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Review Article

Deciphering the vital roles and mechanism of m5C modification on RNA in cancers

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Abstract: 5-methylcytosine (m5C modification) plays an essential role in tumors, which affects different types of RNA, the expression of downstream target genes, and downstream pathways, thus participating in the tumor process. However, the effect of m5C modification on RNA in tumors and the exact mechanism have not been systematically reviewed. Therefore, we reviewed the status and sites of m5C modification, as well as the expression pattern and biological functions of m5C regulators in tumors, and further summarized the effects and regulation mechanism of m5C modification on messenger RNA (mRNA), ribosomal RNA (rRNA), transfer RNA (tRNA), long non-coding RNA (lncRNA) and other RNA in tumors. Finally, we summed up the interaction network, potential application, and value in clinical diagnosis and treatment of tumors. Taken together, this review benefits revealing the mechanism of m5C modification in tumor progression and provides new strategies for tumor diagnosis and treatment.

Keywords: m5C modification, RNA, target gene, m5C regulator, tumor progression

Introduction

Posttranscriptional modifications of RNA have attracted growing attention in tumors [1-5]. Among them, m5C modification regulates the expression of downstream target genes and exerts different biological functions by affecting the stability and translation of different types of RNA, such as mRNA, rRNA, tRNA, and ncRNA [6-8], to further participate in carcinoma [9-13]. The acting elements of m5C modification contain m5C methyltransferase (m5C writers), m5C demethyltransferase (m5C erasers), and a binding protein of m5C methylation (m5C readers), among which m5C writers are mainly NSUN family (NSUN1-NSUN7) and TRDMT1 (DNMT2) [14-17], while ALKBH1 and TET families belong to m5C erasers, ALYREF and YBX1 mainly serve as m5C readers [18-20].

Researchers have reported that m5C modification plays a key role in regulating the occurrence and malignant progress of many tumors, such as breast cancer, liver cancer, prostate cancer, lung cancer, colon cancer, glioma, and other tumors [21-26], which is crucial in tumor invasion, metastasis, metabolism, and immune escape of tumor [27-30]. In this review, the biological function, regulatory mechanism, interaction network, and clinical significance of m5C modification are described systematically from the aspects of RNA types and m5C regulators in tumors, to provide important strategies for revealing the carcinogenic mechanism and clinical significance of m5C modification.

Abnormal m5C modification is closely related to tumorigenesis

High modification of m5C exists in tumors

Changes in m5C modification are closely related to tumorigenesis, and the overall abnormal m5C modification exists in tumors. For examp-

Table 1. The abnormal modification of m5C in tumors

Cell types	State	Factors	Ref
Urothelial carcinoma of the bladder	Up	Malignant progression	[31]
Retinoblastoma	Up	Malignant progression	[32]
Non-small cell lung cancer	Up	Drug-resistance	[41]
Lung adenocarcinoma	Up	Drug-resistance	[42]
Osteosarcoma	Up	DNA damage repair	[89, 90]
Gastric cancer	Up	NSUN2 overexpression	[33]
Cervical cancer	Up	NSUN2 overexpression	[36]
Bladder cancer	Up	NSUN2 overexpression	[37]
Uveal melanoma	Up	NSUN2 overexpression	[35]
Esophageal squamous-cell carcinoma	Up	NSUN2 overexpression	[38]
Hepatocellular carcinoma	Up	NSUN2 overexpression	[39]
Prostate cancer	Up	NSUN2 overexpression	[14]

le, the m5C modification on mRNA in UCB samples and retinoblastoma (RB) cells was higher than that in normal tissues or cells [31, 32]. At the same time, changes in regulators that regulate m5C modification affect the whole m5C modification in tumors. For example, knockout of the NSUN2 gene significantly reduced the overall level of the m5C modification on RNA in gastric cancer (GC), cervical cancer (CC), uveal melanoma (UM) cell lines, bladder cancer (BCa), prostate cancer (PCa) and so forth [14, 33-39]. In addition, miR-124a also affects the expression of m5C regulator, thus regulating the overall level of m5C modification in UM cells [35].

Meanwhile, the abnormalities of m5C modification in some transcripts also exist in tumors. The acting elements of m5C modification may play a key role in tumors by affecting the m5C modification of target genes. For example, the m5C modification on PKM2 mRNA has decreased due to the absence of NSUN2 in BCa [37]. Similarly, abnormalities of m5C modification are also found in p57KIP2 mRNA of gastric cancer, CTNNB1 mRNA in UM cells, and FOXC2 mRNA in GC [33, 35, 40]. In addition, drug resistance of cells, which is the key reason for chemotherapy resistance, also has an impact on the modification of m5C, thus participating in the process of tumorigenesis. For example, the expression of m5C modification is higher in non-small cell lung cancer drug-resistant cells compared with sensitive cells [41]. Studies reported that the level of m5C modification on YAP mRNA in AZD9291-resistant cells of lung adenocarcinoma (LUAD) was significantly higher than that in non-drug-resistant cells of LUAD [42]. All of these abnormalities of m5C modification in tumors are summarized in **Table 1**.

Sites of m5C modification in tumors

Different locations of m5C modification on RNA have different influences, resulting in diverse biological effects in tumors. Recent studies have proven that m5C modification sites are often located in CG-rich environment, mainly in 3'-UTR and CDS regions of mRNA, and less m5C modification on 5'-UTR of mRNA [31, 38, 43]. For example, the sites of m5C modification in pancreatic cancer cells were mainly located in the CDS region and 3'-UTR, and few in 5'-UTR of mRNA. The absence of NSUN2 reduced the m5C modification in the CDS region and increased the m5C-modified sites in 3'-UTR, while the m5C-modified sites of 5'-UTR are still in a low state [44]. mRNA 3'-UTR is a common region of the m5C locus. For example, the m5C modification in 3'-UTR of TEAD1 mRNA, YAP mRNA, and GRB2 mRNA were modified by NSUN2 [38, 42, 45]. Similarly, m5C reader ALYREF can also combine with 3'-UTR on PKM2 mRNA to stabilize PKM2 mRNA in BCa [37]. m5C modification also exists in the CDS region of the transcript. The CDS near the 3'-UTR and 3'-UTR regions of KRT13 mRNA contain m5C modification in CC [34].

m5C regulators have binding specificity to m5C loci. For example, NSUN6 had the characteristics of substrate specificity and preferentially binds to methylation sites in hairpin rings with stem-ring structure. A study identified that the specific methylation of NSUN6 depended on the site of CTC[CT]A, and NSUN6 mainly targeted the CTCCA motif of 3'-UTR [46]. The m5C sites modified by NSUN2 were often enriched in the consensus sequence ([A/G]GGG) of 3'-UTR [44]. In addition, m5C reader YBX1 with cold shock domain (CSD) specifically recognized CA(U/C)C motif in RNA and preferentially bound to m5C-modified RNA oligomer (5'-UCAU(m5C)U-3'), resulting that NSUN2 and

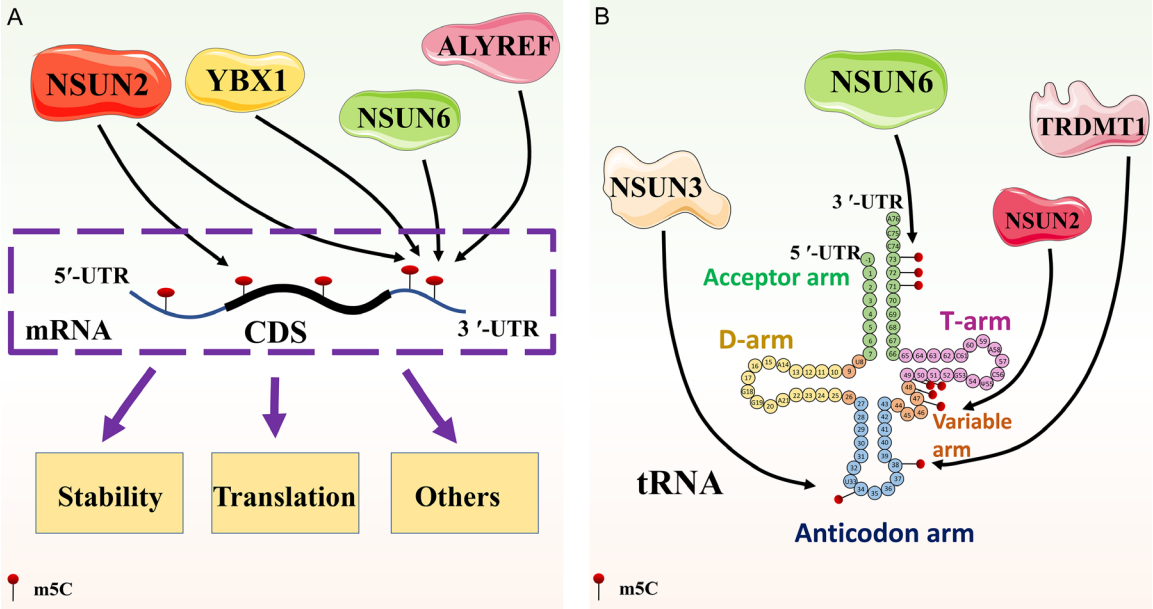

Figure 1. m5C sites on RNA. A: m5C sites on mRNA. B: m5C sites on tRNA. 3'-UTR, 3'-untranslated region; 5'-UTR, 5'-untranslated region; CDS: coding sequences. mRNA, messenger RNA; tRNA: transfer RNA. m5C, 5-methylcytosine.

YBX1 specifically regulated the m5C modification of HDGF mRNA 3'-UTR [31]. m5C-modified sites also exist in tRNA, and the sites at 38, 47, and 48 are three known m5C sites at $tRNA^{Asp}$ (GUC) [47]. Besides, NSUN6 specifically methylated $tRNA^{Thr}$ (TGT), (AGT), (CGT), $tRNA^{Thr}$ (ACG), and $tRNA^{Cys}$ (GCA) sites [46] (**Figure 1**).

Abnormal expression and regulation of m5C regulators in tumors

Abnormal expression of m5C regulators in tumors: The regulators of m5C modification show abnormal expression in different tumor types. Researchers have found that high expression of m5C writers NSUN1 and NSUN2 were a common carcinogenic event. NSUN1 was highly expressed in most tumors, such as colorectal cancer and ovarian cancer [48, 49]. The expression level of NSUN2 was high in HPSCC, ESCC, UM cell lines, PCa, OV, CESC, UCBs, hepatocellular carcinoma, and breast cancer [14, 31, 33-35, 38, 39, 43, 45, 50, 51]. NSUN3 was highly expressed in OSCC, HNSCC, LUSC datasets and LIHC [52-55], and NSUN4 was highly expressed in ccRCC, hepatocellular carcinoma (HCC) and LUSC [54, 56, 57]. Other members of the NSUN family also have abnormal expressions. The mRNA and protein levels of NSUN5 were up-regulated in hepatocellular carcinoma, but suppressed in glioma. Besides, the mRNA level and protein expression of NSUN6 significantly decreased in pancreatic cancer tissues and cell lines [58, 59]. NSUN7 seems to play the role of tumor suppressor gene [60].

m5C erasers display abnormal expression in tumors. TET2 was upregulated in prostate cancer and glioma, but downregulated in ccRCC [56, 61, 62]. Moreover, TET3 was upregulated in HCC and prostate cancer, and TET3 had a poor prognosis [23, 63].

YBX1 and ALYREF, as m5C readers, also have abnormal expression in tumors. For example, YBX1 was highly expressed in tumor tissues compared with normal tissues in UCBs [31]. The expression of ALYREF was also up-regulated in BCa tissues and urothelial bladder cancer [37, 64], suggesting that the change of the m5C regulator plays a key role in tumors.

Abnormality of upstream regulation of m5C in tumors: Abnormal upstream regulation of m5C modification might be the primary reason for triggering changes in m5C modification and promoting tumorigenesis. A growing body of evidence attests that m5C writers have abnormal upstream regulation, and the upstream

molecules regulate the expression of NSUN2 at the gene level. For example, E2F1 and oncogene MYC positively induced NSUN2 expression at the gene level [38, 65]. Androgen receptor (AR) also bound to the upstream sequence of transcriptional initiation of NSUN2 promoter and stimulated the expression of NSUN2 in PCa [14]. DNMT1 methylated the promoter of NSUN2 and inhibited the expression of NSUN2 in osteosarcoma [66]. Upstream regulators regulate the expression of NSUN2 during the transcriptional process. The knockout of the STAT1 gene inhibits the transcriptional and protein expression of NSUN2 in KYSE70 cells (AA type) and KYSE450 (GA type) in ESCC [50]. The regulator of m5C modification is also regulated by microRNA. Previous studies demonstrated that miR-124 regulates 3'-UTR of NSUN2 mRNA in UM, thus inhibiting the expression of NSUN2 at the protein level [35]. Moreover, upstream molecules also regulate the expression of NSUN2 during translational and posttranslational modification. RPL6 interacted with NSUN2 and partially regulated the translation process of NSUN2, promoting the occurrence of gallbladder carcinoma [67]. FOXC2-AS1 (lncRNA) induced the expression of NSUN2 in GC [40]. SUMO-2/3 bound to the SIM region of NSUN2, 236-240 aa of NSUN2, and NSUN2 can be sumoylated by SUMO-2/3 to further regulate the nuclear transport process of NSUN2. The decrease of SUMO-2/3 significantly reduced the level of NSUN2 in the nucleus, which partially inhibited the promoting effect of NSUN2 in gastric cancer [43]. Furthermore, long noncoding RNA DIAPH2-AS1 interacted with NSUN2 and inhibited the ubiquitin degradation of NSUN2 in gastric cancer [68]. In addition, other substances, such as glucose, also bound to and activated NSUN2 [69]. The molecules regulating the m5C eraser have not been reported in the study of m5C modification in tumors.

m5C readers also have abnormal upstream regulation to affect the modification of m5C. SIAH1 and YBX1 shared a common location and were bound to each other in ovarian cancer. SIAH1 not affected YBX1 mRNA, but shorted the half-life of YBX1 protein at the post-transcriptional level and ubiquitinated YBX1 at position K304 of YBX1 through the protease pathway, which reduced protein stability and negatively regulated protein expression of YBX1 [70]. In addition, hypoxia-inducible factor-1α (HIF-1α) bound to the region of ALYREF promoter through the HRE site (5'-ACGTGC-3') in BCa. Increased HIF-1α induced by hypoxia transactivated the promoter of ALYREF and further enhanced the transcriptional and protein expression of ALYREF [37] (**Table 2**; **Figure 2**).

The functions of m5C regulators in tumors

Writers

NSUN1: The regulators of m5C modification play a crucial biological role in tumors [71, 72]. NSUN1 seems to play an oncogene in a variety of tumor types and promotes the progression of cancers. For example, NSUN1 was a prognostic marker for breast cancer and promoted the NIH/3T3 cells to form tumors in nude mice [73]. In high-grade serous ovarian cancer (HGSOC), NSUN1 promoted the proliferation, migration, and tumor invasion [49].

NSUN1 is significantly crucial for the processing of rRNA. In budding yeast, NSUN1 methylated 25s rRNA, regulated pre-rRNA processing, and promoted the formation of the snoRNP complex, thus regulating rRNA processing [73].

NSUN2: NSUN2, functioning as an oncogene, affects the process of cell proliferation, migration, and tumor growth in an m5C modification-depedent manner [74]. For example, NSUN2 promoted the proliferation, clone formation, migration, and tumor growth in vivo of GC, HPSCC, prostate cancer, CC, ESCC and hepatocellular carcinoma cells [14, 33, 36, 38, 39, 43, 45].

NSUN2 has an effect on the cell cycle in tumors. NSUN2 affected the cell cycle progression of gastric cancer cells, and the absence of NSUN2 affected the level of m5C modification, resulting in the differential expression of genes related to the cell cycle in GC. Besides, NSUN2 reduced the percentage of the G1/G0 phase, and knockout of NSUN2 gene induced cell cycle arrest of G1/G0 phase [33, 43]. In UM cell lines, the absence of NSUN2 inhibited the arrest of UM cells through the G1/S checkpoint [35]. Knockout NSUN2 led to fewer G1 and S phase cells, and more G2 and M phase cells in hepatocellular carcinoma [39].

Meanwhile, NSUN2 impacts the process of cell differentiation. NSUN2 promoted angiogenesis

Table 2. The abnormal upstream regulators of m5C modification in tumors

Cell types	Upstream	Regulate	Regulators/Role/State	Functions	Ref
Esophageal squamous-cell carcinoma	E2F1	Positive	NSUN2/Writer/Up	Promote cell proliferation, migration and tumor growth	[38]
Gastric cancer	E2F1	Positive	NSUN2/Writer/Up	Promote metastasis	[124]
Squamous-cell-carcinoma	Myc	Positive	NSUN2/Writer/Up	Promote cell proliferation and growth	[65]
Prostate cancer	AR	Positive	NSUN2/Writer/Up	Promote cell proliferation, migration and tumor growth	[14]
Gallbladder carcinoma	RPL6	Positive	NSUN2/Writer/Up	Promote cell proliferation	[67]
Esophageal squamous-cell carcinoma	STAT1	Positive	NSUN2/Writer/Up	Promote tumor proliferation and progression	[50]
Gastric cancer	FOXC2-AS1	Positive	NSUN2/Writer/Up	Promote cell proliferation, invasion and migration	[40]
Gastric cancer	SUMO-2/3	Positive	NSUN2/Writer/Up	Promote cell proliferation, migration, cell cycle and tumor growth	[43]
Gastric cancer	DIAPH2-AS1	Positive	NSUN2/Writer/Up	Promote cell invasion	[68]
Osteosarcoma	DNMT1	Negative	NSUN2/Writer/Up	Promote the resistance of cell apoptosis	[66]
Uveal melanoma	miR-124	Negative	NSUN2/Writer/Up	Impact on cell cycle	[35]
Hep3B/PC3	Glucose	Positive	NSUN2/Writer/Up	Resist anti-PDL1 immunotherapy	[69]
Urothelial carcinoma of the bladder	ALYREF	Positive	NSUN2/Writer/Up	Promote progression	[64]
Ovarian cancer	SIAH1	Negative	YBX1/Reader/Up	Enhance the resisitance of DDP and promote cell proliferation, invasion and migration	[70]
Bladder cancer	HIF-1α	Positive	ALYREF/Reader/Up	Promote glycolysis and cell proliferation	[37]

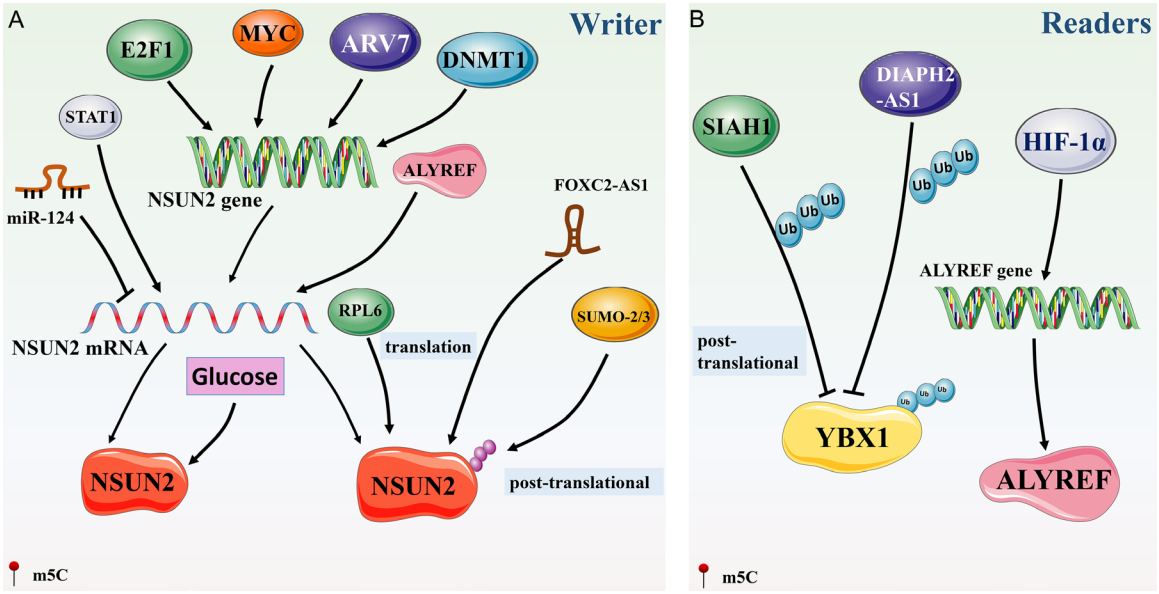

Figure 2. Abnormality of upstream regulation of m5C in tumors. A: The abnormal upstream of m5C writers. B: The abnormal upstream of m5C readers. m5C, 5-methylcytosine.

and poor differentiation of HCC cells via impacting m5C modification in lncRNA H19 [39]. The absence of NSUN2 increased the expression of mucin, regulated the cell polarity of tumorigenesis, and promoted the differentiation of ducts [44].

DNA damage and drug resistance of tumor cells are impacted by NSUN2. For example, the removal of NSUN2 promoted the process of DSBs, thus destroying the integrity of the genome [44]. Similarly, NSUN2 upregulated the m5C modification of LRRC8A mRNA, thereby inhibiting cell apoptosis, promoting survival, and promoting cisplatin resistance of cervical cancer cells [36]. The highly expressed NSUN2 also led to gefitinib resistance and tumor recurrence in non-small cell lung cancer [41].

NSUN2 is also necessary in the metabolism and tumor microenvironment [75, 76]. Taking osteosarcoma (OS) as an example, NSUN2 engaged in and enhanced the process of fatty acid metabolism [76]. Moreover, the high expression of NSUN2 in tumors was accompanied by $CD4^+$ immunoosmosis in the microenvironment, which affected the progression of tumors [44]. NSUN2 also upregulated the m5C modification and protein expression of ATX in endothelial cells and promoted the secretion of ATX. The ATX further interacted with integrin α4 on T cells and promoted the occurrence of FAK/Src-RhoA axis, thus increasing the infiltration of T cells, especially $CD3^+$ T cells, and promoting the occurrence of abdominal aortic aneurysm (AAA) [77].

NSUN3: NSUN3 is mainly located in mitochondria [78]. The m5C modification mediated by NSUN3 is closely related to the function of mitochondria. NSUN3 can affect the synthesis and translation of proteins in mitochondria. The deletion of NSUN3 reduced the synthesis of mitochondrial protein and oxygen consumption, which affected mitochondrial oxidative phosphorylation and respiratory coupling, thus reducing mitochondrial activity [78]. NSUN3 also regulated the shape and structure of mitochondria and influenced the translation of mitochondria [52, 79, 80].

NSUN3 affects metastasis by acting on mitochondria. The deletion of NSUN3 reduced the metastasis of the tumor by reducing the m5C modification of mitochondria. Methylation inhibition showed an increase in an upregulation of glucose transporter 1 (GLUT1) and glycolysis, thus reducing the metastasis [52].

NSUN3 is also related to the immune process. In head and neck squamous cell carcinoma, NSUN3 regulated immune infiltration and promoted tumor growth. The abundance of immune cells with high expression of NSUN3, such

as T cells CD8, and NK cells, is low. The knockout of NSUN3 decreased the number of M2 macrophages but increased the number of M1 macrophages [53]. And relate to that infiltration of immune cells. NSUN3 was closely related to $CD8^+$ T cells [54].

NSUN4: NSUN4 methylated Sox9 mRNA 3'UTR in a m5C-dependant manner, regulated translation reprogramming of Sox9, and promoted cartilage formation [81]. Moreover, NSUN4 was closely related to the infiltration of immune cells, especially neutrophils in LUSC [54].

NSUN4 has an important influence on rRNA. On the one hand, NSUN4 stabilized 16S rRNA together with MTERF4 and formed a complex with MTERF4 to promote subunit assembly [82]. On the other hand, NSUN4 methylated mitochondrial 12S rRNA and promoted the rearrangement of rRNA to form a peptidyl transferase center [83].

NSUN5: NSUN5, as a member of the NSUN family, can also influence m5C modification in RNA. NSUN5 affects the methylation status of rRNA and is expressed in a state of inhibition due to hypermethylation of CpG island in the promoter, to serve as a suppressor in glioma. The inhibited NSUN5 promoted the growth of glioma cells in vivo [59]. Some researchers proved that NSUN5 promoted the proliferation of hepatocellular carcinoma cells in vivo and in vitro [58]. Similarly, NSUN5 had higher expression in colorectal cancer compared with normal tissues and promoted cell growth. Moreover, NSUN5 regulated the cell cycle of colorectal cancer cells and regulated the expression of CDK4 and CCNE1, leading to engaging in tumorigenesis [84]. However, the biological functions of NSUN5 are whether owing to the change of m5C modification in hepatocellular carcinoma and colorectal cancer are needed to further verified.

NSUN6: NSUN6 seems to act as a tumor suppressor gene in tumors. NSUN6 did not impact cell apoptosis, but NSUN6 promoted the expression of CDK10, thus inhibiting the proliferation of pancreatic cancer cells [85]. Similarly, NSUN6 inhibited the proliferation of HCC cells [46].

NSUN6 also plays an important role in the process of development. The deletion of NSUN6 affected the mesoderm to further impact the differentiation of embryonic stem cells by reducing the level of targeted mRNAs in a m5C modified manner. Besides, NSUN6 influenced the development of embryos.

NSUN7: NSUN7 also seems to serve as a tumor suppressor gene. NSUN7 had a good prognosis in ewing sarcoma (ES) [60]. In liver cancer, the hypermethylation of NSUN7 promoted the occurrence of liver cancer [86]. In addition, NSUN7 was also associated with immune infiltrating cells [87].

NSUN7 also impacts metabolism. NSUN7 affected the stability of eRNA by affecting the m5C modification on eRNA, thus regulating metabolism in vivo [88].

TRDMT1 (DNMT2): TRDMT1, as another writer of m5C modification, matters in the tumor. TRDMT1 increased the m5C modification on mRNA in DNA: RNA complex and enhanced the resistance of U2OS cells to IR and breast cancer cells to PARPI olaparib. Further studies demonstrated that TRDMT1 promoted the repair of DNA damage of homologous recombination (HR), which might be the reason for resistance to IR and drugs [89, 90].

Erasers

TET1/2/3: The studies about demethyltransferases of m5C modification are few in tumors, and the currently widely recognized erasers are mainly the TET family, including TET1, TET2, and TET3 [91].

The remover of m5C modification plays a key role in the process of m5C-mediated tumors. For example, dCas13b-TET1/2 with gPEBP1 removed the m5C modification on PEBP1 mRNA and shorted the half-life of PEBP1 mRNA, further reducing the stability of mRNA and decreasing the expression of PEBP1, thus inhibiting the malignant biological process of renal cell carcinoma [92].

The eraser of m5C modification also plays an important role in DNA damage repair. When oxidative damage existed, TET1 was recruited to the damage site at the DNA: RNA complex and removed the m5C modification on mRNA, thus promoting the completion of HR and increasing

the radiation resistance of breast cancer cells [89].

ALKBH1: As another eraser of m5C modification, ALKBH1/ABH1 is mainly located in mitochondria, which affects m5C modification on tRNA including mtRNA [79, 93]. The RNA expression level of ALKBH1 was upregulated in OSCC [52]. ALKBH1 affected the translation of mitochondria [79]. ALKBH1 oxidized m5C modification at the 34th position of mtRNA, which led to f5C modification, thus affecting mitochondrial translation [94].

Readers

YBX1: m5C readers are inseparable from the role in the biological process of tumor formation [95]. Studies have demonstrated that m5C reader YBX1 is related to tumor growth, invasion, and metastasis. Taking UCBs as an example, knockout YBX1 significantly reduced the growth of subcutaneous tumors in a m5C-dependent manner, inhibited the ability of invasion and metastasis in vivo and in vitro, and reduced the formation of lung metastatic nodules in vivo [31]. YBX1 also participated in the process of drug resistance of tumor cells. YBX1 enhanced cisplatin resistance in ovarian cancer cells [70]. Moreover, the expression of YBX1 was also higher in drug-resistant cells treated with gefitinib than sensitive cells in non-small cell drug-resistant cells [41].

ALYREF: ALYREF, as another m5C reader, is significant in the biological process of carcinoma. For example, knockout ALYREF decreased the binding to the m5C sites on PKM2 mRNA and reduced cell growth and colony formation in vitro, and inhibited the growth of subcutaneous tumors in vivo in BCa [37]. The coexpression of ALYREF and NSUN2 also reduced the activity of caspase3/7 and promoted the increase of tumor volume in lung adenocarcinoma [42]. Moreover, ALYREF and NSUN2 genes also promoted the drug resistance of lung adenocarcinoma cells to AZD9291 [42].

In addition, ALYREF is also associated with exosome secretion and tumor metabolism. ALYREF and NSUN2 promoted the expression of MYCN and SOX10 mediated by YAP in H1299 and A549 LUAD cells, thus promoting the ability of exosome secretion of LUAD cells to promote transcription system in a m5C-dependent manner [42]. Meanwhile, ALYREF is involved in the metabolic process of tumors. ALYREF promoted glycolysis of tumor cells in BCa. The removal of ALYREF affected the glucose utilization of BCa cells, as well as the intracellular ATP level and the amount of lactic acid, thus affecting the cell glycolysis process [37].

The mechanism of m5C modification in different RNA types

mRNA

m5C modification on mRNA affects the stability of transcripts: m5C modification on RNA affects the function of RNA. m5C modification on mRNA can regulate mRNA and affect the effector genes, to participate in the process of carcinoma [96-98]. For example, NSUN1 regulated the expression of RAPGEF4 in an m5C-dependent manner and promoted the progression of HGSOC [49]. NSUN2 methylated the CDS region of SCRIB mRNA in pancreatic cancer and TEAD1 mRNA 3'-UTR in HPSCC, increased m5C modification, and promoted the expression, thus regulating cell epithelial differentiation and tumorigenesis [44, 45]. In addition, NSUN2 regulated the expression of downstream target genes PCYT1A and PIK3R1 in an m5C-dependent manner and promoted GC progression through m5C-dependent and independent mechanisms [43]. NSUN2 also regulated the NTN1 mRNA via increasing m5C modification to promote the development of neural invasion [68].

m5C modification on mRNA can impact the stability of mRNA, which might be the reason for the influence on mRNA. On the one hand, the transcripts of oncogenes become stable via the modification of m5C, thus driving tumor development and progression. NSUN2 promoted the increase of m5C modification on downstream TREX2 mRNA, promoted the expression of TREX2 mRNA and protein, and then inhibited the activation of cGAS/STING and promoted the occurrence of tumor [69]. FABP5 mRNA can be stabilized by NSUN2-mediated modification of m5C in OS [76]. YBX1 has been shown to regulate the stability of mRNA and promote the expression of downstream genes E2F5, YY1, and Rcc2, further promoting the progression of ovarian cancer [70]. In different types of tumors, NSUN2 and YBX1 can stabilize oncogene transcripts together. m5C methylation of

HDGF mRNA was catalyzed by NSUN2, and the interaction between YBX1 and ELAVL1 can stabilize HDGF mRNA. The increased m5C modification further enhanced the mRNA expression of some UCB-related oncogenes, leading to the malignant biological process of UCBs [31]. The stability of FOXC2 mRNA, KRT13 mRNA, LRRC8A mRNA, and TIAM2 mRNA were also affected by m5C modification which NSUN2 and YBX1 increased and combined, which promoted the expression of mRNA and protein. LRRC8A further promoted the PI3K-AKT signal pathway and enhanced the ability of tumor invasion and metastasis [34, 36, 40]. NSUN2 and ALYREF can also stabilize oncogene transcripts together. For example, NSUN2 increased and ALYREF recognized the m5C modification on YAP mRNA 3'-UTR in LUAD, which inhibited the degradation of YAP mRNA and stabilized YAP mRNA, and further increased the transcription and protein expression of YAP. At the same time, stable YAP mRNA prevented miR-582-3P from binding to YAP mRNA 3'-UTR, thus stabilizing YAP mRNA [42]. Similarly, the two regulators jointly mediated m5C modification in PKM2 mRNA 3'-UTR in BCa and PFAS mRNA in retinoblastoma cells, thus stabilizing mRNA in a m5C-dependent method and upregulating the protein expression [32, 37]. In addition, NSUN2 can work with LIN28B to stabilize transcripts. NSUN2 targeted and increased the m5C modification of GRB2 mRNA 3'-UTR. Similar to YBX1, LIN28B, had a cold shock domain (CSD), acted as a reader of m5C modification, and recognized and bound m5C sites on GRB2 mRNA, as well as stabilized mRNA and promoted PI3K/AKT and ERK/MAPK pathway, leading to the carcinogenic process of ESCC [38].

On the other hand, m5C modification reduces the transcriptional stability of tumor suppressor genes and promotes tumor progression. NSUN2 targeted the m5C modification of p57KIP2 mRNA 3'-UTR in GC and shorted the half-life of p57KIP2 transcripts, making p57KIP2 mRNA unstable and inhibiting the expression of p57KIP2, leading to involvement in the carcinogenic process of GC [33] NSUN7 seems to act as a tumor suppressor gene. In liver cancer, the hypermethylation of NSUN7 promoter led to the epigenetic inactivation of NSUN7, promoted the hypomethylation of CCDC9B mRNA C1322-1324, thus destroying the stability of the mRNA and reducing the expression of CCDC9B protein, thus reducing the interaction between CCDC9B protein and IVNS1ABP protein, promoting the up-regulation of IVNS1ABP protein, and thus promoting the occurrence of liver cancer [86] (**Table 3**; **Figure 3**).

m5C modification on mRNA affects translation: m5C modification on mRNA can affect translation and further impact effector genes, resulting in participating in tumorigenesis and development [99]. Researchers proved that specific methylation of NSUN6 was associated with translation termination, which influenced the stop codon bias in the process of mRNA translation, and the translational termination site marked by NSUN6 was CTCCA [46], suggesting that NSUN6 regulated the target mRNA at the translation level. Similarly, the removal of NSUN2 did not affect the level of downstream target mRNA of CTNNB1 in UM cells, but regulated the protein level of CTNNB1. Further studies found that NSUN2 acted on the m5C modification in the CDS region of CTNNB1 mRNA and increased the expression of CTNNB1, thus mediating the expression of downstream target genes c-Myc and CyclinD1 of CTNNB1 and promoting the occurrence of UM [35]. Moreover, NSUN2 targeted the downstream gene QSOX1 and increased the m5C modification on QSOX1 mRNA CDS. Then, YBX1 recognized the modification and enhanced the translation of QSOX1, promoting drug resistance [41]. Similarly, NSUN2 regulated the m5C modification site C2756 in the 3'-UTR region of ATX mRNA, and ALYREF interacted with m5C modification on ATX mRNA to promote the nuclear output of ATX mRNA, which promoted the expression of ATX at the translational level, further promoted the expression of LPA and promoted the migration of U87 cells [100] (**Table 3**; **Figure 3**).

m5C modification at DNA: RNA complex affects DNA repair: m5C modification of DNA: RNA complex can promote the process of DNA repair. In the presence of oxidative damage, TRDMT1 methylated the transcribed mRNA at the DNA double-strand break, increased the m5C modification of mRNA, and triggered the repair signal [89, 90]. At the same time, FMRP interacted with TRDMT1 through its N-terminal domain and K homology (NKH) domain, recruited and bound to m5C modified mRNA. In addition, FMRP removed R-loop and worked with TET1 to promote TET1 to remove m5C modifica-

Table 3. The mechanism of m5C modification in tumors

Cell types	Regulators/Role/State	RNA type/Target gene	Functions	Ref
Ovarian cancer	NSUN1/Writer/Up	mRNA/RAPGEF4	Promote proliferation, migration and invasion	[49]
Pancreatic cancer	NSUN2/Writer/Up	mRNA/SCRIB	Inhibit the DSBs, decrease the differentiation, and impact on immune infiltration	[44]
Hypopharyngeal squamous cell carcinoma	NSUN2/Writer/Up	mRNA/TEAD1	Promote proliferation, migration and growth	[45]
Gastric cancer	NSUN2/Writer/Up	mRNA/PCYT1A and PIK3R1	Promote proliferation, migration and growth	[43]
Gastric cancer	NSUN2/Writer/Up	mRNA/NTN1	Promote neural invasion	[68]
Hep3B/PC3	NSUN2/Writer/Up	mRNA/TREX2	Resist anti-PDL1 immunotherapy	[69]
Osteosarcoma	NSUN2/Writer/Up	mRNA/FABP5	Promote fatty acid metabolism	[76]
Gastric cancer	NSUN2/Writer/Up	mRNA/p57KIP2	Impact on cell cycle	[33]
Esophageal squamous-cell carcinoma	NSUN2/Writer/Up	mRNA/GRB2	Promote proliferation, migration and growth	[38]
Uveal melanoma	NSUN2/Writer/Up	mRNA/CTNNB1	Impact on cell cycle	[35]
Prostate cancer	NSUN2/Writer/Up	mRNA/AR	Promote proliferation, migration and growth	[14]
Cervical cancer	NSUN2/Writer/Up	mRNA/LRRC8A	Resist to DDP, inhibit apoptosis and promote proliferation, migration and growth	[36]
Cervical squamous cell carcinoma	NSUN2/Writer/Up	mRNA/KRT13	Promote invasion and migration	[34]
Pancreatic cancer	NSUN6/Writer/Down	mRNA/CDK10	Inhibit proliferation and growth	[85]
Liver cancer	NSUN6/Writer/Down	mRNA/unkown	Inhibit proliferation and affect the development of the embryo	[46]
Liver cancer	NSUN7/Writer/Down	mRNA/CCDC9B	Promote progression	[86]
Ovarian cancer	YBX1/Reader/Up	mRNA/E2F5	Enhance the resistance of DDP	[70]
Renal cell carcinoma	YBX1/Reader/Up	mRNA/PEBP1	Promote cell invasion and migration	[92]
Urothelial bladder cancer	ALYREF/Reader/Up	mRNA/RABL6/TK1/NSUN2	Promote cell proliferation	[64]
Bladder cancer	NSUN2 and YBX1/Writer and reader/Up	mRNA/HDGF	Promote growth and aggressiveness, and reduce the growth of lung nodules	[31]
Gastric cancer	NSUN2 and YBX1/Writer and reader/Up	mRNA/FOXC2	Promote cell proliferation, invasion and migration	[40]
Pancreatic cancer	NSUN2 and YBX1/Writer and reader/Up	mRNA/TIAM2	Promote cell proliferation, invasion and migration	[129]
Non-small cell lung cancer	NSUN2 and YBX1/Writer and reader/Up	mRNA/QSOX1	Promote drug resistance	[41]
Gastric cancer	NSUN2 and YBX1/Writer and reader/Up	mRNA/ORAI2	Promote metastasis	[64]
Bladder cancer	NSUN2 and ALYREF/Writer and reader/Up	mRNA/PKM2	Promote glycolysis and engage in metabolic process, and promote proliferation and growth	[37]
Retinoblastoma	NSUN2 and ALYREF/Writer and reader/Up	mRNA/PFAS	Promote purine synthesis	[32]
Lung adenocarcinoma	NSUN2 and ALYREF/Writer and reader/Up	mRNA/YAP	Enhance the resistance of AZD9291, promote secretion of exosome and proliferation and growth	[42]
Glioma	NSUN2 and ALYREF/Writer and reader/Up	mRNA/ATX	Promote cell migration	[100]
Osteosarcoma	TRDMT1/Writer	DNA: RNA/unkown	Promote DNA damage repair of HR	[89, 90]
Osteosarcoma	TET1 and TRDMT1/eraser and writer	DNA: RNA/unkown	Promote HR and enhance the resistance to IR	[89]
Glioma	NSUN5/Writer/Down	rRNA/NQO1	Inhibit cell growth	[59]
/	NSUN1/Writer	rRNA/unkown	Promote the process of rRNA	[73]
Osteosarcoma	NSUN2/Writer	tRNA/unkown	Not affect mitochondrial activity	[109]
Oral squamous cell carcinoma	NSUN3/Writer	tRNA/unkown	Affect the translation process of mitochondrial mRNA and regulate mitochondrial activity	[52]
/	NSUN6/Writer	tRNA/unkown	Affect the translation and synthesis of proteins	[46]
/	TRDMT1/Writer	tRNA/unkown	Regulate the process of protein translation	[118]
/	YTHDF2/Reader	tRNA/unkown	Promote the process and maturation of rRNA	[105]
Gastric cancer	NSUN2/Writer/Up	lncRNA/NR_033928	Promote progression	[121]

Esophageal squamous cell carcinoma	NSUN2/Writer/Up	lncRNA/lncNMR	Promote progression	[123]
Cholangiocarcinoma	NSUN2 and YBX1/ Writer and reader/Up	lncRNA/lncNKILA	Promote distant metastasis of lymph nodes	[122]
Hepatocellular carcinoma	NSUN2/Writer/Up	lncRNA/lncH19	Promote differentiation, angiogenesis, and migration and growth, and impact on cell cycle	[39]
/	NSUN7/Writer	eRNA/Pfkl, Sirt5, Idh3b, and Hmox2	Regulate metabolism	[88]

tion catalyzed by TRDMT1, leading to promoting the completion of transcription-coupled HR and increasing the sensitivity of breast cancer cells to radiotherapy and promoting cell survival [89]. Similarly, when the DNA damage appeared in U-2 osteosarcoma (U2OS) cells, m5C modification in transcript increased by TRDMT1 promoted the binding of RAD52 and DNA: RNA complex and promoted the process of DSB repair [90] (**Table 3**; **Figure 3**).

rRNA

m5C modification not only has different effects on mRNA in tumors, but also plays a key role in the function of ribosomal RNA [101]. Previous studies have shown that m5C modification was located in the cytosine C3761 and C4417 sites of 28S rRNA in human pancreatic cancer cells [44]. The structural characteristics of NSUN4 may be related to the m5C modification of rRNA in mitochondria [102, 103].

m5C modification on rRNA affects the translation process [104]. Methyltransferase NSUN5 had little effect on the transcript, but NSUN5 methylated the C3782 of 28S rRNA. On the one hand, the deletion of NSUN5 reduced the overall translation of glioma cells. On the other hand, NSUN5 activated the process of protein translation in response to stress, such as promoting the translation of stress-activating protein NQO1, thus promoting the occurrence of glioma [59].

The modification of m5C on rRNA also affects the processing or maturation of rRNA. Studies testified that NSUN1 specifically targeted rRNA and increased the m5C modification at position 4447 of 28s rRNA. NSUN1 also formed a complex with boxC/D snoRNAs to act on the processing of rRNA [73]. Moreover, NSUN4 methylated the 911 site of 12S rRNA, increased the m5C modification of 12S rRNA, and promoted the rearrangement of rRNA [83].

In addition, although YTHDF2 acts as the reader of m6A, YTHDF2 can also bind to the m5C site and serve as the reader of m5C modification. YTHDF2 recognized the m5C locus by conserved residue Trp432. On the one hand, knockout YTHDF2 reduced the m5C modification on mRNA. On the other hand, the deletion of YTHDF2 increased the overall m5C modification level of cells and increased the m5C modification sites on 18S rRNA and 28S rRNA, which played an important role in the processing and maturation of rRNA [105] (**Table 3**; **Figure 3**).

tRNA

In the posttranscriptional modification of tRNA, m5C modification is predominant [47, 106, 107]. NSUN6 of the NSUN family was used as a methyltransferase of tRNA, and NSUN2 also methylated the positions 48, 49, and 50 of mitochondrial RNA [108-111]. Studies have proven that when NSUN2, DNMT2, and NSUN6 were knocked out at the same time, the level of m5C modification on tRNA was significantly decreased [46, 108, 112]. m5C modification on tRNA can affect the translation and synthesis of proteins. TRDMT1 not only plays a role in DNA, but also acts as the methyltransferase of tRNA [113-116]. TRDMT1 methylated the position in C38 of tRNA (Asp), thus regulating the process of protein translation with the Asp sequence [117, 118]. In the presence of oxidative damage, TRDMT1 was bound to aminoacyl-tRNA synthetases, including glycyl-tRNA synthetases [89]. At the same time, the anticancer drug azacytidine inhibited m5C modification on tRNA, which was dependent on the methylation of TRDMT1 [17]. Moreover, NSUN2 affected the m5C modification of tRNA, thus affecting protein synthesis [119].

m5C modification on tRNA also affects the translation process of mitochondrial RNA, which may affect the activity of mitochondria and tumor metabolic reprogramming. The removal

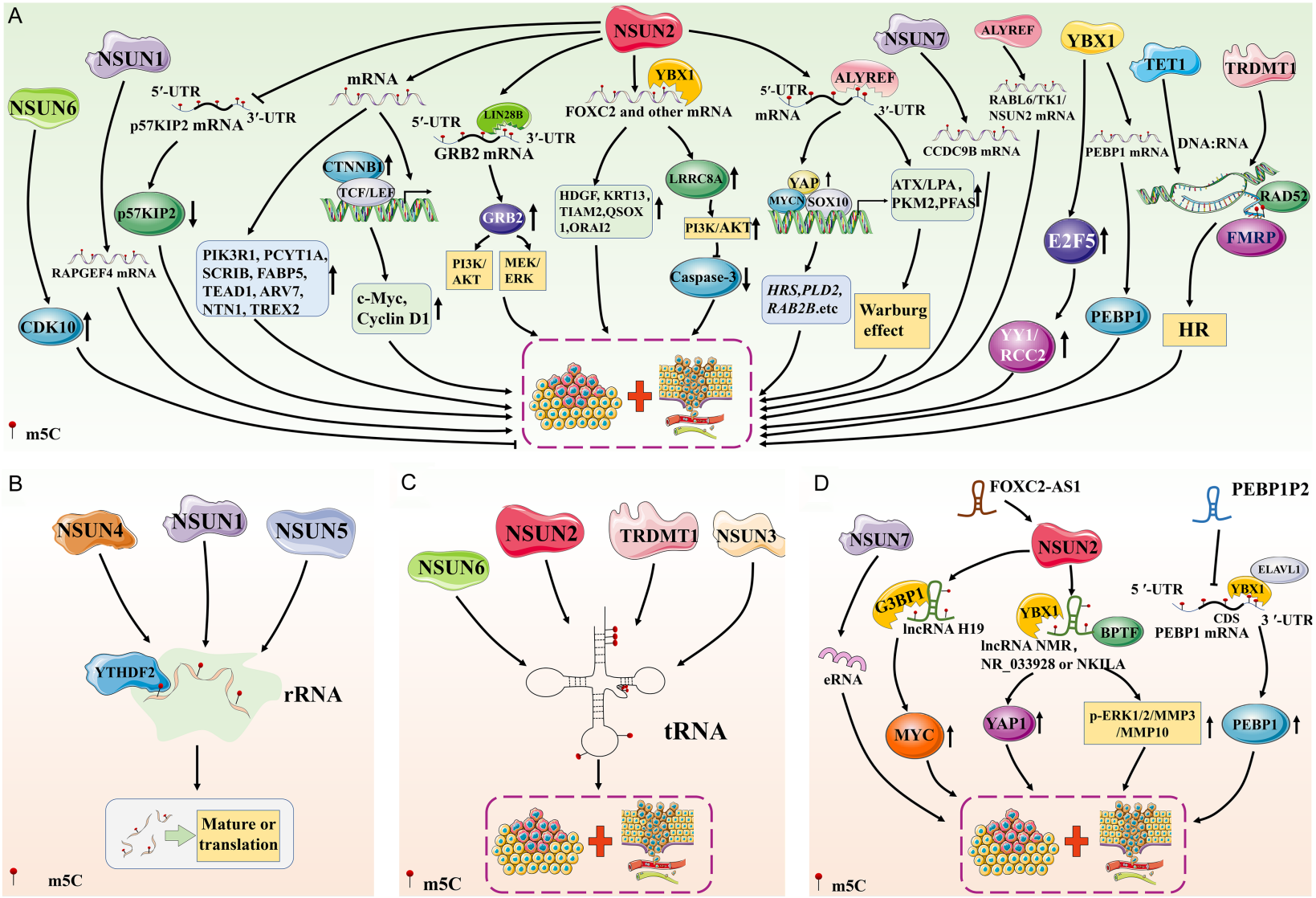

Figure 3. The mechanism of m5C modification in different RNA types. A: The mechanism of m5C regulators in mRNA. B: The mechanism of m5C regulators in rRNA. C: The mechanism of m5C regulators in tRNA. D: The mechanism of m5C regulators in lncRNA. 3'-UTR, 3'-untranslated region; 5'-UTR, 5'-untranslated region; CDS: coding sequences. m5C, 5-methylcytosine.

of NSUN3 significantly reduced the m5C modification of the $tRNA^{Met}$C34 site in OSCC, which reduced the level of protein encoded by mitochondrial RNA and the metabolite level of TCA, thus regulating the activity of mitochondria. At the same time, the decrease of $tRNA^{Met}$ increased the glycolysis process and inhibited the metastasis of tumor cells [52, 78, 80]. Moreover, NSUN2 was mainly located in the mitochondrial matrix and catalyzed the m5C modification of tRNA. Knocking out NSUN2 reduced the m5C modification in the V-loop region of the mtRNA in the U-2 osteosarcoma (U2OS) cell line [109] (**Table 3**; **Figure 3**).

Other ncRNA

Except for coding RNA, the regulation of m5C modification by non-coding RNA is also extremely important [88, 120]. LncRNA acts on the regulators of m5C modification, thus regulating the m5C modification of target genes. Studies have confirmed that lncRNA FOXC2AS1 was directly related to NSUN2 and enhanced the combination of FOXC2 and NSUN2, increasing in the m5C level of FOXC2 mRNA, thus stabilizing FOXC2 transcripts [40]. Pseudogene PEBP1P2 prolonged the half-life of PEBP1 mRNA by m5C modification, and stabilized m5C modification on PEBP1 mRNA CDS and 3'-UTR through the participation of YBX1 and ELAVL1 proteins, thus promoting the expression of PEBP1 at transcriptional and translational levels, and preventing the invasion and metastasis of ccRCC [92]. Suggesting that lncRNA plays an important role in regulating m5C modification.

LncRNA itself also has m5C modification sites, which can be directly catalyzed by m5C writers, and then further participated in tumorigenesis. NSUN2 targeted the C986 site of lncRNA H19. NSUN2-dependent m5C modification prolonged the half-life of lncRNA H19 and increased the stability of H19, which recruited and bound to oncoprotein G3BP1, and further promoted the enrichment of MYC, resulting in poor differentiation and malignant phenotype of hepatocellular carcinoma [39]. Moreover, NSUN2 methylated and upregulated NR_033928 (LncRNA), and NR_033928 interacted with IGF2BP3/HUR complex, which promoted the expression of GLS and the occurrence of gastric cancer [121]. In addition, NSUN2 interacted with long noncoding NKILA which was highly expressed in cholangio carcinoma (CCA), and increased the m5C modification on NKILA. Subsequently, YBX1, as the reader of NKILA mRNA, recognized and acted on the m5C sites of NKILA. Then, NKILA further targeted YAP1, thus promoting the formation of CCA [122]. LncRNA NMR methylated by NSUN2 may also inhibit the level of m5C modification in PLOD3, Col4A5, Lamb1, and HSPG2. At the same time, the combination of lncRNA NMR and BPTF activated the ERK pathway and upregulated the expression of MMP10 and MMP3, thus promoting the occurrence of ESCC [123] (**Table 3**; **Figure 3**).

m5C modifications also exist in eRNA. The decrease of NSUN7 reduced the expression of Pfkl, Sirt5, Idh3b and Hmox2 mRNA. At the same time, NSUN7 correspondingly reduced the expression of Pfkl, Sirt5, Idh3b, and Hmox2 eRNAs. When the expression of NSUN7 increased, m5C modifications on eRNA also increased, which indicated that m5C modification on eRNAs can affect the stability of eRNAs, thus regulating metabolism in vivo [88].

The regulation networks of m5C modification in tumors

For the regulators of m5C modification, E2F1, MYC, RPL6, STAT1, SUMO-2/3, lncRNA FOXC2-AS1, DIAPH2-AS1, AR and glucose regulated and promoted the expression of NSUN2. MiR-141, DNMT1, and MDV3100 which was an AR antagonist inhibited the expression of NSUN2. Moreover, SIAH1 regulated the expression of m5C reader YBX1, and ALYREF was also positively regulated by HIF-1α. Subsequently, NSUN1 regulated the expression of RAPGEF4, and CCDC9B was regulated by NSUN7. Moreover, the expression of NSUN2 regulated the expression levels of PIK3R1, PCYT1A, CTNNB1, p57KIP2, QSOX1, and ATX. NSUN2 also promoted the expression of SCRIB mRNA, TEAD1 mRNA, NTN1 mRNA, TREX2 mRNA, FABP5 mRNA, and GRB2 mRNA, and regulates the m5C level of lncRNA NKILA, H19, NR_033928, and lncRNA NMR. At the same time, NSUN6 positively regulated the expression of CDK10. In addition, YBX1 promoted the expression of E2F5 and regulated the level of PEBP1 mRNA. YBX1 also regulated and promoted the expression of FOXC2 mRNA, AR mRNA, HDGF mRNA,

KRT13 mRNA, LRRC8A mRNA, and TIAM2 mRNA together with NSUN2. ALYREF regulated and promoted the expression of YAP mRNA, PKM2 mRNA, and PFAS mRNA together with NSUN2. The regulated downstream genes were then involved in PI3K-AKT signaling pathways, cell cycle related pathways, DNA repair, RNA metabolism, regulation of cell morphogenesis, and immune response pathways [33, 36, 44, 45, 50], which further promoted biological functions such as proliferation, invasion and resistance to chemotherapeutic drugs, thus participating in the process of tumor formation. Meanwhile, TRDMT1 regulated the m5C modification of tRNA and the transcripts in DNA: RNA complex, and YTHDF2, NSUN1, NSUN3, NSUN4 and NSUN5 regulated the m5C modification of rRNA. NSUN2, NSUN3, NSUN6 and TRDMT1 regulatded the m5C methylation of tRNA. The change of rRNA modification affects its own processing or maturation process, and the change of tRNA modification may further affect the activity of mitochondria, affect metabolism, and further have an influence on the occurrence and development of tumors.

However, downstream targeting genes also regulate the regulators of m5C modification, thus forming a feedback loop and participating in carcinoma. Taking prostate cancer as an example, NSUN2 affected the activity of AR and promoted the expression of AR mRNA and protein. The combination of YBX1 and AR mRNA did not affect the nucleocytoplasmic output of AR mRNA, but impacted the stability of AR mRNA and promoted the expression of AR mRNA. In addition, NSUN2 regulated AR at the pre-mRNA level and promoted the expression of AR splice variant 7 (AR-V7), while AR-V7 in turn enhanced the transcription and positively regulated the expression of NSUN2, thus further affecting the tolerance of C4-2 cells to MDV3100 (Enzalutamide) and promoting the progression of prostate cancer [14]. The interaction between NSUN2 and AR forms a positive feedback loop. Similarly, NSUN2 targeted the m5C modification of CTNNB1 mRNA in gastric cancer, and promoted the expression of CTNB1 and MYC. However, MYC also directly targeted the NSUN2 gene and promoted the expression of NSUN2. The interaction between NSUN2 and MYC also formed a positive feedback loop. In addition, ALYREF targeting m5C modification promoted the splicing of RABL6 and TK1 mRNA through its K171 domain, thus increasing the stability of the mRNA. At the same time, ALYREF also recognized m5C modification on NSUN2 mRNA, promoted the expression of NSUN2 in UCB, and promoted the occurrence of UCB [64]. NSUN2 increased and YBX1 recognized m5C modification on ORAI2 mRNA, which promoted the expression of ORAI2, thus promoting the metastasis of gastric cancer. After GC cells ingested fatty acids from omental adipocytes, the transcription factor E2F1 was upregulated through the AMPK pathway, and further promoted the expression of NSUN2 through cis-element [124], which formed a positive loop to promote carcinoma. Studies suggest that regulators of m5C modification and downstream target genes interact with each other to form a positive feedback loop and jointly promote the occurrence of tumors (**Figure 4**).

Application and potential value of m5C modification in tumor diagnosis and treatment

m5C modification is closely connected with tumorigenesis. Studies have shown that the upregulated expression of NSUN2 that plays the role of oncogenes in most tumors was associated with poor prognosis in ESCC, PCa, pancreatic cancer, UCBs, GC, HPSCC, and OS [14, 38, 43-45, 50, 76]. Studies on hepatocellular carcinoma have reported that the upregulation of NSUN1 and NSUN5 were related to the poor prognosis of the tumor [55, 58], but the prognosis of NSUN5 in glioma is good [59]. NSUN6 seems to work as a tumor suppressor gene in some tumors [46]. The high expression of NSUN6 was related to the good prognosis of PC [85]. At the same time, TET3 had a poor prognosis [23, 63]. Moreover, YBX1 and ALYREF of m5C readers are also significantly associated with the prognosis of patients. For example, YBX1 was related to T, N stage, and tumor grade, and was also the cause of poor DFS in patients in UCBs [31]. Similarly, the upregulated ALYREF in BCa was also connected with lymph node metastasis and poor OS and RFS in patients [37]. As a result, the m5C modification was related to the prognosis of tumors and the change of m5C modification seemed to be the trend of tumor occurrence. This change in the tumor might be a biomarker in clinic diagnosis.

Given the important role of m5C modification in tumors, finding a therapeutic method targeting

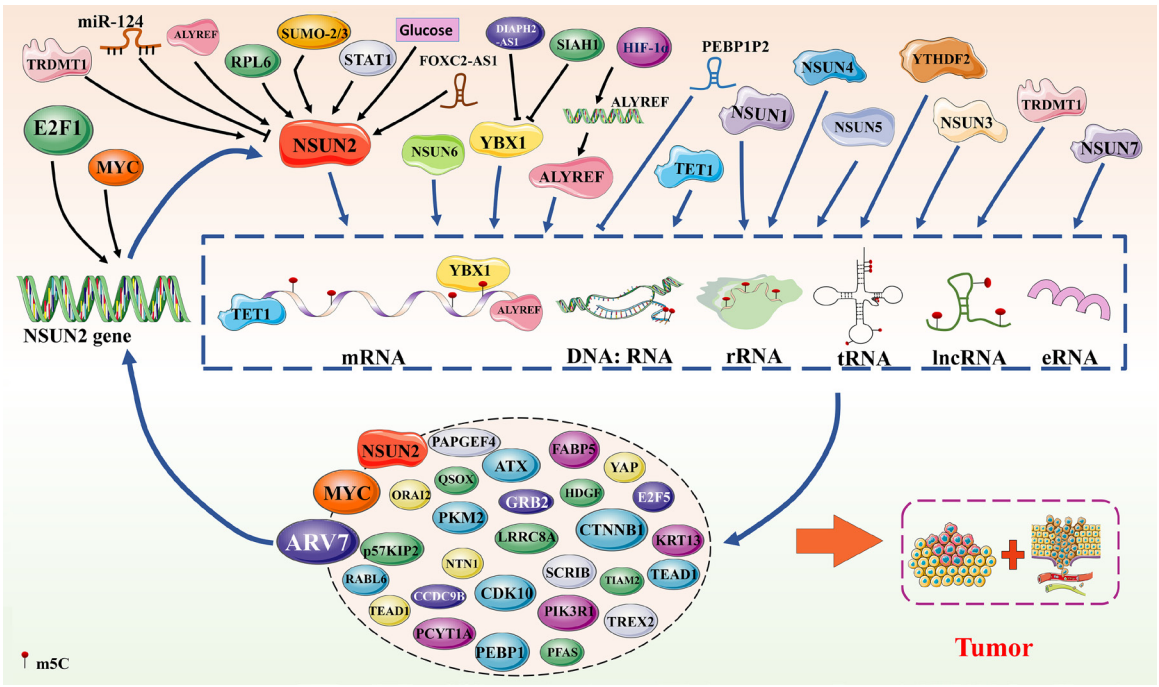

Figure 4. The regulation networks of m5C modification in tumors. The regulators of m5C modification form a positive loop with target genes, driving the process of tumor. m5C, 5-methylcytosine.

m5C modification is extremely significant. Molecular targeted therapy seems to be a good treatment for targeting m5C modification. On the one hand, directly targeting the acting elements of m5C modification can change the level of m5C modification directly, thereby treating tumors. For example, the m5C modification in YAP mRNA was blocked by the change of m5C regulator in LUAD, which helped block the malignant phenotype of cells and resistance to AZD9291, thus better for the treatment of lung adenocarcinoma [42]. On the other hand, by targeting the upstream regulators of m5C modification elements, we can indirectly change the level of m5C modification in tumors and inhibit the development of tumors. For example, targeting SUMO or FOXC2-AS1 in gastric cancer can affect the m5C modification catalyzed by NSUN2, and might be a new therapeutic target for GC [40, 43]. In addition, the combination of drugs or drugs and molecules provide new ideas for the therapy of m5C-mediated tumors [125]. Treatment of oral squamous cell carcinoma with TIG and DOX effectively inhibited lymph node metastasis of tumor cells [52]. In gliomas, DNQ and IB-DNQ drugs inhibited NSUN5, which was hypermethylation in cells [59]. The combination of some drugs such as MDV3100 and shNSUN2 can better inhibit tumor growth, and the same as the combination of shNSUN2 and apalutamide [14]. Moreover, the removal of NSUN2 and METTL1 enhanced the toxicity of 5-Fu to Hela cells [126]. In addition, the regulators of m5C modification are closely related to immune infiltration and are important in the tumor microenvironment. For example, the state of CD4 immune infiltration in the tumor microenvironment depended on the gene of NSUN2 in pancreatic cancer [44]. The m5C modification was also associated with immune infiltration in GC and so forth. Therefore, immunotherapy may also become an effective treatment of tumors mediated by m5C modification in clinics [127, 128].

Conclusions

m5C modification affects different types of RNA, which is very important in regulating the stability, translation, and processing maturation of RNA. The abnormal expression patterns of m5C modification exist in tumors, and different regulators have different m5C locus on RNA. Moreover, the biological functions of m5C regulators work distinctly in various tumors and the effects of m5C modification depend on the type of RNA, and different regulators are regu-

lated by various transcription factors, noncoding RNAs, or others, which constructs the complicated regulatory network of m5C modification in tumors. Altogether, m5C modification of posttranscriptional modification is essential in tumors, which provides new strategies for tumor clinical diagnosis and treatment.

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Disclosure of conflict of interest

None.

Abbreviations

mRNA, messenger RNA; rRNA, ribosomal RNA; tRNA, transfer RNA; lncRNA, long non-coding RNA; NSUN2, NOP2/Sun RNA Methyltransferase 2; ALYREF, Aly/REF Export Factor; YBX1, Y-Box Binding Protein 1; TRDMT1, tRNA aspartic acid methyltransferase; ALKBH 1, alpha-ketoglutarate-dependent dioxygenase ABH1; UCB, urothelial carcinoma of the bladder; GC, gastric cancer; CC, cervical cancer; UM, uveal melanoma; BCa, bladder cancer; ESCC, esophageal squamous-cell carcinoma; PCa, prostate cancer; HPSCC, hypopharyngeal squamous cell carcinoma; OV, ovarian cancer; CESC, cervical squamous cell carcinoma; LUAD, lung adenocarcinoma.

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