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Review Article

N7-methylguanosine modification: from regulatory roles to therapeutic implications in cancer

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Abstract: N7-methylguanosine (m7G) is one of the most common post-transcriptional epigenetic modifications. Different m7G methyltransferases (writers) load the m7G-cap at the 5'-terminal or inside the RNAs. For example, writers such as methyltransferase-like 1 (METTL1)/WD repeat domain 4 (WDR4) and Williams-Beuren syndrome chromosome region 22 (WBSCR22) have been reported in mammals to promote cell proliferation, EMT, and chemoresistance in massive quantities of cancers. The underlying mechanism includes modulating the RNA secondary structure, preventing RNA degradation from exonucleases, and improving codon-dependent translation. However, some studies have shown that in colorectal and lung cancers, m7G inhibits tumor progression. m7G binding proteins (readers), such as eukaryotic translation initiation factor 4E (eIF4E), promote the efficiency of cap-dependent translation and accelerate the cell cycle to improve cancer progression. Due to the more profound understanding of m7G regulatory proteins in cancer, numerous studies aim to investigate the clinical efficiency of m7G-targeted therapy. eIF4E antisense oligonucleotide drug (4EASO) and Ribavirin are the most mature trials that competitively inhibit the binding of eIF4E to m7G-cap. These drugs have encouraging results in halting cancer progression and improving prognosis, including AML and non-small cell lung cancer, which provide a promising perspective for developing more m7G-targeted drugs. In the future, we look forward to an ongoing investigation into the role of m7G modification in tumors and drug resistance to m7G-related therapies to be solved. Therefore, the clinical application would be put into practice as soon as possible.

Keywords: N7-methylguanosine modification, cancer, cancer therapy, RNA metabolism

Introduction

RNA epigenetic modification regulates gene expression at the post-transcriptional level, significantly affecting RNA properties [1]. To date, more than 150 different RNA modifications have been identified, the most common ones of which are N1-methyladenosine (m1A), 3-methylcytidine (m3C), 5-methylcytosine (m5C), N6-methyladenosine (m6A) and N7-methylguanosine (m7G) modification [2]. m7G modification, methylation in the seventh N on RNA guanylate, is widely present in mRNA, rRNA, tRNA, sRNA, telomerase RNA (TERC), microRNA (miRNA), and long non-coding RNA (lncRNA) [3-5]. However, small cytoplasmic RNA (scRNA), small interfering RNA (siRNA), and circular RNA (circRNA) have not been reported to contain m7G modification. Two main types of m7G modification are classified according to their locations, which are m7G-cap at 5'-terminal and internal m7G modification (**Figure 1**) [6, 7]. And at the N2 site inside the m7G-cap, guanylate can be further hypermethylated to m2,2,7G-trimethylguanosine (TMG) caps [8]. Like other epigenetic modifications, m7G modifications are dynamically regulated by RNA methyltransferases and m7G recognition proteins. Although, Alkane monooxygenase (AlkB) has a general function in demethylating almost all the RNA modifications, including m7G, which is utilized to detect m7G sites inside RNAs [9]. However, despite our curiosity about m7G demethylase, we still did not find any clues on the specific enzyme to the best search. Indeed, we hope that more studies will explore the potential enzymes that demethylase m7G modification and their function in tumor progression.

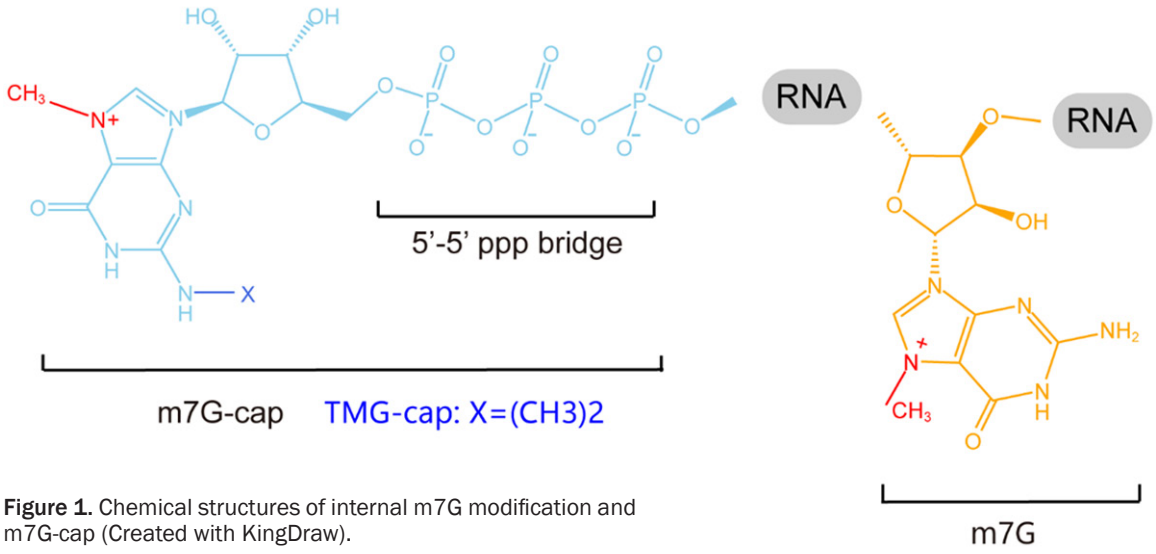

Figure 1. Chemical structures of internal m7G modification and m7G-cap (Created with KingDraw).

As a conserved RNA modification in the genome, m7G modification is dynamically regulated under different physiological and pathological conditions. Firstly, m7G modification in ischemic stroke also shows dynamic changes, from the hypomethylation in an ischemic state to the METTL1-mediated hypermethylation during ischemia-reperfusion injury [10]. m7G regulation is not only reflected in the global methylation level but also in the sites of internal methylation, which typically occurs in the GA/GG enriched region in the 5'-untranslated region (UTR) [11]. While in response to oxidative and heat stress, m7G modifications are increased and dynamically accumulate in the coding sequence (CDS) and 3'-UTR [12, 13]. Secondly, the abnormal self-renewal and differentiation of cells during nervous system development are usually accompanied by the change of m7G methylation, which affects the expression of stem cell gene signature [14]. Significant differences in m7G methylation are shown in malignant transformation and response to chemotherapeutic drugs [15, 16].

Interestingly, whether the methylation is upregulated or downregulated depends on the specific disease type. In the following text, we will explain the role of m7G modification and related regulators in the occurrence and development of various tumors. Hopefully, we aim to provide new insights into cancer diagnosis and treatment.

The history of m7G modification research

As early as 1975, around 14 articles reported the m7G modification in viral RNAs, mRNAs, tRNAs, and rRNAs [17, 18]. Hefti E was the first scientist to identify m7G-cap in 5' nucleotide sequence in sindbis viral RNA [19]. The first m7G methyltransferase in the vaccinia virus was solubilized in 1975 by M J Ensinger [20]. m7G-cap and inner m7G modification and their methyltransferases in diverse RNAs were subsequently discovered after that [21, 22]. Three years later, the successful development of m7G-specific antibodies significantly promoted the research of m7G modification [23]. m7G reader was first reported in 1991 as eif4E bound to the m7G base through hydrogen-bond pairing [24]. Later in 2007, the m7G reader, eIF4E, was found to be closely related to various malignant tumors, which opened a new era of exploring the mechanism of cancer progression based on m7G modification [25]. Interestingly, there was an academic debate on the existence of m7G modification in human let-7e in colon cancer in 2020 [26, 27]. Jeppe Vinther and Luca Pandolfini had a heated discussion on m7G detection methods (M7G-RIP-seq, BoRed-seq) and analysis methods (MS/MS analysis, etc.). This provides a deeper thinking for scientists devoted to RNA epigenetic modification. Since then, the research on m7G modification has experienced explosive growth. More and more scientists are committed to exploring

Table 1. M7G regulators for targeted RNAs

RNA	*m7G types*	*Organisms*	*guanine-N7 MTase*	*Reference*
tRNA	Internal m7G	Eubacteria	TrmB*	[133]
	Internal m7G	Yeast	Trm8*/Trm82	[134]
	Internal m7G	Mammalian	METTL1*/WDR4	[20]
	TMG-cap	Yeast	TGS1	[39]
mRNA	Internal m7G	Mammalian	METTL1*/WDR4	[34]
	m7G cap	vaccina virus	Abd1 (vD1*/vD2)	[135]
	m7G cap	Mammalian	RNMT (HCMT1)	[37]
miRNA	Internal m7G	Mammalian	METTL1*/WDR4	[33]
16S rRNA	Internal m7G	Streptomyces tenebrarius	KgmB	[40]
18S rRNA	Internal m7G	Yeast	Bud23-Trm112	[41]
	Internal m7G	Mammalian	WBSCR22	[23]
23S rRNA	Internal m7G	E. coli	YcbY (RlmKL)	[46]
sRNA	TMG-cap	Yeast, Mammalian	TGS1	[8]
TERC	TMG-cap	Mammalian	TGS1	[24, 25]

*Indicating catalytic subunits in complexes.

the relationship between m7G modification and tumorigenesis.

Regulators of m7G modification

Writers: guanine-N7 methyltransferases (MTase)

Most of the m7G methyltransferases belong to the Adomet-dependent methyltransferase family [28, 29]. The m7G writers differ in species and RNA substrates (**Table 1**). *TrmB* is the first identified m7G writer in E. coli to catalyze m7G modification at the G46 site in tRNA [30]. m7G46, located in a variable region within tRNA, is the most common methylation site in prokaryotic and eukaryotic tRNAs [31]. In yeast, the writer is a complex of catalytic subunit *Trm8* and auxiliary subunit *Trm82* [32]. In mammals, METTL1/WDR4 complex is responsible for m7G modification in tRNAs, mRNAs, and miRNAs [33, 34]. The latest study unraveled the core mechanism of METTL1 catalyzing m7G, that the disordered N-terminal region of METTL1 is the nexus for methyltransferase activity, which can be regulated by Ser27 phosphorylation [35]. WDR4 acts as a scaffold for METTL1 and tRNA T-arm, activating m7G methylation in the presence of S-adenosylmethionine or S-adenosylhomocysteine [36].

The m7G writer for the cap structure at the 5' end is a complex of RNMT and RNMT-activated small protein (RAM) [37]. Furthermore, CDK1-cyclinB1 activates RNMT by direct phosphorylation and indirectly blocking the inhibitory protein, nuclear protein subunit α2 (KPNA2) [38]. As for TMG-cap, TGS1 is responsible for its biosynthesis in tRNA, sRNA, and telomerase RNA [39]. For the 16S rRNA in *Streptomyces tenebrarius*, *KgmB* writes the m7G modification at the G1405 site [40]. For 18S rRNA in yeast and mammals, Bud23/TRMT112 [41, 42] and WBSCR22 [43-45] are identified as the responsible writers. m7G modification of the large subunit in 23S rRNA requires *YcbY (RlmKL)* [46].

Readers

RNA epigenetic modifications must be bound and recognized by specific proteins before performing corresponding functions [41]. Known readers include the YTH family proteins for identifying m6A modifications [47] and the nuclear regulatory protein ALYREF for m5C modifications [48]. There are mainly two m7G readers, CBC and eIF4E. CBC consists of a nuclear cap-binding protein subunit 2 (NCBP2/CBP20) and an adaptor protein subunit 1 (NCBP1/CBP80) [49]. After the m7G-cap is recognized, RNAs are translocated from the nucleus to the cytoplasm [50]. The interaction between the negative π-electron clouds from two aromatic residues in eIF4E and the positive charge in the m7G cap makes them available to bind to each other [51]. Then, translation initiation complexes are recruited to initiate mRNA

translation [52]. Notably, the nuclear localization of eIF4E mediated by importin 8 is essential to maintain its pro-oncogenic activity [53]. When m7G-cap is hypermethylated, snuportin-1 and other factors, such as importin-β, recognize the TMG-cap structure and mediate the translocation of modified RNA from the cytoplasm to the nucleus [54]. Recent studies have revealed that eIF4E directly binds the methyltransferase domain in RNMT to form an m7G cap-eIF4E-RNMT complex and promotes m7G-cap assembly [55]. As mentioned above, various proteins regulate the spatial location of RNAs and RNA biological functions by recognizing m7G modification.

The impact of m7G modification on transcription and translation

m7G modification regulates almost all events in RNA post-transcriptional regulation, including post-transcriptional splicing, RNA cytoplasmic localization, and RNA stability [56]. In addition, the TMP cap in TERC inhibits telomerase assembly and restricts elongation [57]. In precursor tRNAs, G-quadruplex formation is interrupted, and canonical stem-loop structure formation is promoted through m7G modification [33]. m7G-cap enhances the plasticity of RNA cytosolic localization by preparing a docking site for CBC to mediate nuclear export [54]. Conversely, TMG-cap is recognized by snuportin-1 to translocate RNAs from the cytoplasm to the nucleus [58]. m7G modification enhances the stability of RNAs through various mechanisms [59]. Firstly, positive charge or zwitterion is introduced onto the nucleobase by m7G modification [60]. On the other hand, the endogenous m7G46 in tRNA forms a stable tertiary base pair with C13-G22 to maintain the tRNA geometry in the secondary structure [61]. Loss of the m7G modification makes tRNA more susceptible to the rapid tRNA degradation (RTD) pathway, especially under heat stress [62]. In addition, the m7G cap and TMG-cap impart a protective layer at the 5' end of the RNAs, which effectively prevents 5' exonuclease activities [63, 64]. Thus, m7G modifications contribute to RNA stability, simultaneously enhancing the expression of oncogenes and promoting malignant transformation.

The initiation and process of mRNA translation undergo the regulation of m7G modification [52]. m7G-cap recruits initiation factors like eukaryotic translation initiation factor E (eIF4E) and eIF4F [65]. Additionally, m7G-cap facilitates the "RNA-looping" to generate the recognition of translation initiation codon by 40S ribosome at the 5'-end [66]. Besides, tRNA (m7G46) modification adjusts the translation process by altering tRNA codon decoding ability and ribosome-pausing mechanism [15]. Codon recognition dependent mechanism introduces a theory that tRNAs with higher frequencies of m7G modification enjoys higher translation efficiency [67]. However, whether m7G modification has a direct biological role on ribosomes needs to be further explored. In summary, we listed the regulatory function of m7G modification on RNA metabolism (**Figure 2**).

Roles of m7G modification in normal cells

Dysregulation of m7G modification directly affects cell function. In the HeLa cell line, CBC, accompanied by ARS2, promotes RNA stability by recognizing m7G-cap in RNAs to enhance cell proliferation [68]. WDR4 mutation and METTL1 dysfunction are closely related to abnormal cell self-renewal and neural differentiation. These regulators have been reported to participate in various neural developmental diseases, including microcephalic primordial dwarfism, Down syndrome, and multiple sclerosis [14]. As observed in Embryonic Stem cells, METTL1 alteration significantly affected the m7G tRNA methylome. METTL1 knockdown dramatically affected tRNA function, mRNA translation, and ribosome pausing in the "RAGGU" motif region [69]. Global m7G profile in pluripotent stem cells (hiPSCs) reveals that reduced m7G modification interferes with pluripotency by promoting embryoid body (EB) formation and slower cell cycling and finally results in mesoderm differentiation [34].

Additionally, m7G modification regulates angiogenesis. It has been reported to be associated with ischemic disorders, idiopathic pulmonary fibrosis, and pulmonary arterial hypertension [70]. There was a general decrease in m7G modification inside mRNA during ischemia. However, in post-ischemic injury, the involvement of METTL1 promotes the proliferation, migration, and tube formation of HUVECs by the m7G-VEGFA pathway, resulting in the increase of local angiogenesis and the recovery of blood circulation [10]. However, as a new RNA modifi-

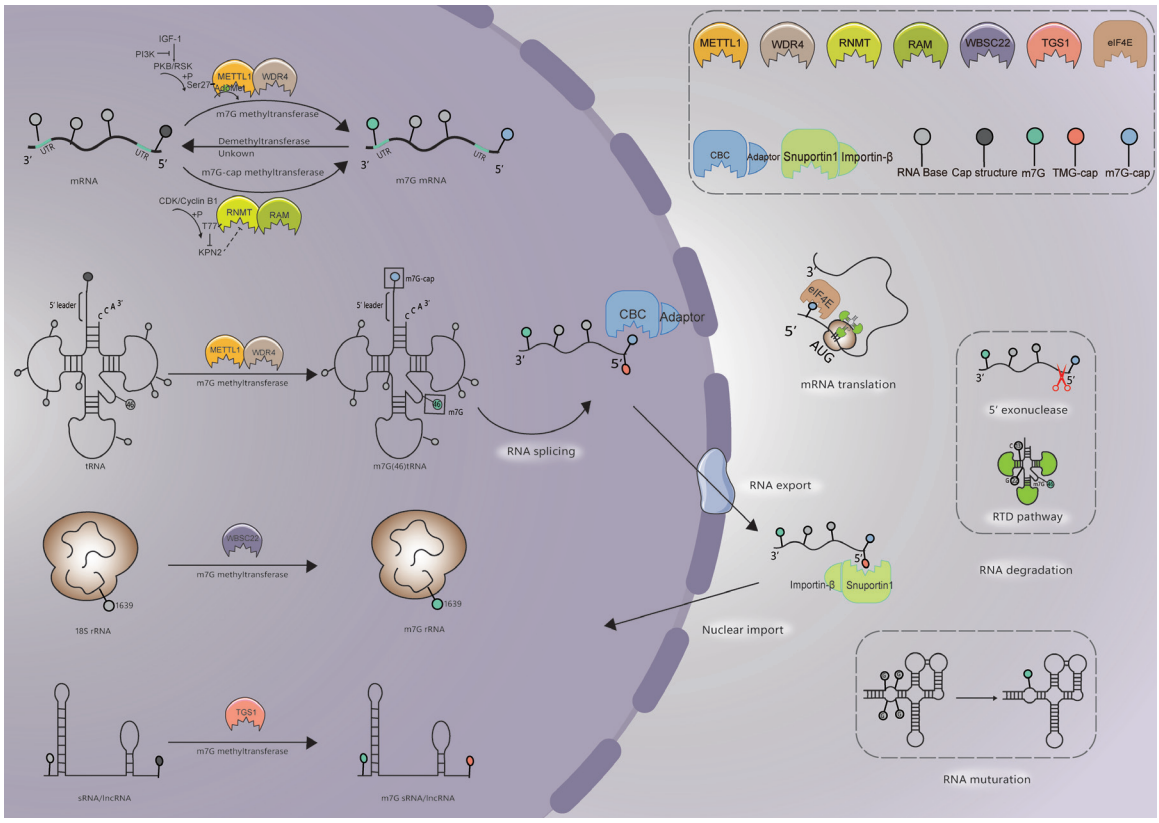

Figure 2. The process and molecular functions of m7G modification in RNA metabolism. Different methyltransferases were essential for introducing internal m7G modification and cap m7G modification at 5'-termial in human, including METTL1/WDR4, RNMT/RAM, WBSC22 and TGS1, relative locations are marked with numbers. In the nucleus, m7G-cap could be recognized by CBC to generate RNA nuclear exportation. In the cytoplasm, the binding of TMG-cap and Snuportin1 associated with importin-β enables RNA to be imported into the nucleus. m7G regulates RNA degradation by maintaining RNA geometry structure and protecting RNAs from 5' exonucleases and RTD pathway. m7G promotes RNA maturation by affecting secondary structures. m7G regulates mRNA translation through eIF4E recognition, interference of ribosome pausing and tRNA decoding.

cation, the mechanism for regulating angiogenesis is still unclear. Furthermore, bioinformatics analysis showed that the m7G-related gene signature (NUDT16, NUDT4, CYFIP1, LARP1, and DCP2) was closely related to the progression of heart failure [71]. In addition, WDR4 was listed as one of the eight RNA epigenetic modification-related proteins that affect osteoarthritis disease [72].

Roles of m7G regulators in cancer

As a post-transcriptional RNA modification, there is no uniformity in the role of m7G modification on tumors. Most studies suggest that m7G modification promotes the expression of proto-oncogenes to drive the initiation and progression of tumors [69]. Five m7G-related lncRNAs (LINC00924, LINC00944, LINC00865, LINC00702, and ZFAS1) screened out gastric cancer patients who might benefit from immunotherapy, which provides a reference for the treatment and prognosis prediction of cancer [73]. However, some studies indicate an anticancer function of m7G modification in lung and colon cancer [74]. Herein, we briefly summarize the biological roles of m7G-related proteins in various tumors (**Table 2** and **Figure 3**).

METTL1/WDR4 complex

METTL1/WDR4 complex has been widely reported to be a parameter of malignant transformation in multiple malignancies, such as breast cancer, glioblastoma, hepatocellular carcinoma (HCC), intra-hepatic cholangiocarcinoma (IHC), acute myeloid leukemia (AML), head and neck squamous cell carcinoma (HNSCC), Nasopharyngeal carcinoma (NPC), bladder cancer, lung adenocarcinoma, and cutaneous melanoma [75-77]. Deleting the Trm8-Trm82 gene in yeast increases its sensitivity to 5-fluorouracil

Table 2. The role of m7G regulators in cancer

Molecule	Cancer	Role in cancer	Molecular Mechanism	Phenotype	Reference
RNMT	Breast cancer	+	RNMT-PI3K^{MUT}	Proliferation, migration and tumorigenesis	[104]
RNMT	Glioma	+	B7-H6-c-Myc-RNMT	Proliferation, migration and tumorigenesis	[106]
METTL1/WDR4	Glioblastoma	+	Arg-TCT-4-1↑/cell cycle	Proliferation, migration and tumorigenesis	[16]
METTL1/WDR4	Hepatocellular carcinoma	+	PTEN↓-AKT	Proliferation and migration	[136]
		+	CCNB1-P53/PI3K-AKT	Proliferation, migration and chemorisistance	[82]
		+	m7G tRNA↑-oncogene mRNA↑	Proliferation, migration and tumorigenesis	[67]
		+	SLUG/SNAIL and TGF-β2-PMN-MDSC	tumor metastasis after (IRFA)	[85, 86]
METTL1/WDR4	Cholangio-carcinoma	+	m7G tRNA↑-oncogene mRNA↑	Proliferation, migration and tumorigenesis	[83]
METTL1/WDR4	Bladder cancer	+	EGFR↑/EFEMP	Proliferation and migration	[89]
METTL1/WDR4	Cervical cancer	+	tRNA stability	Chemoresistance	[79]
CBC	Cervical cancer	+	CBC/ARS2-let7	Proliferation	[68]
METTL1/WDR4	Neck and head squamous cancer	+	m7G tRNA↑-PI3K-AKT-MTOR	Proliferation, migration and tumorigenesis	[87]
METTL1/WDR4	Nasopharyngeal carcinoma	+	ARNT-METTL1/WDR4↑-WNT-β-catenin	Proliferation, migration, tumorigenesis and therapeutic resistance	[80]
METTL1/WDR4	AML	+	Arg-TCT-4-1↑-cell cycle	Proliferation, migration and tumorigenesis	[16]
METTL1/WDR4	Esophageal squamous cell carcinoma	+	MTORC1/RPTOR-ULK/autophagy	Reduced autophagy	[88]
METTL1/WDR4	Lung cancer	+	tRNA function	Proliferation, migration and tumorigenesis	[77]
	Colon cancer	-	miR-149-3p↓/S100A4↑/p53↑	Chemoresistance	[74]
METTL1/WDR4	Lung cancer and colorectal cancer	-	let-7e↓-HMGA2/RAS/MYC↑	Proliferation, migration and tumorigenesis	[5]
eIF4E	AML	+	NF-κB/eIF4E1↑ and eIF4E3↓	Proliferation, migration and tumorigenesis	[123]
eIF4E	Prostate cancer	+	MTOR/Mnk1/2-eIF4E↑	Proliferation, migration, tumorigenesis and therapeutic resistance	[96]
CBC (NCBP1)	Lung cancer	+	NCBP1↑-CUL4B↑	Proliferation, migration and tumorigenesis	[111]

(-) for suppressor gene; (+) for oncogene.

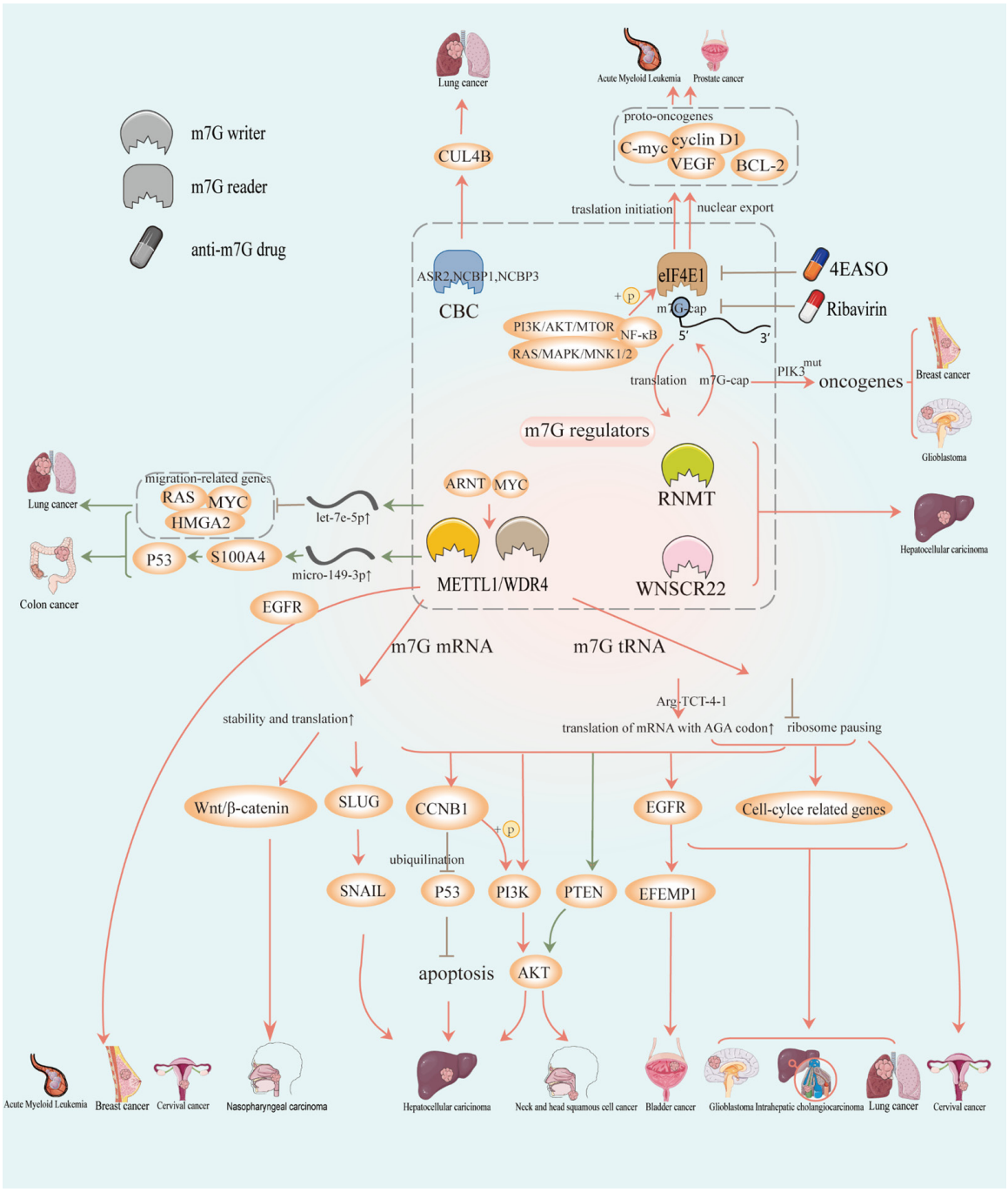

Figure 3. The role of m7G regulators in human cancers. m7G regulators are associated with various kinds of cancers including hepatocellular carcinoma, intrahepatic cholangiocarcinoma, bladder cancer, prostate cancer, cervical cancer, glioblastoma, pancreatic cancer, acute myeloid leukemia, colon cancer, lung cancer, etc. The schema was prepared with object images from Servier Medical Art (https://smart.servier.com).

[78]. In the cervical cancer cell (HeLa cells) and NPC, the human homologous METTL1/WDR4 complex induces cell resistance to the chemotherapeutic drugs (paclitaxel and cisplatin) [79, 80]. In HCC, high expression of METTL1 is significantly correlated with serum alpha-fetoprotein (AFP) levels and hematogenous metastasis [81]. WDR4 promotes the epithelial-mesenchymal transition (EMT) and confers resistance to sorafenib and lenvatinib in HCC cells [82]. In

HCC and IHC, m7G tRNA and mRNA modification endow higher translation efficiency of oncogenes like EGFR [67, 83, 84]. Targeting METTL1/WDR4-m7G-SLUG/SNAIL pathway and TGF-β2-PMN-MDSC may provide a compensatory address to tumor metastasis after insufficient radiofrequency ablation (IRFA) [85, 86]. In the above cancers, METTL1/WDR4 ultimately affects cell cycle-related genes (such as cyclin A2, cyclin D2, cyclin-dependent kinase 4 (CDK4), CDK6, and CDK8) through mRNA "translatome" regulation, to promote the G1/S phase transition. Meanwhile, other proto-oncogenes are simultaneously affected, including epidermic growth factor receptors (EGFR), High Mobility Group at-hook 2 (HMGA2), ASH2 like (ASH2L), Set Domain Bifurcate d Histone Lysine Method Transfer 1 (SETDB1), Ubiquitin Conferring Enzyme E2 t [16]. Regulated by C-MYC, WDR4 activates the PI3K/AKT pathway and accelerates the ubiquitination-mediated degradation of P53 by enhancing the stability and translation of CCNB1 RNA [82]. In NPC, under the regulation of ARNT, METTL1 promotes EMT through the WNT/β-catenin signaling pathway [80]. In HNSCC, apart from influencing the intracellular interaction between malignant epithelial cells and stromal cells, METTL1/WDR4 also dramatically impacts the immune microenvironment [87]. In esophageal squamous cell carcinoma, METTL1/WDR4 impedes MTORC1 and RPTOR-mediated ULK/autophagy pathway in an m7G-tRNA-related codon-dependent manner [88]. The METTL1-m7G-EGFR/EGF-containing Fibulin Extracellular Matrix Protein (EFEMP1) axis is essential in promoting bladder cancer progression [89]. Besides promoting RNA expression, METTL1 has also been proposed to negatively regulate the tumor suppressor PTEN and activate the AKT pathway in liver cancer [81].

Although METTL1/WDR4 has been reported to be a positive driver in most cancers, it has also been reported to have a negative regulatory effect on certain tumors. For example, in colorectal cancer, the overexpression of METTL1 increases drug sensitivity through the miR-149-3p/S100A4/p53 axis in cisplatin-resistant cell lines [74]. Some research revealed an inhibitory role of METTL1 in lung cancer, in contrast with the previous study. Both in lung and colorectal cancers, it has been reported that METTL1 promotes the m7G methylation of the GG-enriched region in the miRNA precursor to stabilize its secondary structure and inhibit cell migration [5, 90-92]. Among the regulated miRNAs, let-7e plays a significant role in inhibiting genes such as HMGA2, RAS, and MYC [5].

eIF4E

The overactivation of m7G "reader" eIF4E was observed in 30% malignancies, like M4/M5 subtypes of AML, pancreatic cancer, and prostate cancer [93, 94]. Under the regulation of nuclear factor kappa-B (NF-κB), eIF4E enhances proto-oncogene expression by recognizing m7G-cap [95]. In addition, both PI3K/Akt/mTOR pathway and the Ras/MAPK/MNK1/2 pathway contribute to the phosphorylation of eIF4E to increase its binding capacity to m7G-cap [96]. In AML and bladder cancer, the final impact of eIF4E aggregates on the oncogenes like VEGF, whereas implies a slight effect on the constructive ones, like GAPDH [97]. In addition, there is simultaneous overexpression of eIF4E1 and loss function of eIF4E3 in AML. Different amino acid residue sequence of eIF4E3 makes it bind to m7G-cap by electrostatic force and Van der Waals force, thus playing an antagonistic role against eIF4E1. Overexpressing eIF4E3 results in decreased expression of eIF4E1-regulated proto-oncogenes VEGF, MYC, CCND1, and NBN. In pancreatic ductal adenocarcinoma, the MAPK interacting protein kinases (MNK)/eIF4E pathway was reported to be related to chemotherapy drug resistance [98]. And SOX2 was later discovered to be the downstream target of eIF4E to promote tumor proliferation [99]. In prostate cancer, eIF4E can significantly enhance the transcription of certain genes, such as MYC and BCL-2 [100].

Promyelocytic leukemia (PML) protein was a tumor suppressor in a spectrum of cancers, including acute promyelocytic leukemia (APL) [101]. The really interesting new gene (RING) domain of PML has an overwhelming advantage in binding the m7G-cap of mRNAs to eIF4E by over 100-fold, thus reducing the affinity of eIF4E to m7G-cap [102]. The expression of genes, including cell cycle-related genes (such as cyclin D1), is significantly decreased, and the cell cycle was arrested in the G1/S phase [103]. Consequently, it might be declined that blocking eIF4E-mediated translation initiation may play an antioncogenic role.

Other m7G regulators

RNMT can play a specific role in PIK3CA mutant breast cancer to promote tumor growth and proliferation, yet not in PIK3CA wild-type breast cancer [104]. The transcription factor c-Myc/E2F3 was later demonstrated to participate in the positive regulation of RNMT [105]. In addition, there is positive feedback for RNMT recognizing the 5'-cap structure in its mRNA to promote stability and translation efficiency [55]. Besides, through B7-H6/c-Myc/RNMT pathway, the proliferation level of glioma stem cells is significantly increased [106]. Otherwise, WBSCR22 has been reported to induce hypermethylation phenotype in breast cancer, melanoma, and HCC [107, 108]. In colorectal cancer, it was demonstrated to be correlated with tumor stemness [109] and drug resistance [110]. However, the concrete regulatory mechanism has not been clarified. In lung cancer, NCBP1/cullin 4B (CUL4B) is significantly upregulated and is correlated with EMT, tumor cell invasion and migration [111].

To sum up, there have been plenty of reports on the role of METTL1/WDR4 and eIF4E as m7G regulators in cancer. Most experiments have proved that they exert a positive role in promoting tumor progression, except for lung cancer and colon cancer. However, more scientific work needs to be conducted for more cognition on other regulators.

Therapeutic strategy based on m7G

The role of m7G modification in cancer provides new perspectives into exploring effective therapeutic strategies for cancer treatment [112]. Although the importance of METTL1/WDR4 in regulating m7G modification and tumor malignancy is self-evident [112], seldom researches has been on developing METTL1/WDR4-targeted therapy in tumor treatment. However, scientists have constructed a 3D model of the METTL1/WDR4 heterodimeric complex, which is expected to facilitate an understanding of the crucial function residues and the development of bioactive inhibitors [75].

Since eIF4E directs RNA into the cytoplasm and initiates translation, blocking the binding between eIF4E and m7G may exert antioncogenic effects. The efficacy of eIF4E inhibitors has an "addictive" effect; that is, cancer cells with high eIF4E levels are more susceptible to eIF4E inhibitors [107]. This "addiction" effect has also been observed in other known oncogenes, such as Her2 and PI3K [112-114]. Therefore, an increasing number of studies are screening patients with high eIF4E expression for phase I/II clinical trials of eIF4E-specific antisense oligonucleotide (4EASO) in combination with conventional chemotherapeutic agents. The therapeutic effect of 4EAS0 drugs has been confirmed to promote apoptosis and cell cycle arrest in mesothelioma and prostate cancer [115, 116]. The application of 4EAS0 in non-small cell lung cancer significantly overcomes the cancer resistance to gemcitabine [117]. However, another type of 4EAS0, LY2275796, does not have significant antitumor efficacy in patients with stage IV tumors [118]. ISIS 183750 is a second-generation antisense oligonucleotide drug. Phase I/II clinical trials indicated that co-administration of ISIS 183750 in irinotecan-refractory colorectal cancer reduced the therapeutic dosage to 160 mg/m^2 twice weekly [119]. However, whether it can be further applied in clinical practice still needs more laboratory and clinical experiments.

Ribavirin, a commonly used antiviral drug, has been recently found to have antitumor effects as an m7G cap analog that inhibits cell proliferation [120]. The efficacy of ribavirin in AML also depends on the level of eIF4E expression. In tumors with high eIF4E levels (i.e., M4/M5 subtypes of AML), ribavirin has a significant impediment effect. In contrast, it has a limited impact on low-expressing cells (i.e., normal myeloid cells and M1/M2 AML) [112]. Six clinical trials have been conducted to explore the therapeutic effects of ribavirin in cancer treatment with or without combined adjuvant chemotherapy [121]. These clinical trials cover solid tumors, including advanced prostate cancer, head and neck squamous cell carcinoma, recurrent hepatocellular carcinoma, and hematologic malignancies such as AML and lymphoma (ClinicalTrials.gov). A phase II clinical trial in AML showed that ribavirin resulted in clinical remission in 3/11 patients, stabilization in 4/11 patients, and progression in 3/11 patients (NCT00559091). Another clinical trial showed that the combination of ribavirin and low-dose Ara-C was therapeutically effective, but patients eventually met unavoidable ribavirin

resistance [122]. Another mechanism of ribavirin is to prevent eIF4E binding to importin 8 and reduce eIF4E nuclear localization. Thus, the accumulated eIF4E in the cytoplasm is later transported back to the nucleus after withdrawal of the drug, making it a possible cause of disease relapse and recrudescence [123]. Therefore, combined inhibition of importin 8 may help to overcome tumor resistance [53]. Recently, studies have found that the active metabolite of ribavirin, ribavirin triphosphate (RTP), could bind eIF4E in a concentration-dependent manner, and its affinity is close to the upper limit of m7G. These studies show that RTP can re-associate to eIF4E at the cap-binding site [124]. In summary, ribavirin has potent anti-cancer and chemo-sensitizing effects. However, the clinical indications for ribavirin application and drug resistance are still urgent issues to be addressed [125].

Conclusion and perspective

RNA epigenetics is a heated spot in scientific research [126]. The m7G modification regulates oncogenes on the post-transcriptional and translational levels efficiently. Dysregulation of m7G modification contributes to tumor pathogenesis and progression. Attention to m7G modification leads to a deeper understanding of its biofunction and underlying molecular mechanism [127]. However, our knowledge of it is far from enough. The exact "erasers" for the m7G modification process remained a mystery. The mechanisms of m7G modification in tumors could be interpreted into two main aspects, one is METTL1/WDR4-mediated m7G modification within tRNAs and mRNAs, and another is eIF4E-related recognition of m7G-cap in the 5'-terminal on mRNAs. m7G-lncRNAs participate in the disease progression in gastric cancer, thyroid cancer, hepatocellular carcinoma, endometrial carcinoma and cutaneous melanoma [76, 128-130]. Unfortunately, the role of m7G modification in other RNAs and other m7G modification sites in cancer has few reports. Apart from regulating tumor cells, the impact of m7G modification on tumor microenvironment and tumor immunity is unveiled by multi-omics data [131, 132], yet the underlying mechanism requires further investigation. The function of m7G modification varies in tumors. Except for colon cancer and lung cancer, m7G modification generally promotes tumor progression. There are also several clinical studies that intend to investigate the antitumor effect of 4EASO and m7G-analogue, ribavirin. So far, most of them have achieved encouraging results, but also demonstrate the emergence of drug resistance issues. In the end, we look forward that more m7G-related drugs would be applied in cancer therapy practice and drug resistance would be conquered.

Acknowledgements

Kingdraw is used for mapping **Figure 1**. Servier Medical Art (http://smart.servier.com/) is used for drawing **Figure 3**, licensed under a Creative Common Attribution 3.0 Generic License (https://creativecommons.org/licenses/by/3.0/). The authors gratefully acknowledge the financial support by the National Natural Science Foundation of China (8220-3216), the Natural Science Foundation of Jiangsu Province (BK20220724) and Postgraduate Research & Practice Innovation Program of Jiangsu Province (SJCX22-0682).

Disclosure of conflict of interest

None.

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