Role of m5C methylation in digestive system tumors (Review)

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Abstract. Currently, the incidence of digestive system tumors has been increasing annually, thus becoming a prevalent cause of cancer-related mortalities. Although significant strides have been made in targeting the molecular mechanisms that underpin the development of these tumors, their treatment and prognosis still pose substantial challenges. This is primarily due to the ambiguity of early diagnostic indicators and the fact that most digestive system tumors are detected at an advanced stage. However, epigenetic modifications are capable of altering the expression of oncogenes and regulating biological processes in cancer. In recent years, the study of methylation in relation to tumor pathogenesis has become a focus of prominent research. Among the various types of methylation, 5-methylcytosine (m5C) methylation plays a crucial role in the development of digestive system tumors and is anticipated to serve as a novel therapeutic target. However, to date, a comprehensive and systematic review concerning the role of m5C methylation in digestive system tumors is lacking. Consequently, the present study reviewed the role of m5C methylation in digestive system tumors such as esophageal cancer, gastric cancer and hepatocellular carcinoma, with the aim of providing a valuable reference for future research endeavors.

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1. Introduction

Cancer remains the leading cause of premature mortality and reduced life expectancy in a number of countries around the world and is a heavy health burden (1). Cancer of the digestive system is one of the most common types of malignant tumor, with esophageal, gastric, colorectal, liver and pancreatic cancers ranking among the top cancers of the digestive system in terms of incidence and mortality rates (2). The majority of types of cancer of the digestive system are in the middle-to-late stages of progression when they are detected and diagnosed; these patients have a poor prognosis so they have become among the most commonly discussed public health problems (3). Epigenetics is the stable, heritable alteration of gene function and expression levels without changes to the nucleotide sequence and epigenetics mainly includes DNA methylation, histone modification, noncoding RNA regulation and chromatin remodeling (4-6). Epigenetic modifications can regulate the biological processes of cancer and thus influence the progression of diseases such as tumors. RNA 5-methylcytosine (m5C) methylation is at the forefront of epitranscriptomics and is one of the most important epigenetic modification mechanisms in RNA posttranscriptional regulation. Dynamic RNA modifications have emerged as key posttranscriptional regulators of genetic information during embryonic development and disease progression (7). Among them, RNA m5C methylation is one of the most important epigenetic modification mechanisms in RNA posttranscriptional regulation. m5C is a common RNA modifier that has received widespread attention for its key regulatory role in mRNA metabolism (8). m5C modification was first identified in DNA and later shown to mediate RNA methylation (9). m5C modifications are one of the most common posttranscriptional modifications of RNA, along with N6-methyladenosine and pseudouridine (Ψ) (10) and are found in a wide variety of RNA molecules, including transfer (t)RNAs, ribosomal (r) RNAs, mRNAs and noncoding (nc)RNAs (11). The level of posttranscriptional modification of RNA methylation regulates a variety of biological processes, such as splicing, nuclear export, stability and translation of RNA (12), which in turn affects physiological processes such as cell differentiation,

embryonic development and learning and memory; however, it also plays an important role in the onset and development of a number of diseases, including tumors (13,14). In digestive system tumors, RNA m5C modification plays a key role in the pathogenesis of esophageal, gastric, liver and pancreatic cancers (15-28) (Table I). However, there is no systematic review summarizing the role of RNA m5C modification in digestive system tumors. Therefore, the present study reviewed the role and regulatory mechanisms of RNA m5C methylation and its regulators in digestive system cancers, such as esophageal, gastric, hepatocellular and pancreatic cancers, with the aim of providing new ideas for precise tumor prevention, intervention and potential therapeutic targets.

2. Mechanisms associated with RNA m5C methylation

The m5C modification in which a methyl group is attached to the fifth carbon of the DNA or RNA cytosine ring, is a reversible type of epigenetic modification. This modification process was first identified in DNA (29) and was later shown to mediate RNA methylation and is a ubiquitous posttranscriptional modification of RNA along with N6-methyladenosine (m6A) and Ψ (10). There are various types of RNA methylation, such as m6A, N1-methyladenosine (m1A), m5C, N7-methylguanosine (m7G) and 2'-O-methylribosidine (Um) (10). m6A methylation is a methylation that occurs at the sixth N atom of adenine and is the most common and abundant chemical modification of eukaryotic mRNAs, accounting for ~60% of the total (30). m1A is the result of the methylation of adenosine at position 1 and is found mainly in tRNAs, rRNAs, mRNAs and lncRNAs (31,32). N7-methylguanosine (m7G) is an RNA methylation that occurs at the N7 position of guanine and accounts for ~0.4% of all guanine residues (33). m7G methylation occurs in mRNA, tRNA and rRNA and is catalyzed by the methyltransferase METTL1-WDR4 complex (34,35). Research on RNA methylation has focused mainly on m6A and less so on m5C methylation. However, in recent years, m5C methylation has been shown to markedly affect a variety of biological processes, including cell proliferation, differentiation, migration and apoptosis (36,37). As a result, research in oncology has received increasing attention. m5C modifications exist in a wide range of RNA molecules, including tRNAs, rRNAs, mRNAs and ncRNAs (11). The m5C methylation process mainly involves relevant methyltransferases (writers), demethylases (erasers) and binding proteins (readers). The m5C methylation uses S-adenosylmethionine (SAM) as a methyl donor. Through the action of methyltransferases, m5C methylation is initiated. The methylated RNA subsequently binds to binding proteins to exert biological effects (Fig. 1) (38). This process also involves the action of demethylases, making it a dynamic and reversible process. In summary, through the interaction of the aforementioned three types of proteins, m5C methylation widely affects gene expression and various biological processes at multiple levels, although the specific mechanism remains unclear.

m5C methylation-associated methyltransferases (writers). The methyltransferases of m5C are mainly composed of the nucleolar protein NSUN (NOL1/NOP2/SUN domain family) family and DNA methyltransferase member 2 (DNMT2) (39). DNMT2 mainly regulates the m5C methylation of tRNAs and miRNAs and can catalyze the C38 methylation of aspartic acid transporter RNA (tRNA-Asp) (40). This process is associated with the primary sequence and tertiary structure of tRNAs (41). In mammals, the NSUN enzyme family is composed of NSUN1-7 (42). However, m5C is characterized mainly by NSUN family proteins, among which NSUN2 has been the most thoroughly studied. It is widely acknowledged that RNA m5C is catalyzed mainly by NOP2/Sun RNA methyltransferase family member 2 (NSUN2). As an essential m5C 'writer', NSUN2 participates in a broad spectrum of biological processes (43). However, the ultimate consequences of post-transcriptional regulation rely largely on m5C 'readers'. These 'readers' can recognize m5C modifications and exert crucial influences on mRNA output, stability and translation initiation (12,44). NSUN2 is a methyltransferase that depends on two cysteine sites. C321 catalyzes the methylation of cytosine by binding to the cytosine pyrimidine ring and forming a covalent bond, whereas C271 mediates the release of methylated RNA. The methylation process mediated by NSUN2 mainly involves leucine at the variable loop swing position of the majority of tRNAs. Additionally, it methylates mRNAs, ncRNAs and lncRNAs (42). NSUN2 can methylate cytosine through binding to the cytosine ring and forming a covalent bond. In addition, the intracellular localization of NSUN2 varies with different stages of the cell cycle. Specifically, during the G_1 phase, NSUN2 is located mainly in the nucleus; during the S phase, it is positioned between the nucleolus and nucleoplasm; during the G_2 phase, it is in the cytoplasm; and during the M phase, it is in the centromere (45,46). NSUN2 participates in a diverse range of biological processes, including cell differentiation, proliferation and migration (47-49). In addition, it is highly expressed in numerous types of cancers, such as gastric cancer, esophageal cancer, hepatocellular carcinoma (HCC), pancreatic cancer, prostate cancer and kidney cancer (15-18,50-52). NSUN1 (NOP2) is a protein specific to the nucleolus. It can catalyze the methylation of yeast 25SrRNA, 60S ribosomal subunit and 26SrRNA. Additionally, it can catalyze methylation at the cytosine 4447 position of human 28S rRNA and it stabilizes the structure of rRNA (53). NSUN3 is located mainly in the mitochondrial matrix within human cells. It can recognize the anticodon loop of the mitochondrial methionine transfer RNA (tRNA-Met) and methylate C34. In addition, it is essential for the formation of 5-formyl-2'-cytidine (f5C) (54). The absence of NSUN3 leads to mitochondrial dysfunction (55). NSUN4 functions mainly on 12S rRNA in eukaryotic mitochondria and participates in the methylation of rRNA at the C911 position (56). It is abnormally expressed in lung adenocarcinoma, HCC and renal clear-cell carcinoma (26). NSUN5 contains a m5C site at C3782 of 28S rRNA in human cervical cancer cells, which regulates the protein translation process (57). In colorectal cancer, NSUN5 mainly acts by modifying the second m5C methyltransferase in eukaryotic rRNA. It is associated with ribosomes and can change the total protein content (57) as well as regulate the cell cycle to promote tumor development (21). NSUN6 is localized to the Golgi apparatus in the cytoplasm of human cells and is a methylation transferase with strong substrate properties for mRNAs; it is enriched in the 3'UTR and associated with translational termination (58). It methylates

Table I. Role of RNA m5C methylation in digestive system cancers.

First author/s, year	Tumor type	Expression	RNA m5C level	Target molecule	Effect	(Refs.)
Li, 2018	Esophagus carcinoma	NSUN2 ↑	↑	lncRNA↑	Migration +, Invasion +	(19)
Su, 2021		NSUN2 ↑	↑	GRB2↑	Proliferation +, Migration +, Invasion +	(15)
Zou, 2020	Gastric carcinoma	NSUN2 ↑	Unknown	PIK3R1, PCYT1A ↑	Proliferation +, Migration +, Invasion +	(14)
Mei, 2020		NSUN2 ↑	↑	$p57^{Kip2}$ ↑	Proliferation +	(20)
Yan, 2021		NSUN2 ↑, YBX1 ↑	↑	FOXC2 mRNA ↑	Migration +, Invasion +	(18)
Sun, 2020	Hepatocellular carcinoma	NSUN2 ↑	↑	H19 lncRNA	Migration +	(17)
Zhang, 2020		NSUN5 ↑	↑	Unknown	Migration +	(21)
He, 2020		NSUN4 ↑	Unknown	Unknown	Unknown	(22)
Xue, 2023		ALYREF ↑	Unknown	Unknown	Proliferation +	(23)
Chen, 2022	Pancreatic carcinoma	NSUN2 ↑	Unknown	Unknown	Proliferation +	(24)
Yang, 2021		NSUN6 ↑	Unknown	Unknown	Proliferation -	(25)
Gao, 2019	Gall bladder carcinoma	NSUN2 ↑	Unknown	RPL6	Proliferation +, Migration +, Invasion +	(26)
Zheng, 2022	Bile ducts carcinoma	NSUN2 ↑	↑	Unknown	Proliferation +, Migration	(27)
Yin, 2022	Colorectal cancer	NSUN5 ↑, YBX1 ↑	↑	Unknown	Proliferation +,	(28)

↑, up; ↓, down; +, promotion; -, inhibition. m5C, 5-methylcytosine; NSUN, NOL1/NOP2/SUN domain family.

threonine transporter RNA (tRNAThr) and cysteine transporter RNA (tRNACys) (59). NSUN7 may act on eukaryotic eRNAs (60) and is associated with shorter survival (61,62). These related transferase enzymes play important roles in various physiological activities of organisms.

m5C methylation-associated demethylases (erasers). Demethylases mediate RNA demethylation, which is induced by the tet methylcytosine dioxygenase (TET) family of proteins and the nature of TET-induced m5C demethylation is to replace the modification by catalytically promoting the oxidation of 5mC (63,64). At present, a definitive RNA m5C demethylase has not yet been identified, but TET2 in the TET family of proteins can further oxidize tRNA m5C to α-ketoglutarate in reaction to form 5-hydroxymethylcytosine (hm5C) (65). In addition, TET was found to mediate the specific enrichment of hm5 C in intracellular tRNAs, a process that may destabilize the m5 C-binding protein by disrupting its binding to RNA (66,67). Thus, TET2 can mediate C oxidation via m5C methylation of tRNA to promote translation *in vitro* (67). hm5C is oxidized to f5C in mitochondrial tRNA by α-ketoglutarate-dependent ALKB homodimeric dioxygenase 1 (ALKBH1). However, the mechanism through which f5C is reduced in tRNA remains unclear (68).

m5C methylation-associated binding proteins (readers). The binding proteins associated with m5C RNA methylation that have been identified are the RNA methyltransferase Aly/REF export factor (ALYREF) and Y-box binding protein 1 (YBX1). ALYREF is a 'reader' protein situated in the nucleus. It directly recognizes and binds to the m5C site within RNA, thus facilitating the export of RNA from the nucleus to the cytoplasm (12). YBX1, a DNA/RNA-binding protein and a m5C 'reader', can stabilize m5c-modified messenger ribonucleic acid (69). It often regulates the stability of mRNAs by specifically binding to response elements in various mRNA transcripts, such as those encoding IL-6, VEGF and heat shock protein 70 (70,71). It is highly associated with tumor cell proliferation, drug resistance, metastasis and prognosis (72). ALYREF and YBX1 can exert their biological effects by recognizing and binding to the m5C site (22). In addition, ALYREF can recognize m5C-methylated mRNAs and mediate their nucleocytoplasmic shuttling process (73). YBX1 preferentially recognizes and binds to m5C-modified mRNAs via its cold shock protein structural

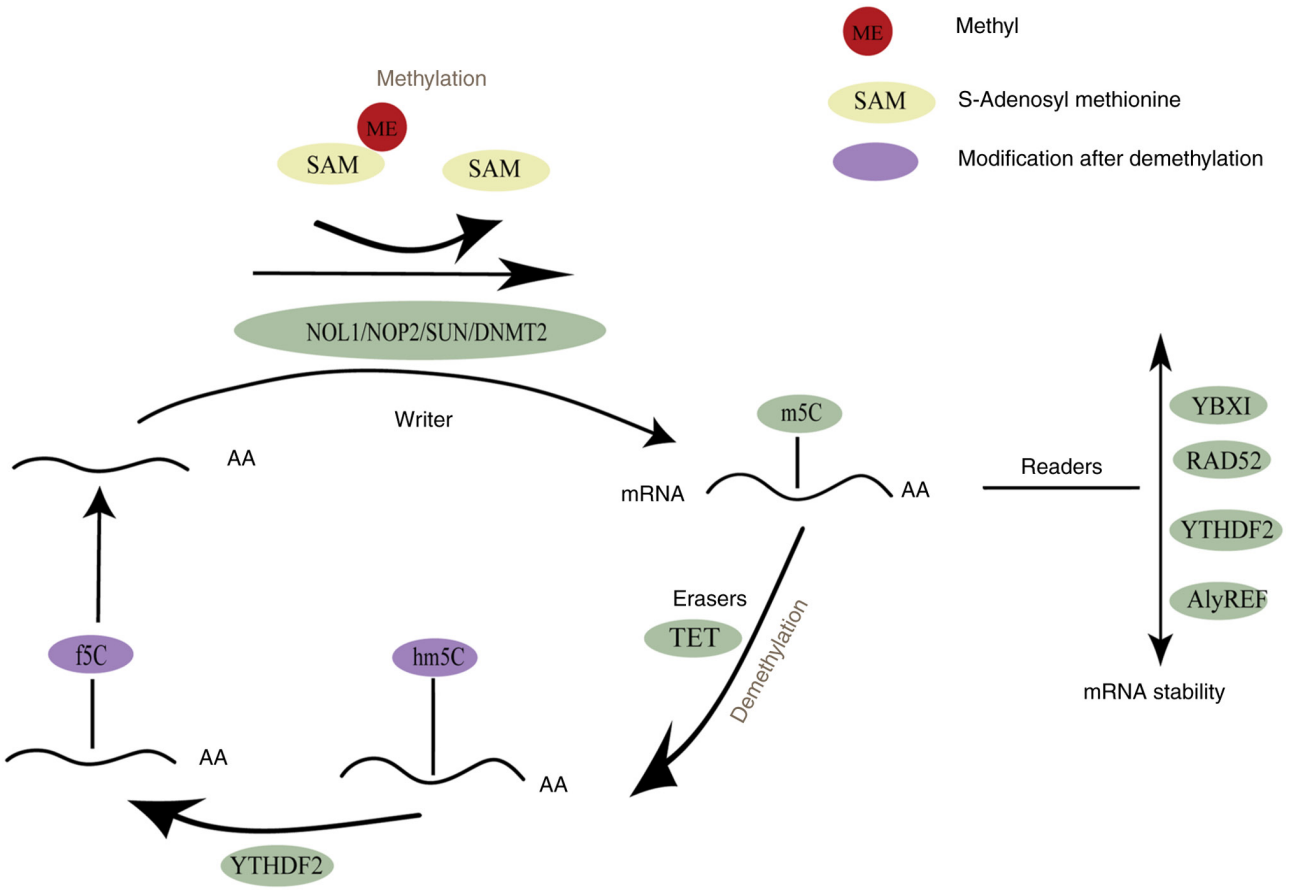

Figure 1. m5C methylation process: m5C methyltransferase uses SAM as a methyl donor, which transfers the methyl group to the fifth carbon atom of cytosine to promote m5C methylation. Upon m5C modification of RNA, m5C recognition proteins specifically recognize and bind m5C modification sites. m5C demethylases mediate RNA demethylation, reflecting the dynamic reversibility of m5C methylation. ME, methyl; SAM, S-adenosyl methionine; NSUN, NOL1/NOP2/SUN; DNMT2, DNA (cytosine-5)-methyltransferase 2; m5C, 5-methylcytosine; TET, ten-eleven translocation; ALKBH1, AlkB homology 1; ALYREF, Aly/REF export factor; YBX1, Y-box binding protein 1; RAD52, radiation sensitive 52; YTHDF2, YTH N6-methyl-adenosine RNA binding protein 2; f5c, 5-formylcytidine; hm5C, 5-hydroxymethylcytidine.

domain to regulate mRNA stability in the cytoplasm. It is overexpressed in gastric cancer tissues and is associated with hepatic metastasis and poor prognosis in advanced gastric cancer patients. In addition, it promotes gastric carcinogenesis, angiogenesis and drug resistance (18,74). It has been shown that the level of nuclear mRNA chromosomes increases when AlYREF expression is reduced and plays the opposite role when it is overexpressed and that this phenomenon does not occur in m5 C-binding-deficient Al YREF types, suggesting that AlYREF may be involved in facilitating the nuclear egress process of mRNA by binding to the m5C-binding site of the mRNA and that NSUN2 is involved in regulating the nuclear egress process mediated by AlYREF. NSUN2 is involved in the regulation of AlYREF-mediated nucleation (12).

3. m5C methylation in RNA and tumors of the digestive system

m5C modifications in mRNAs have important physiological functions and are involved in various biological processes of RNA, including RNA export, translation and ribosome assembly (75,76). It can affect the stability, splicing and nucleocytoplasmic shuttling process of mRNAs (12). In addition, it affects posttranscriptional gene expression and protein synthesis (18,19). It is also involved in various biological processes, such as DNA damage repair (77); cell proliferation and migration (78); and the development, differentiation and reprogramming of stem cells (14). Among various RNA molecules, the m5C modification of tRNAs is involved in neural development and cell differentiation processes, the m5C modification of rRNAs regulates oxidative stress and the m5C modification of mRNAs is associated with the growth, development and aging processes of organisms (42,79). In digestive system tumors, studies have shown that RNA m5C methylation is involved in the occurrence and development of digestive system cancers such as esophageal cancer, gastric cancer, liver cancer and pancreatic cancer (15-18). This may be due to the action of RNA methyltransferases, which change the abundance of m5C modifications in the RNA of oncogenes or tumor suppressor genes. The modification sites are further recognized by RNA m5C-binding proteins, thereby regulating the function and expression level of tumor-related genes. As a result, the homeostasis of the internal environment is unbalanced, promoting or inhibiting the formation of tumors.

RNA m5C methylation and esophageal cancer. Esophageal cancer (ESCC) is an aggressive tumor with rapid growth and a high rate of lymph node metastasis (80). Esophageal

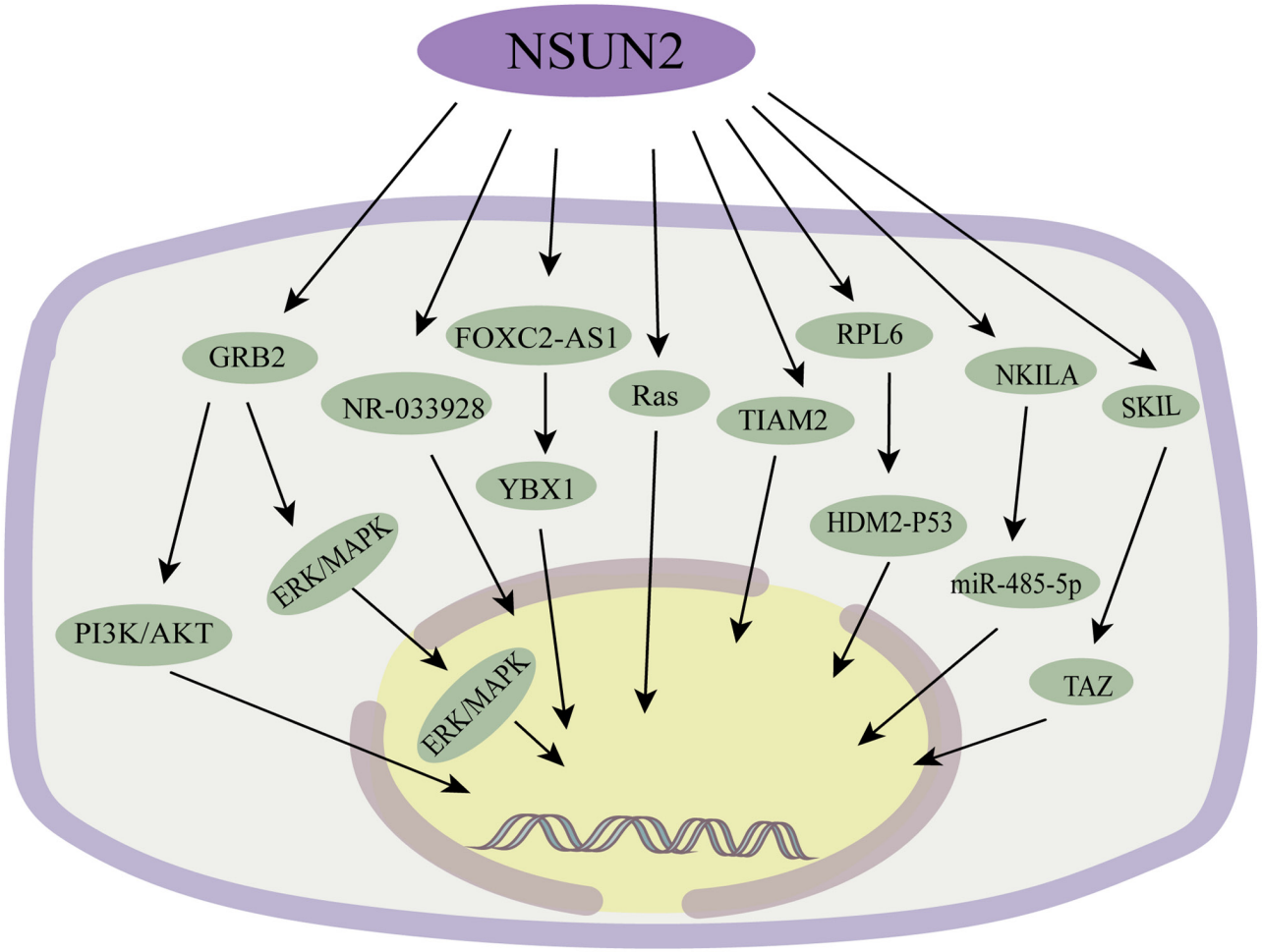

Figure 2. The m5C methyltransferase NSUN2 is involved in the development of esophageal cancer, gastric cancer, hepatocellular carcinoma, gallbladder cancer, cholangiocarcinoma, pancreatic cancer and colorectal cancer. m5C, 5-methylcytosine; NSUN, NOL1/NOP2/SUN domain family.

squamous cell carcinoma and adenocarcinoma are the two main histologic subtypes, of which ESCC accounts for ~90% of cases (81). The m5C methyltransferase NSUN2 is overexpressed in ESCC, m5C methylation is increased in ESCC tumors and the higher the expression of NSUN2 is, the worse the prognosis of ESCC patients (15). Furthermore, in the NSUN2 knockout mouse model of ESCC constructed in that study, the tumorigenesis and progression of ESCC were inhibited. Mechanistically, NSUN2 induces m5C modification of growth factor receptor-binding protein 2 (GRB2) and increases its stability. This process is mediated by a novel m5C-mediated RNA-binding protein, lin-28 homolog B (LIN28B). GRB2 transcripts are dependent on LIN28B for stabilization and increased levels of GRB2 activate the PI3K/AKT/ERK/MAPK signaling pathway, which promotes esophageal squamous cell carcinoma progression (Fig. 2). These results indicate that NSUN2 indirectly activates the oncogenic PI3K/AKT and ERK/MAPK signaling pathways through m5C, promoting the occurrence and progression of ESCC and providing a promising targeted therapeutic strategy for ESCC (15). Similarly, a study showed that high expression of NSUN2 leads to an increase in the level of m5C-modified mRNA in ESCC cells. The m5C 'reader' YBX1 binds to spermine oxidase (SMOX) mRNA and enhances its stability in an NSUN2-mediated m5C-dependent manner, thereby accelerating the proliferation and metastasis of ESCC cells (82). These findings further confirm the m5C-mediated epigenetic regulatory mechanism and that the YBX1/m5C-SMOX-mTOCR1 axis is involved in the occurrence and development of ESCC. In summary, these findings suggest that NSUN2 can mediate the tumorigenesis and development of ESCC through multiple signaling pathways. However, the specific mechanism has not been fully elucidated, so further research is still needed. Nevertheless, m5C methylation can serve as a potential therapeutic target in the treatment of esophageal cancer and further research can be conducted on the PI3K/AKT, ERK/MAPK and YBX1/m5C-SMOX-mTOCR1 axes.

RNA m5C methylation and gastric cancer (GC). GC is the fifth most common cancer globally and the fourth leading cause of cancer-related mortality (83). Studies have demonstrated that NSUN2 is highly expressed in gastric cancer cells and tissues (16,18,84). NSUN2 promotes the proliferation of gastric cancer cells and the growth of tumors (16). Cyclin-dependent kinase inhibitor 1C (CDKN1C) $p57^{Kip2}$ is a tumor suppressor (85). In addition, $p57^{Kip2}$ is also an important downstream gene regulated by NSUN2. After NSUN2 is knocked out, the level of m5C on the 3'UTR of p57Kip2 mRNA decreases, which undermines the mRNA stability of p57Kip2 and downregulates its protein level and the proliferation ability

of gastric cancer cells is enhanced (20). That is, NSUN2 can promote the proliferation of cancer cells in a m5C-dependent manner by inhibiting p57Kip2. Hu *et al* (16) report that small ubiquitin-like modifier 2 and 3 directly interact with NSUN2 by stabilizing it and mediating its nuclear translocation to promote its oncogenic activity. The expression of the m5C RNA methyltransferase NSUN2 is upregulated in gastric cancer tissues, which promotes the proliferation, migration and invasion of gastric cancer cells and is associated with a poor prognosis. Hu *et al* (16) also report that PIK3R1 and PCYT1A may be m5C target genes and that knockdown of NSUN2 reduces the m5C of PIK3R1 and PCYT1A, decreases their expression levels and markedly lowers RNA m5C levels in gastric cancer cells. NSUN2 has also been shown to maintain stability and upregulate the expression of a methylated lncRNA, NR-033928, in a m5C-dependent manner, which correlates with poor prognosis in patients with gastric cancer (86). Mechanistically, NSUN2 catalyzes the m5C modification of NR-033928, which promotes GC proliferation and inhibits apoptosis by increasing glutaminase expression (86). Li *et al* (87) experimentally explored lncRNA profiles in gastric cancer neuroinvasion (GC-NI) and reported the upregulation of DIAPH2-AS1 in NI-positive GC tissues. A further study revealed that DIAPH2-AS1 interacted with NSUN2 and stabilized NSUN2 from ubiquitin-proteasome pathway-mediated degradation. The protective effect of DIAPH2-AS1 on NSUN2 is enhanced by m5C modification to increase the stability of NTN1 mRNA, which ultimately induces GC-NI (87). Their study reveals that in GC-NI, DIAPH2-AS1 is a new oncogenic lncRNA and validated the DIAPH2-AS1-NSUN2-NTNI axis as a potential NI-positive GC therapeutic target, providing a new diagnostic biomarker. In addition, FOXC2 antisense RNA 1 (FOXC2 antisense RNA 1, FOXC2-AS1) is a newly identified functional lncRNA that is highly expressed in gastric cancer tissues and cells and promotes gastric cancer cell proliferation, migration and invasion; it is associated with poor prognosis in gastric cancer patients (18). Specifically, FOXC2-AS1 recruits NSUN2 to FOXC2 mRNA and increases its m5C level, followed by binding of the m5C-binding protein YBX1 to FOXC2 mRNA to increase FOXC2 mRNA stability. In conclusion, FOXC2-AS1 mediates the oncogenic effects of the m5c modification of FOXC2 via NSUN2 and YBX1 in gastric cancer cells, providing a new target for gastric cancer therapy.

RNA m5C methylation and HCC. HCC is the sixth most common cancer and the third leading cause of cancer mortality worldwide (83). Surgery is a common treatment, but owing to late detection and easy metastasis, most patients are not suited for surgical treatment. Therefore, research on the role of HCC development and its mechanism has focused on finding effective targets for the treatment of HCC and thus improving the prognosis of HCC patients. The m5C methyltransferase NSUN2 is highly expressed in HCC tissues (88). Hypermethylated target genes (GRB2, AATF and RNF115) are involved in oncogenic pathways. The expression of genes such as GRB2, RNF115, AATF, ADAM15, RTN3 and HDGF is positively associated with the expression of NSUN2. These findings indicate that hypermethylated genes associated with NSUN2 are involved in the development of tumors. Transcriptome analysis revealed that hypermethylated genes are involved mainly in phosphokinase signaling pathways, such as the Ras and PI3K-Akt pathways. NSUN2 affects the sensitivity of HCC cells to sorafenib by regulating the activity of the Ras pathway (88). NSUN4, which has been less studied, has also been reported to be involved in the poor prognosis of HCC patients (22). ALYREF may be involved in hepatocellular carcinogenesis by affecting the methylation levels of target genes (23). As aforementioned, NSUN5 is associated with lower overall survival. NSUN5 mRNA and protein expression levels are upregulated in HCC tissues and the overexpression of NSUN5 promotes the proliferation and migration of HCC cells although the exact mechanism remains unclear (21). Although the mechanism involved in the aforementioned experimental studies has not yet been elucidated, compared with the less-studied NSUN4 and NSUN5, they have also been proven to be involved in the progression of liver cells. High expression of NSUN2, NSUN4, NSUN5 and ALYREF is associated with poor prognosis in patients with HCC and all of these genes can be used as biomarkers for the diagnosis and prognosis of HCC. These findings indicate that both methyltransferases and related binding proteins involved in m5C methylation are involved in the occurrence and development of HCC, which provides directions for subsequent relevant research.

RNA m5C methylation and gallbladder cancer (GBC). GBC is the most common biliary tract malignancy (89) and is highly invasive (90). However, owing to the lack of early symptoms, almost all GBC patients are diagnosed at an advanced stage and the treatment method is mostly surgical resection (91). Although certain achievements have been made in exploring oncogenes and tumor suppressor genes that promote tumors in GBC, there is still a lack of independent biomarkers that can be routinely used in clinical practice (92,93). Therefore, identifying new factors that may serve as new diagnostic biomarkers and therapeutic targets for the treatment of GBC patients is crucial.

Gao *et al* (26) reported that NSUN2 is highly expressed in GBC tissues and cell lines and that silencing NSUN2 inhibits GBC cell proliferation and tumorigenesis (26). By contrast, the upregulation of NSUN2 promotes GBC cell growth. Ribosomal protein L6 (RPL6), which regulates the HDM2-p53 pathway, inhibits cell growth (94).

In their further experiments, they reported that RPL6 closely interacts with NSUN2. GBC cells grew markedly slower in the absence of RPL6 and grew relatively normally in the presence of NSUN2 (26). Therefore, the synergistic effect of NSUN2 and RPL6 promotes GB occurrence. In summary, the function of NSUN2 in GBC provides new mechanistic insights and targeting NSUN2 may be a potentially effective treatment for GBC as well as a diagnostic biomarker, which, of course, needs to be supported by more definitive clinical studies. In any case, NSUN2 is indeed involved in the development of GBC, which provides direction both in terms of finding inhibitors of methylation and in terms of pathways.

RNA m5C methylation and cholangiocarcinoma (CCA). CCA is a biliary epithelial malignancy. It is the second most common type of primary HCC and accounts for ~15% of mortalities from hepatobiliary malignancies (95). Early diagnosis of CCA

is difficult and most patients are already in the locally progressive stage or have distant metastases when they present to the doctor (96). CCA is difficult to treat and even if a few patients are able to undergo surgical treatment, there is a high rate of recurrence and early local or distant metastasis following surgery, whereas the 5-year survival rate is <10% and the 1-year recurrence rate is ≤50% (97). Therefore, there is an urgent need to elucidate the underlying mechanisms of CCA progression to develop new therapeutic strategies. NF-κB-interacting lncRNA (NKILA) is a functional lncRNA and a study showed that dysregulation of NKILA expression is associated with the malignant behavior of cancer cells (98). Similarly, NKILA expression is upregulated in CCA patients and NKILA expression is associated with advanced TNM stage, lymph node and distant metastasis in CCA patients. NKILA promotes CCA proliferation and metastasis both *in vitro* and *in vivo* and this process is associated with NSUN2 and increases its m5C level (27). NKILA promotes CCA proliferation and metastasis both *in vitro* and *in vivo* and this process is associated with NSUN2 and increases its m5C level. This study suggests that NKILA functions as an oncogenic lncRNA in regulating the growth and metastasis of CCA and is a promising therapeutic target for CCA patients. The current experimental study is far from sufficient and more experimental studies are needed to elucidate the mechanism by which NKILA promotes CCA progression through m5C methylation.

RNA m5C methylation and pancreatic cancer (PC). PC is a malignant tumor of the digestive system characterized by a high degree of metastasis, ranking 12th among the most common cancers in the world, with an increasing annual incidence (83,99). In recent years, despite advances in diagnosis and treatment, treatment outcomes remain suboptimal and are the 7th leading cause of cancer-related mortality (100). High morbidity and mortality pose a great threat to human health and have become an enormous global burden. Therefore, the need to clarify the molecular mechanisms of PC progression and identify promising and effective therapeutic targets is urgent. Previous studies have demonstrated that NSUN2 is highly expressed in PC tissues compared with normal tissues and that its elevated expression portends a poor prognosis (100). Silencing NSUN2 reduced the proliferation, migration and invasive ability of PC cells and inhibited the growth and metastasis of xenograft tumors. Conversely, the overexpression of NSUN2 promoted PC growth and metastasis (101). TIAM2 has been shown to promote the proliferation and migration of cancer cells (102,103). TIAM2 is associated with poor prognosis in PC patients, but its regulatory mechanism is unclear (104). Thus, they identified the downstream targets of NSUN2 by m5C sequencing and RNA sequencing and the results revealed that NSUN2 deletion resulted in reduced m5C modification levels, along with reduced TIAM2 mRNA expression. Further validation revealed that NSUN2 silencing accelerated TIAM2 mRNA decay in a YBX1-dependent manner. In addition, NSUN2 exerts its oncogenic function in part by enhancing TIAM2 transcription. More importantly, disruption of the NSUN2/TIAM2 axis suppresses the malignant phenotype of PC cells by blocking EMT. This study highlights the critical function of NSUN2 in PC and provides novel mechanistic insights into the NSUN2/TIAM2 axis as a promising therapeutic target.

Pancreatic ductal adenocarcinoma (PDAC) is one of the deadliest human malignancies, with a low overall 5-year survival rate and is the seventh leading cause of cancer mortality in men and women worldwide (83). PDAC has no obvious symptoms in the early stage and most patients are diagnosed with locally advanced or metastatic tumors; thus, the mortality rate is high (105). Therefore, it is particularly important to discover new targeted drugs that can diagnose and treat PDAC at an early stage. There are few studies on m5C and PDAC and there are only a few score on m5C prognosis. Yun *et al* (106) obtained a negative correlation of m5C scores with overall survival for predicting prognosis in patients with PDAC by integrating m5C-related differentially expressed genes. Similarly, high m5c expression has been shown to predict poor prognosis in PDAC patients and their response to immunotherapy (107,108). Pancreatic and pancreatic ductal adenocarcinomas are both associated with m5C methylation and even more clearly, in the case of pancreatic cancer, tumor growth and development can be promoted through the NSUN2/TIAM2 axis. Disruption of this axis inhibits tumor growth, suggesting directions for the treatment of pancreatic cancer.

RNA m5C methylation and colorectal cancer. Colorectal cancer (CRC) has the third highest incidence and the second highest mortality rate among all cancers worldwide. Surgery is a common treatment, but most of the treatments are unsatisfactory (83,109). Therefore, finding effective therapeutic targets is particularly important. SnoN, also known as SKIL, is a negative regulator of TGF-β signaling (110,111). NSUN2 is highly expressed in colorectal cancer. Further knockdown of NSUN2 in mice revealed the oncogenic role of NSUN2-mediated RNA m5C modification in colorectal cancer. The methylation of NSUN2 promotes the stability of SKIL mRNA in a YBX1-dependent manner, which ultimately activates and upregulates the expression of TAZ, thus promoting the development of CRC. Although few studies have investigated m5C methylation in CRC, the NSUN2-m5C-SKIL-TAZ axis provides a clear direction for the treatment of CRC.

4. m5C methylation and digestive system tumor-related therapy

m5C methylation plays a crucial role in the development and progression of various digestive tumors, offering a potential target for the development of novel therapeutic strategies. However, research on m5C methylation remains in the basic stage, lacking specific intervention strategies and therapeutic approaches for clinical application. The following analysis is based on existing studies and potential future research directions.

Potential of m5C methylation as a diagnostic and prognostic marker. Alterations in m5C methylation levels are closely linked to the occurrence, progression and prognosis of numerous digestive tumors. For instance, NSUN2 is highly expressed in multiple cancers and is associated with a poor prognosis (15-18). In addition, changes in the stability of m5C-modified RNA molecules, such as lncRNAs and mRNAs, in tumor cells have paved the way for the development of diagnostic markers. For example, m5C-modified H19 lncRNA is associated with poor differentiation in HCC (17),

while FOXC2-AS1 promotes invasion and metastasis in gastric cancer by stabilizing FOXC2 mRNA through m5C modification (18). These findings suggest that m5C-modified RNA molecules could serve as potential diagnostic and prognostic markers for the early detection and monitoring of tumor progression.

Development of m5C methylation inhibitors. Although no specific m5C methyltransferase inhibitors have been developed for clinical use yet, certain drugs such as azacitidine have been shown to inhibit cancer cell proliferation by non-specifically inhibiting RNA and DNA methylation (112). Future research will need to focus on developing m5C methyltransferase inhibitors that are specific to m5C methyltransferase, aiming to minimize side effects on normal cells. Additionally, studies targeting m5C demethylating enzymes, such as TET family proteins, may also offer insights for the development of new therapeutic agents.

Prospects of m5C methylation in immunotherapy. In recent years, significant advancements have been made in the application of m5C methylation in tumor immunotherapy (113). For example, Segovia *et al* (114) successfully induced apoptosis and immunogenic cell death in cancer cells using a combination of m5C inhibitors and immune checkpoint inhibitors. Another study revealed that m5C-modified mRNAs reprogram tumor-associated macrophages or anticancer T cells, inducing antitumor immunity and promoting tumor regression (115). Currently, the m5C-associated risk score is an independent prognostic factor for patients with colon cancer and the score can be used to predict the prognosis, immunotherapy response and drug sensitivity of colon cancer patients (116). In addition, the methyltransferase complex component RBM15B- and the m6A 'card reader' IGFBP2-mediated glutathione peroxidase 4 may be novel modulators of cancer immunotherapy through activation of the cyclic GMP-AMP synthase-interferon signaling pathway in colorectal adenocarcinoma, which has emerged as a novel modulator of cancer immunotherapy (117). These findings imply that m5C methylation modification could be a potential target for immunotherapy and future studies could further explore its application in other digestive tumors. Regarding future research directions, they can concentrate on clinical data integration. They should strengthen the integration with clinical medical data and verify the diagnostic and prognostic value of m5C methylation markers through large-scale clinical sample analysis. Drug development is another important direction. Developing specific m5C methyltransferase and demethylase inhibitors and exploring their application in clinical treatment are essential. Multiomics research can also be applied. By combining transcriptomics, proteomics and metabolomics, we can comprehensively elucidate the mechanism of m5C methylation in tumorigenesis and development. Further translational medicine research can be conducted to explore the application of m5C methylation in personalized medicine, providing theoretical support for precision therapy.

5. Conclusion

The present study reviewed the role of m5C methylation in digestive system tumors and its potential as a therapeutic target. Although m5C methylation has been shown to play a significant role in the development of a number of digestive system tumors, such as esophageal squamous cell carcinoma, HCC, breast cancer and thyroid cancer, current research is still in the basic stage, lacking specific intervention strategies and therapeutic approaches for clinical application. Future studies need to strengthen the integration with clinical medical data, develop specific m5C methylation inhibitors and explore their potential application in tumor immunotherapy. Through these efforts, m5C methylation is expected to become a new target for the diagnosis and treatment of digestive system tumors, providing a new direction for improving patient prognosis.

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Availability of data and materials

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Authors' contributions

LZ and JY made substantial contributions to the conception and design of the present study. SY, GW, JA, HJ and BT were involved in revising the manuscript critically for important intellectual content. Data authentication is not applicable. All authors read and approved the final manuscript for publication.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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