated lncRNAs predict overall survival in lung adenocarcinoma and affect the immune microenvironment [104]. In pancreatic cancer, three m5C-related lncRNAs

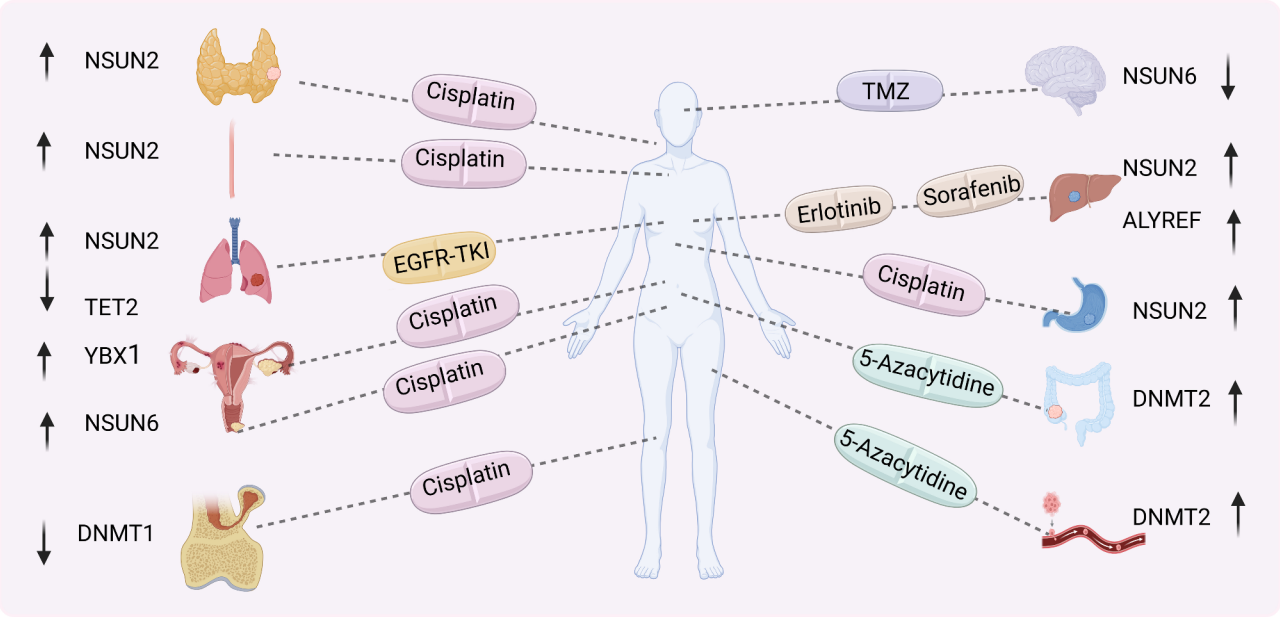

Fig. 3 m5C modification induced specific drug resistance. Specific chemotherapy drug resistance associated with m5C and related regulators in cancers

demonstrate prognostic value. Identifying m5C's specific immune checkpoint molecular targets could facilitate the development of targeted therapies in combination with checkpoint blockade immunotherapies, enhancing cancer management [105]. Investigating novel m5C epigenetic alterations in cancer is crucial for revealing underlying molecular and immunological mechanisms, paving the way for new therapeutic strategies.

m5C and diseases other than cancer

Epigenetic modifications, including m5C methylation, play a crucial role in the intricate modulation of gene expression, which in turn influences cellular functions [102, 106]. Aberrations in epigenetic modifications can thus lead to the pathogenesis of a variety of diseases [107, 108]. The dysregulation of RNA processing and the metabolism of non-coding RNAs is a central aspect of the etiology underlying these conditions [109, 110].

m5C has been implicated in the DNA damage response (DDR) pathway, which is vital for preserving genomic integrity and a key player in the suppression of mutations associated with numerous human diseases, such as neurodegenerative disorders. RNA modifications may contribute to the pathogenesis of neurodegenerative disorders through two mechanisms: by modulating gene expression and by impacting the DNA repair processes and genomic stability [111]. In cardiovascular and cerebrovascular diseases, m5C methylation is involved in the regulation of cardiomyocyte necroptosis. Heart necroptosis-associated piRNAs (HNEAP) have been identified to modulate this process by targeting DNMT1-mediated methylation of m5C, thereby activating Atf7 mRNA transcripts [112]. These studies underscore the significance of m5C methylation in the broader spectrum of disease pathogenesis beyond cancer, offering novel perspectives for potential therapeutic targets in cardiovascular and neurodegenerative conditions.

Discussions

The mechanisms for tumor resistance are complex. In this review, we focused on the association of m5C methylation with tumor resistance (Fig. 3). The role of m5C modification in tumor drug resistance is multifaceted, involving multiple aspects such as gene expression, cell survival, DNA repair, and tumor microenvironment. Considering the association of m5C RNA modifications with various malignancies, therapeutic interventions that modulate the levels of m5C by altering the gene sequences of key m5C methyltransferases present a promising avenue for cancer progression intervention [113]. While gene therapies show limitations of non-specificity, the development of specific inhibitors to reduce the activity of certain m5C methyltransferases and reverse tumor advancement represents an alternative approach with enhanced safety. While drugs that inhibit m6A methylation have been used in cancer treatment, to date, no specific inhibitors targeting RNA m5C methyltransferases have been developed. Several compounds initially designed to disrupt DNA m5C methylation may also interfere with RNA methylation. One study showed that nitrogenous nucleoside analogs effectively reduce DNMT2-mediated tRNA m5C levels in cancer cells, inhibiting cancer cell proliferation [83]. Nonetheless, the potential lack of selectivity of these compounds and

the subsequent effects on methylation at multiple sites introduces the risk of off-target effects and unknown liabilities.

Despite the encouraging advancements described above, there are still numerous challenges associated with the clinical application of strategies targeting m5C methylation. It is important to note that these applications predominantly focus on a single m5C modification and its corresponding regulators. The effects of m5C methylation on certain cancers also remain unknown. More systematic studies are needed to explore the mechanisms of m5C methylation in tumor resistance. A growing body of evidence indicates that RNA modifying proteins hold significant promise as pharmacological targets or diagnostic markers. Nonetheless, our understanding of the interactions between various RNA modifications and their modifiers is still in its infancy, which raises some uncertainties regarding the clinical application of strategies targeting RNA modifying proteins.

Numerous m5C regulators are overexpressed in tumors, contributing to drug resistance primarily by inhibiting apoptosis, promoting autophagy and epithelial-mesenchymal transition (EMT), enhancing cellular stemness, and activating various signaling pathways such as ERK1/2, STAT3, Notch, and NF-κB. For these regulators that promote tumor progression and drug resistance, targeted inhibition can effectively increase tumor sensitivity to chemotherapy. A recent study identified an effective small molecule inhibitor of NSUN2 that reduces the enzyme's function and the expression of its downstream targets. This discovery offers a novel and promising therapeutic option for CRC immunotherapy [114]. By targeting the YBX1 signaling cascade, it is possible to overcome tumor immune evasion and multidrug resistance, indicating its potential as an effective therapeutic approach to combat tumor chemotherapy resistance [115]. While these inhibitors have demonstrated some efficacy in laboratory studies, they have not entered the clinical stage. Thus, it is essential to conduct further research to investigate the performance and mechanisms of these inhibitors under diverse conditions, with the aim to offer patients more effective treatment options. Moreover, further research into m5C modifications in immune responses may lead to new tumor immunotherapy and drug resistance strategies. Several companies are already focusing on RNA modifications. As the preclinical success of these drugs becomes evident, RNA epigenetic drug development will advance to a new level.

Conclusions

The regulation of RNA fate by m5C modification plays a crucial role in cancer therapeutic resistance, providing potential targets for new strategies for overcoming resistance and optimizing cancer treatments. Although significant progress has been made in understanding the mechanisms and clinical implications of m5C modification, our knowledge remains limited. Further development of new methodologies, large-scale and in-depth studies, and increased focus on the potential of emerging immunotherapies are needed to advance this field.

Abbreviations

HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
hm5C	5-hydroxylmethylcytosine
m5C	5-methylcytosine
m5C-RIP-seq	m5C RNA immunoprecipitation sequencing
m6A	N6-methyladenosine
RNA-BisSeq	RNA bisulfite sequencing
TMZ	Temozolomide
UBS-seq	Ultrafast bisulfite sequencing
OC	Ovarian cancer
ESCC	Esophageal squamous cell carcinoma
GC	Gastric cancer
CC	Cervical cancer
ATC	Anaplastic thyroid cancer

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Author contributions

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Data availability

Not applicable.

Declarations

Ethics approval and consent to participate

Not applicable.

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Competing interests

The authors declare that they have no competing interests.

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References

1. 1 Orsolic I, Carrier A, Esteller M. Genetic and epigenetic defects of the RNA modification machinery in cancer. Trends Genet. 2023;39:74–88.
2. 2 Wang C, Hou X, Guan Q, Zhou H, Zhou L, Liu L, et al. RNA modification in cardiovascular disease: implications for therapeutic interventions. Signal Transduct Target Ther. 2023;8:412.
3. Barbieri I, Kouzarides T. Role of RNA modifications in cancer. Nat Rev Cancer. 2020;20:303–22.
4. Han D, Xu MM. RNA modification in the Immune System. Annu Rev Immunol. 2023;41:73–98.

5. An Y, Duan H. The role of m6A RNA methylation in cancer metabolism. Mol Cancer. 2022;21:14.
6. Liu C, Cao Y, Zuo Y, Zhang C, Ren S, Zhang X et al. Hybridization-based discovery of novel quinazoline-2-indolinone derivatives as potent and selective PI3Kalpha inhibitors. J Adv Res 2024.
7. Sun Y, Shen W, Hu S, Lyu Q, Wang Q, Wei T, et al. METTL3 promotes chemoresistance in small cell lung cancer by inducing mitophagy. J Exp Clin Cancer Res. 2023;42:65.
8. Liu L, Li H, Hu D, Wang Y, Shao W, Zhong J, et al. Insights into N6-methyladenosine and programmed cell death in cancer. Mol Cancer. 2022;21:32.
9. Qin S, Mao Y, Wang H, Duan Y, Zhao L. The interplay between m6A modification and non-coding RNA in cancer stemness modulation: mechanisms, signaling pathways, and clinical implications. Int J Biol Sci. 2021;17:2718–36.
10. Lin Z, Niu Y, Wan A, Chen D, Liang H, Chen X, et al. RNA m(6) a methylation regulates sorafenib resistance in liver cancer through FOXO3-mediated autophagy. EMBO J. 2020;39:e103181.
11. Li M, Tao Z, Zhao Y, Li L, Zheng J, Li Z, et al. 5-methylcytosine RNA methyltransferases and their potential roles in cancer. J Transl Med. 2022;20:214.
12. Nombela P, Miguel-Lopez B, Blanco S. The role of m(6)A, m(5)C and psi RNA modifications in cancer: novel therapeutic opportunities. Mol Cancer. 2021;20:18.
13. Gu X, Ma X, Chen C, Guan J, Wang J, Wu S, et al. Vital roles of m(5)C RNA modification in cancer and immune cell biology. Front Immunol. 2023;14:1207371.
14. Wu J, Zhao Q, Chen S, Xu H, Zhang R, Cai D, et al. NSUN4-mediated m5C modification of circERI3 promotes lung cancer development by altering mitochondrial energy metabolism. Cancer Lett. 2024;605:217266.
15. Zhang T, Zhao F, Li J, Sun X, Zhang X, Wang H, et al. Programmable RNA 5-methylcytosine (m5C) modification of cellular RNAs by dCasRx conjugated methyltransferase and demethylase. Nucleic Acids Res. 2024;52:2776–91.
16. Qiu L, Jing Q, Li Y, Han J. RNA modification: mechanisms and therapeutic targets. Mol Biomed. 2023;4:25.
17. Yang X, Yang Y, Sun BF, Chen YS, Xu JW, Lai WY, et al. 5-methylcytosine promotes mRNA export - NSUN2 as the methyltransferase and ALYREF as an m(5)C reader. Cell Res. 2017;27:606–25.
18. Han X, Wang M, Zhao YL, Yang Y, Yang YG. RNA methylations in human cancers. Semin Cancer Biol. 2021;75:97–115.
19. Shinoda S, Kitagawa S, Nakagawa S, Wei FY, Tomizawa K, Araki K, et al. Mammalian NSUN2 introduces 5-methylcytidines into mitochondrial tRNAs. Nucleic Acids Res. 2019;47:8734–45.
20. Van Haute L, Lee SY, McCann BJ, Powell CA, Bansal D, Vasiliauskaite L, et al. NSUN2 introduces 5-methylcytosines in mammalian mitochondrial tRNAs. Nucleic Acids Res. 2019;47:8720–33.
21. Liu K, Xu P, Lv J, Ge H, Yan Z, Huang S, et al. Peritoneal high-fat environment promotes peritoneal metastasis of gastric cancer cells through activation of NSUN2-mediated ORAI2 m5C modification. Oncogene. 2023;42:1980–93.
22. Hu Y, Chen C, Tong X, Chen S, Hu X, Pan B, et al. NSUN2 modified by SUMO-2/3 promotes gastric cancer progression and regulates mRNA m5C methylation. Cell Death Dis. 2021;12:842.
23. Feng J, Xu T, He M, Li J, Yao P, Ma C, et al. NSUN2-mediated m5C modification of HBV RNA positively regulates HBV replication. PLoS Pathog. 2023;19:e1011808.
24. Delaunay S, Pascual G, Feng B, Klann K, Behm M, Hotz-Wagenblatt A, et al. Mitochondrial RNA modifications shape metabolic plasticity in metastasis. Nature. 2022;607:593–603.
25. Zhang Y, Zhang X, Shi J, Tuorto F, Li X, Liu Y, et al. Dnmt2 mediates intergenerational transmission of paternally acquired metabolic disorders through sperm small non-coding RNAs. Nat Cell Biol. 2018;20:535–40.
26. Zimmermann RA, Fischer TR, Schwickert M, Nidoieva Z, Schirmeister T, Kersten C. Chemical Space virtual screening against hard-to-drug RNA methyltransferases DNMT2 and NSUN6. Int J Mol Sci 2023; 24.
27. Li H, Liu H, Zhu D, Dou C, Gang B, Zhang M, et al. Biological function molecular pathways and druggability of DNMT2/TRDMT1. Pharmacol Res. 2024;205:107222.
28. Arguello AE, Li A, Sun X, Eggert TW, Mairhofer E, Kleiner RE. Reactivity-dependent profiling of RNA 5-methylcytidine dioxygenases. Nat Commun. 2022;13:4176.
29. Kawarada L, Suzuki T, Ohira T, Hirata S, Miyauchi K, Suzuki T. ALKBH1 is an RNA dioxygenase responsible for cytoplasmic and mitochondrial tRNA modifications. Nucleic Acids Res. 2017;45:7401–15.
30. Li Y, Xue M, Deng X, Dong L, Nguyen LXT, Ren L, et al. TET2-mediated mRNA demethylation regulates leukemia stem cell homing and self-renewal. Cell Stem Cell. 2023;30:1072–e10901010.
31. Shen H, Ontiveros RJ, Owens MC, Liu MY, Ghanty U, Kohli RM, et al. TET-mediated 5-methylcytosine oxidation in tRNA promotes translation. J Biol Chem. 2021;296:100087.
32. Yu T, Zhang Q, Yu SK, Nie FQ, Zhang ML, Wang Q, et al. THOC3 interacts with YBX1 to promote lung squamous cell carcinoma progression through PFKFB4 mRNA modification. Cell Death Dis. 2023;14:475.
33. Liu Y, Yang Y, Wu R, Gao CC, Liao X, Han X, et al. mRNA m(5)C inhibits adipogenesis and promotes myogenesis by respectively facilitating YBX2 and SMO mRNA export in ALYREF-m(5)C manner. Cell Mol Life Sci. 2022;79:481.
34. Chen H, Yang H, Zhu X, Yadav T, Ouyang J, Truesdell SS, et al. M(5)C modification of mRNA serves a DNA damage code to promote homologous recombination. Nat Commun. 2020;11:2834.
35. Zhao Y, Xing C, Peng H. ALYREF (Aly/REF export factor): a potential biomarker for predicting cancer occurrence and therapeutic efficacy. Life Sci. 2024;338:122372.
36. Chen YS, Yang WL, Zhao YL, Yang YG. Dynamic transcriptomic m(5) C and its regulatory role in RNA processing. Wiley Interdiscip Rev RNA. 2021;12:e1639.
37. Liu X, Wei Q, Yang C, Zhao H, Xu J, Mobet Y, et al. RNA m(5)C modification upregulates E2F1 expression in a manner dependent on YBX1 phase separation and promotes tumor progression in ovarian cancer. Exp Mol Med. 2024;56:600–15.
38. Cai Y, Li N, Li H. YBX2 modulates mRNA stability via interaction with YTHDF2 in endometrial cancer cells. Exp Cell Res. 2023;427:113586.
39. Dai X, Gonzalez G, Li L, Li J, You C, Miao W, et al. YTHDF2 binds to 5-Methylcytosine in RNA and modulates the maturation of ribosomal RNA. Anal Chem. 2020;92:1346–54.
40. Yang H, Wang Y, Xiang Y, Yadav T, Ouyang J, Phoon L, et al. FMRP promotes transcription-coupled homologous recombination via facilitating TET1-mediated m5C RNA modification demethylation. Proc Natl Acad Sci U S A. 2022;119:e2116251119.
41. Ma HL, Bizet M, Soares Da Costa C, Murisier F, de Bony EJ, Wang MK, et al. SRSF2 plays an unexpected role as reader of m(5)C on mRNA, linking epitranscriptomics to cancer. Mol Cell. 2023;83:4239–e42544210.
42. Zhang Y, Zhang LS, Dai Q, Chen P, Lu M, Kairis EL, et al. 5-methylcytosine (m(5)C) RNA modification controls the innate immune response to virus infection by regulating type I interferons. Proc Natl Acad Sci U S A. 2022;119:e2123338119.
43. Smallwood SA, Lee HJ, Angermueller C, Krueger F, Saadeh H, Peat J, et al. Single-cell genome-wide bisulfite sequencing for assessing epigenetic heterogeneity. Nat Methods. 2014;11:817–20.
44. Zhang Z, Chen T, Chen HX, Xie YY, Chen LQ, Zhao YL, et al. Systematic calibration of epitranscriptomic maps using a synthetic modification-free RNA library. Nat Methods. 2021;18:1213–22.
45. Gu X, Liang Z. Transcriptome-Wide Mapping 5-Methylcytosine by m(5)C RNA Immunoprecipitation Followed by Deep Sequencing in Plant. Methods Mol Biol. 2019;1933:389–394.
46. Chen YS, Ma HL, Yang Y, Lai WY, Sun BF, Yang YG. 5-Methylcytosine analysis by RNA-BisSeq. Methods Mol Biol. 2019;1870:237–48.
47. Schaefer M. RNA 5-Methylcytosine analysis by Bisulfite Sequencing. Methods Enzymol. 2015;560:297–329.
48. Dai Q, Ye C, Irkliyenko I, Wang Y, Sun HL, Gao Y, et al. Ultrafast bisulfite sequencing detection of 5-methylcytosine in DNA and RNA. Nat Biotechnol. 2024;42:1559–70.
49. Lu L, Zhang X, Zhou Y, Shi Z, Xie X, Zhang X, et al. Base-resolution m(5)C profiling across the mammalian transcriptome by bisulfite-free enzyme-assisted chemical labeling approach. Mol Cell. 2024;84:2984–e30002988.
50. Yu M, Cai Z, Zhang J, Zhang Y, Fu J, Cui X. Aberrant NSUN2-mediated m5C modification of exosomal LncRNA MALAT1 induced RANKL-mediated bone destruction in multiple myeloma. Commun Biol. 2024;7:1249.
51. Xiao Z, Li J, Liang C, Liu Y, Zhang Y, Zhang Y, et al. Identification of M5c regulator-medicated methylation modification patterns for prognosis and immune microenvironment in glioma. Aging. 2023;15:12275–95.
52. Galmarini D, Galmarini CM, Galmarini FC. Cancer chemotherapy: a critical analysis of its 60 years of history. Crit Rev Oncol Hematol. 2012;84:181–99.
53. Byrne JD, Yeh JJ, DeSimone JM. Use of iontophoresis for the treatment of cancer. J Control Release. 2018;284:144–51.
54. Hellmann MD, Li BT, Chaft JE, Kris MG. Chemotherapy remains an essential element of personalized care for persons with lung cancers. Ann Oncol. 2016;27:1829–35.
55. Galluzzi L, Kepp O, Vander Heiden MG, Kroemer G. Metabolic targets for cancer therapy. Nat Rev Drug Discov. 2013;12:829–46.

56. Sarosiek KA, Ni Chonghaile T, Letai A. Mitochondria: gatekeepers of response to chemotherapy. Trends Cell Biol. 2013;23:612–9.
57. Yun D, Yang Z, Zhang S, Yang H, Liu D, Grutzmann R, et al. An m5C methylation regulator-associated signature predicts prognosis and therapy response in pancreatic cancer. Front Cell Dev Biol. 2022;10:975684.
58. Chen D, Gu X, Nurzat Y, Xu L, Li X, Wu L, et al. Writers, readers, and erasers RNA modifications and drug resistance in cancer. Mol Cancer. 2024;23:178.
59. Cheng JX, Chen L, Li Y, Cloe A, Yue M, Wei J, et al. RNA cytosine methylation and methyltransferases mediate chromatin organization and 5-azacytidine response and resistance in leukaemia. Nat Commun. 2018;9:1163.
60. Aggarwal C, Prawira A, Antonia S, Rahma O, Tolcher A, Cohen RB et al. Dual checkpoint targeting of B7-H3 and PD-1 with enoblituzumab and pembrolizumab in advanced solid tumors: interim results from a multicenter phase I/II trial. J Immunother Cancer 2022; 10.
61. Cisplatin. Lancet. 1982;1:374–5.
62. Moon HM, Park JS, Lee IB, Kang YI, Jung HJ, An D, et al. Cisplatin fastens chromatin irreversibly even at a high chloride concentration. Nucleic Acids Res. 2021;49:12035–47.
63. Fuertes MA, Alonso C, Perez JM. Biochemical modulation of cisplatin mechanisms of action: enhancement of antitumor activity and circumvention of drug resistance. Chem Rev. 2003;103:645–62.
64. Browning RJ, Reardon PJT, Parhizkar M, Pedley RB, Edirisinghe M, Knowles JC, et al. Drug delivery strategies for platinum-based chemotherapy. ACS Nano. 2017;11:8560–78.
65. Meng H, Miao H, Zhang Y, Chen T, Yuan L, Wan Y, et al. YBX1 promotes homologous recombination and resistance to platinum-induced stress in ovarian cancer by recognizing m5C modification. Cancer Lett. 2024;597:217064.
66. Gao W, Chen L, Lin L, Yang M, Li T, Wei H, et al. SIAH1 reverses chemoresistance in epithelial ovarian cancer via ubiquitination of YBX-1. Oncogenesis. 2022;11:13.
67. Li Y, Li J, Luo M, Zhou C, Shi X, Yang W, et al. Novel long noncoding RNA NMR promotes tumor progression via NSUN2 and BPTF in esophageal squamous cell carcinoma. Cancer Lett. 2018;430:57–66.
68. Shao D, Liu C, Wang Y, Lin J, Cheng X, Han P, et al. DNMT1 determines osteosarcoma cell resistance to apoptosis by associatively modulating DNA and mRNA cytosine-5 methylation. FASEB J. 2023;37:e23284.
69. Shen X, Sun H, Shu S, Tang W, Yuan Y, Su H, et al. Suppression of NSUN2 enhances the sensitivity to chemosensitivity and inhibits proliferation by mediating cell apoptosis in gastric cancer. Pathol Res Pract. 2024;253:154986.
70. Li P, Wang W, Zhou R, Ding Y, Li X. The m(5) C methyltransferase NSUN2 promotes codon-dependent oncogenic translation by stabilising tRNA in anaplastic thyroid cancer. Clin Transl Med. 2023;13:e1466.
71. Tomar MS, Kumar A, Srivastava C, Shrivastava A. Elucidating the mechanisms of Temozolomide resistance in gliomas and the strategies to overcome the resistance. Biochim Biophys Acta Rev Cancer. 2021;1876:188616.
72. Yu ZC, Li T, Tully E, Huang P, Chen CN, Oberdoerffer P, et al. Temozolomide sensitizes ARID1A-Mutated cancers to PARP inhibitors. Cancer Res. 2023;83:2750–62.
73. Li Y, Gao Z, Wang Y, Pang B, Zhang B, Hu R, et al. Lysine methylation promotes NFAT5 activation and determines temozolomide efficacy in glioblastoma. Nat Commun. 2023;14:4062.
74. Awah CU, Winter J, Mazdoom CM, Ogunwobi OO. NSUN6, an RNA methyltransferase of 5-mC controls Glioblastoma response to temozolomide (TMZ) via NELFB and RPS6KB2 interaction. Cancer Biol Ther. 2021;22:587–97.
75. Shewach DS, Lawrence TS. Antimetabolite radiosensitizers. J Clin Oncol. 2007;25:4043–50.
76. Llorenc V, Nakamura Y, Metea C, Karstens L, Molins B, Lin P. Antimetabolite drugs exhibit distinctive Immunomodulatory mechanisms and effects on the intestinal microbiota in experimental autoimmune uveitis. Invest Ophthalmol Vis Sci. 2022;63:30.
77. Balzarini J. Effect of antimetabolite drugs of nucleotide metabolism on the anti-human immunodeficiency virus activity of nucleoside reverse transcriptase inhibitors. Pharmacol Ther. 2000;87:175–87.
78. Almajidi YQ, Kadhim MM, Alsaikhan F, Turki Jalil A, Hassan Sayyid N et al. Alexis Ramirez-Coronel A. Doxorubicin-loaded micelles in tumor cell-specific chemotherapy. *Environ Res* 2023; 227: 115722.
79. Yarmohammadi F, Rezaee R, Karimi G. Natural compounds against doxorubicin-induced cardiotoxicity: a review on the involvement of Nrf2/ARE signaling pathway. Phytother Res. 2021;35:1163–75.
80. Yang W, Sun Q, Zhang X, Zheng L, Yang X, He N, et al. A novel doxorubicin/CTLA-4 blocker co-loaded drug delivery system improves efficacy and safety in antitumor therapy. Cell Death Dis. 2024;15:386.
81. Ashrafizadeh M, Mirzaei S, Gholami MH, Hashemi F, Zabolian A, Raei M, et al. Hyaluronic acid-based nanoplatforms for Doxorubicin: a review of stimuli-responsive carriers, co-delivery and resistance suppression. Carbohydr Polym. 2021;272:118491.
82. Okamoto M, Fujiwara M, Hori M, Okada K, Yazama F, Konishi H, et al. tRNA modifying enzymes, NSUN2 and METTL1, determine sensitivity to 5-fluorouracil in HeLa cells. PLoS Genet. 2014;10:e1004639.
83. Schaefer M, Hagemann S, Hanna K, Lyko F. Azacytidine inhibits RNA methylation at DNMT2 target sites in human cancer cell lines. Cancer Res. 2009;69:8127–32.
84. Zhu G, Chen X. Aptamer-based targeted therapy. Adv Drug Deliv Rev. 2018;134:65–78.
85. Li F, Lin Y, Li R, Shen X, Xiang M, Xiong G, et al. Molecular targeted therapy for metastatic colorectal cancer: current and evolving approaches. Front Pharmacol. 2023;14:1165666.
86. Crooke ST, Witztum JL, Bennett CF, Baker BF. RNA-Targeted therapeutics. Cell Metab. 2018;27:714–39.
87. Yang Y, Wang J. Exploring the multi-level interaction mechanism between drugs and targets based on artificial intelligence. Cancer Insight. 2022;1:47–51.
88. Ohmori T, Yamaoka T, Ando K, Kusumoto S, Kishino Y, Manabe R et al. Molecular and clinical features of EGFR-TKI-Associated Lung Injury. Int J Mol Sci 2021; 22.
89. Oshima Y, Tanimoto T, Yuji K, Tojo A. EGFR-TKI-Associated Interstitial Pneumonitis in Nivolumab-Treated Patients With Non-Small Cell Lung Cancer. *JAMA Oncol* 2018; 4: 1112–1115.
90. Yu W, Zhang C, Wang Y, Tian X, Miao Y, Meng F, et al. YAP 5-methylcytosine modification increases its mRNA stability and promotes the transcription of exosome secretion-related genes in lung adenocarcinoma. Cancer Gene Ther. 2023;30:149–62.
91. Wang Y, Wei J, Feng L, Li O, Huang L, Zhou S, et al. Aberrant m5C hypermethylation mediates intrinsic resistance to gefitinib through NSUN2/YBX1/QSOX1 axis in EGFR-mutant non-small-cell lung cancer. Mol Cancer. 2023;22:81.
92. Zhang J, Zhao K, Zhou W, Kang R, Wei S, Shu Y, et al. Tet methylcytosine dioxygenase 2 (TET2) deficiency elicits EGFR-TKI (tyrosine kinase inhibitors) resistance in non-small cell lung cancer. Signal Transduct Target Ther. 2024;9:65.
93. Nulali J, Zhang K, Long M, Wan Y, Liu Y, Zhang Q, et al. ALYREF-mediated RNA 5-Methylcytosine modification promotes Hepatocellular Carcinoma Progression Via stabilizing EGFR mRNA and pSTAT3 activation. Int J Biol Sci. 2024;20:331–46.
94. Song D, An K, Zhai W, Feng L, Xu Y, Sun R, et al. NSUN2-mediated mRNA m(5)C modification regulates the progression of Hepatocellular Carcinoma. Genomics Proteom Bioinf. 2023;21:823–33.
95. Gong L, Zhang Y, Liu C, Zhang M, Han S. Application of Radiosensitizers in Cancer Radiotherapy. Int J Nanomed. 2021;16:1083–102.
96. Zhou Z, Guan B, Xia H, Zheng R, Xu B. Particle radiotherapy in the era of radioimmunotherapy. Cancer Lett. 2023;567:216268.
97. Yu M, Ni M, Xu F, Liu C, Chen L, Li J, et al. NSUN6-mediated 5-methylcytosine modification of NDRG1 mRNA promotes radioresistance in cervical cancer. Mol Cancer. 2024;23:139.
98. Du S, Yan J, Xue Y, Zhong Y, Dong Y. Adoptive cell therapy for cancer treatment. Explor (Beijing). 2023;3:20210058.
99. Chen T, Xu ZG, Luo J, Manne RK, Wang Z, Hsu CC, et al. NSUN2 is a glucose sensor suppressing cGAS/STING to maintain tumorigenesis and immunotherapy resistance. Cell Metab. 2023;35:1782–e17981788.
100. Jin S, Li J, Shen Y, Wu Y, Zhang Z, Ma H. RNA 5-Methylcytosine Regulator NSUN3 promotes tumor progression through regulating immune infiltration in head and neck squamous cell carcinoma. Oral Dis. 2024;30:313–28.
101. Lv Z, Xue C, Zhang L, Sun J, Bo C. Elevated mRNA level of Y-Box binding protein 1 indicates unfavorable prognosis correlated with macrophage infiltration and T cell exhaustion in luminal breast Cancer. Cancer Manag Res. 2021;13:6411–28.
102. Chen Z, Zeng C, Yang L, Che Y, Chen M, Sau L et al. YTHDF2 promotes ATP synthesis and immune evasion in B cell malignancies. Cell 2024.
103. Chen H, Ge XL, Zhang ZY, Liu M, Wu RY, Zhang XF, et al. M(5)C regulator-mediated methylation modification patterns and tumor microenvironment infiltration characterization in lung adenocarcinoma. Transl Lung Cancer Res. 2021;10:2172–92.

104. Pan J, Huang Z, Xu Y. m5C-Related lncRNAs predict overall survival of patients and regulate the Tumor Immune Microenvironment in Lung Adenocarcinoma. Front Cell Dev Biol. 2021;9:671821.
105. Liu X, Wang D, Han S, Wang F, Zang J, Xu C et al. Signature of m5C-Related lncRNA for Prognostic Prediction and Immune Responses in Pancreatic Cancer. J Oncol. 2022;2022:7467797.
106. Han QQ, Ren QD, Guo X, Farag MA, Zhang YH, Zhang MQ et al. Punicalagin attenuates hyperuricemia via restoring hyperuricemia-induced renal and intestinal dysfunctions. J Adv Res 2024.
107. Wang L, Yu P, Wang J, Xu G, Wang T, Feng J et al. Downregulation of circ-ZNF609 Promotes Heart Repair by Modulating RNA N(6)-Methyladenosine-Modified Yap Expression. Research (Wash D C). 2022;2022:9825916.
108. Xia K, Wang T, Chen Z, Guo J, Yu B, Chen Q, et al. Hepatocellular SETDB1 regulates hepatic ischemia-reperfusion Injury through Targeting lysine methylation of ASK1 Signal. Res (Wash D C). 2023;6:0256.
109. Lin J, Yang X, Liu S, Luo Z, Chen Q, Sun Y, et al. Long non-coding RNA MFAT1 promotes skeletal muscle fibrosis by modulating the miR-135a-5p-Tgfbr2/Smad4 axis as a ceRNA. J Cell Mol Med. 2021;25:4420–33.
110. Li FQ, Chen WB, Luo ZW, Chen YS, Sun YY, Su XP, et al. Bone marrow mesenchymal stem cell-derived exosomal microRNAs target PI3K/Akt signaling pathway to promote the activation of fibroblasts. World J Stem Cells. 2023;15:248–67.
111. Teng S, Han C, Zhou J, He Z, Qian W. M(5)C RNA methylation: a potential mechanism for infectious Alzheimer's disease. Front Cell Dev Biol. 2024;12:1440143.
112. Wang K, Li FH, Zhou LY, Zhao XM, Gao XQ, Liu CY et al. HNEAP Regulates Necroptosis of Cardiomyocytes by Suppressing the m(5) C Methylation of Atf7 mRNA. Advanced science (Weinheim, Baden-Wurttemberg, Germany). 2023;10:e2304329.
113. Cheng JX, Chen L, Li Y, Cloe A, Yue M, Wei J, et al. Author correction: RNA cytosine methylation and methyltransferases mediate chromatin organization and 5-azacytidine response and resistance in leukaemia. Nat Commun. 2018;9:2286.
114. Chen B, Deng Y, Hong Y, Fan L, Zhai X, Hu H et al. Metabolic Recoding of NSUN2-Mediated m(5)C Modification Promotes the Progression of Colorectal Cancer via the NSUN2/YBX1/m(5)C-ENO1 Positive Feedback Loop. Advanced science (Weinheim, Baden-Wurttemberg, Germany). 2024;11:e2309840.
115. Xu J, Ji L, Liang Y, Wan Z, Zheng W, Song X, et al. CircRNA-SORE mediates sorafenib resistance in hepatocellular carcinoma by stabilizing YBX1. Signal Transduct Target Ther. 2020;5:298.

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