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Running Title: Regulation of RNA modification in cancer

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ABSTRACT

Post-transcriptional regulation is an indispensable cellular mechanism of gene expression control that dictates various cellular functions and cell fate decisions. Recently, various chemical RNA modifications, termed the "epitranscriptome," have been proposed to play crucial roles in the regulation of post-transcriptional gene expression. To date, more than 170 RNA modifications have been identified in almost all types of RNA. As with DNA modification-mediated control of gene expression, regulation of gene expression via RNA modification is also accomplished by three groups of proteins: writers, readers, and erasers. Several emerging studies have revealed that dysregulation in RNA modification is closely associated with tumorigenesis. Notably, the molecular outcomes of specific RNA modifications often have opposite cellular consequences. In this review, we highlight the current progress in the elucidation of the mechanisms of cancer development due to chemical modifications of various RNA species.

INTRODUCTION

Over the past few decades, it has been established that genetic dysregulation underlies various human diseases. For example, genetic errors arising from gene amplification, deletion, mutation, or chromosomal translocation have been associated with numerous cancers (1-3). Meanwhile, increasing evidence suggests that epigenetic modifications of chromatin structure also affect tumor formation and cancer development via abnormal regulation of gene expression (4). Furthermore, the impairment of epigenetic regulation of oncogenes and tumor suppressor genes has been linked to several signaling pathways that lead to cancer development (5-7). Similarly, several recent studies have suggested an important role for RNA modifications, termed the "epitranscriptome", representing a new layer of post-transcriptional gene regulation (8, 9). Although several studies have investigated signaling pathways and transcriptional

regulation in cancer, relatively little is known about the post-transcriptional regulation of cancer. Therefore, a better understanding of the gene regulatory mechanisms controlling tumorigenesis and cancer development will facilitate their therapeutic exploitation (10, 11).

To date, more than 170 chemical RNA modifications have been identified, including *N6*-methyladenosine (m^6A), pseudouridine (ψ), *N1*-methyladenosine (m^1A), 2′-*O*-methylation (Nm), *N5*-methylcytosine (m^5C), and internal *N7*-methylguanosine (m^7G) (12, 13). Each of these modifications has been shown to have a preferential modification site within the mRNA. Similar to DNA methylation in epigenetics, three classes of RNA-binding proteins, broadly classified as writers, readers, and erasers, mediate the regulation of RNA modification (Fig. 1) (14). Writer proteins install the modification, while eraser proteins remove the modification, and reader proteins recognize the modification and regulate the metabolism of the target RNA. The discovery of both writer and eraser proteins indicates that many RNA modifications are likely reversible. Many recent studies have suggested that abnormally regulated RNA modification may lead to tumorigenesis and cancer development (2, 15, 16).

VARIOUS RNA MODIFICATIONS DRIVE TUMORIGENESIS

In this review, we focus on six internal RNA modifications most closely linked to tumorigenesis and discuss the RNA species in which they have been identified, their molecular mechanisms, and evidence of their involvement in cancer (Fig. 1).

N6-methyladenosine (m^6A)

N6-methyladenosine (m^6A) is one of the best-characterized RNA modifications whose importance has recently been highlighted in various biological studies. m^6A modification has been identified in several RNA species, including messenger RNA (mRNA), transfer RNA (tRNA), ribosomal RNA (rRNA), non-coding RNA (ncRNA), and viral RNA genomes (12, 17). It has been reported that m^6A modification is the most prevalent mRNA modification, and

approximately 25% of eukaryotic mRNAs harbor at least one m^6A modified base (8, 18). Although m^6A modification sites vary among different mRNA species and tissues, these sites have been shown to be generally located near translation stop codons in the 3′ untranslated region (UTR) of mRNA (8, 18, 19). However, a study showed that m^6A modifications in ncRNA are dispersed across the gene body without any apparent preferred location (20). Nevertheless, both coding and ncRNAs share a common set of m^6A writer protein complexes. Methyltransferase-like protein 3 (METTL3, also known as MT-A70), which catalyzes m^6A modification, forms a catalytic core complex with METTL14, which recognizes the DRACH motif (D = A, G, or U; R = G or A; and H = A, C, or U) (18, 21). Although several different components of the complex have been discovered, most reports consistently suggested that additional proteins, which lack methyltransferase activity, are required for proper m^6A modification (22-24). m^6A modification is known to be reversed by the action of m^6A eraser proteins. Unlike m^6A writer protein complexes, only two m^6A eraser proteins, fat mass and obesity-associated protein (FTO) and α-ketoglutarate-dependent dioxygenase alk B homolog 5 (ALKBH5), have been identified to date (25, 26). These two eraser proteins do not form a complex but are independently responsible for demethylation of m^6A (25, 26). While several previous studies have reported that FTO depletion leads to global demethylation of m^6A, more recent data indicate that FTO preferentially demethylates 2′-*O*-dimethyladenosine (m^6Am) or *N1*-methyladenosine (m^1A) in tRNA (27). In contrast, ALKBH5 is considered a better m^6A demethylase candidate as it recognizes the m^6A demethylation consensus sequence (26). The m^6A reader proteins have been studied more extensively. m^6A modification is recognized by proteins belonging to the YT521-B homology (YTH) domain family (YTHDF1, YTHDF2, and YTHDF3), YTH domain-containing proteins (YTHDC1 and YTHDC2), heterogeneous nuclear ribonucleoproteins (hnRNPC and hnRNPG), and insulin-like growth factor 2 mRNA-binding proteins (IGF2BP1, IGF2BP2, and IGF2BP3), which regulate almost all the steps of

mRNA metabolism, including splicing, export, translation, and stability (28-32). Interestingly, eukaryotic translation initiation factor 3 (eIF3) has also been shown to function as an m^6A reader protein by directly binding m^6A modification sites in the 5′ UTR of mRNA, resulting in the recruitment of the 43S complex and initiation of translation independently of the cap-binding protein, eIF4E (33-35).

Several studies have investigated the association between m^6A modification and cancer. These studies have revealed that the levels of m^6A mRNA modification in cancer cells are generally elevated and closely correlated with the development of several cancers (36-39). In colorectal cancer (CRC) and gastric cancer, for example, a high degree of m^6A modification is associated with mRNA stability (40, 41). m^6A reader proteins, IGF2BP1, IGF2BP2, and IGF2BP3, recognize m^6A modifications in oncogene mRNAs, preventing mRNA degradation and ultimately promoting cancer development (40, 41). In bladder cancer (BLC) and lung cancer, m^6A modification increases the translation efficiency of oncogenes without affecting mRNA abundance (34, 35, 42). Integrin alpha-6 (*ITGA6*) mRNA exhibits a high level of m^6A modification at the 3′ UTR of mRNA, which is recognized by the reader proteins, YTHDF1 and YTHDF3 in BLC, promoting the translation of *ITGA*6 mRNA (42). In lung cancer, METTL3 has been shown to function as both an m^6A writer and a reader protein. METTL3 recognizes m^6A modifications at the 3′ UTR of mRNAs near the translation stop codon and then interacts directly with eIF3 subunit h (eIF3h) at the 5′ end, promoting translation via ribosome recycling by forming an mRNA loop. Consequently, METTL3-dependent translation enhancement of a large subset of mRNAs triggers tumorigenesis in lung cancer (34, 35). Many studies have revealed a correlation between high levels of m^6A mRNA modifications and development of several cancers, including acute myeloid leukemia (AML), lung cancer, breast cancer (BrC), BLC, and glioblastoma (GBM) (36, 38, 39, 43). However, the m^6A reader proteins involved in specific cancers and their molecular mechanisms are yet to be elucidated.

Cancer development has also been reported to be influenced by m^{6}A modification of ncRNA (15, 44). MicroRNA (miRNA) m^{6}A modification can alter the abundance of miRNA in cells, which in turn regulates the stability of the target mRNA associated with tumorigenesis in BLC and hepatocellular carcinoma (HCC) (15, 44). Specifically, METTL3 has been shown to interact with the microprocessor protein, DGCR8, affecting primary-miRNA (pri-miRNA) processing in BLC (44). A study showed that the knockdown of METTL3 in BLC induces the accumulation of *pri-miR221/222*, whereas the overexpression of METTL3 increases the level of mature *miR221/222* (44). The study further showed that METTL3 accelerates the maturation of *pri-miR221/222*, resulting in the suppression of the transcription of a tumor suppressor gene, phosphatase and tensin homolog (*PTEN*) (44). In addition, m^{6}A modification of long intergenic non-coding RNA (lincRNA) has been implicated in cancer development. In HCC, METTL3-mediated m^{6}A modification increases *LINC00958* stability (15). Consequently, accumulation of *LINC00958* acts as an miRNA sponge by binding and inhibiting *miR-3619-5p*. The loss of *miR-3619-5p* enhances the expression of its target oncogene, hepatoma-derived growth factor (*HDGF*) mRNA, which in turn promotes lipogenesis, cell proliferation, and migration in HCC cells (15).

However, it has been reported that a high level of m^{6}A modifications may also be inhibitory in the same types of cancer, depending on the target mRNAs. Recently, low levels of m^{6}A modifications were identified in CRC and BLC (45-47). Mechanistically, the increase in m^{6}A modification induced by the overexpression of METTL14 is thought to lead to rapid YTHDF2-mediated mRNA degradation of the essential developmental transcription factor, SRY-related high-mobility-group box 4 (*SOX4*). Loss of SOX4 expression inhibits CRC malignancy via SOX4-mediated epithelial–mesenchymal transition (EMT) and PI3K/Akt signal transduction (45). In BLC, METTL14 expression is low, resulting in low m^{6}A modification levels. This, in turn, leads to increased mRNA stability of *Notch1*, which has been shown to play a crucial role

in cell proliferation, self-renewal, and metastasis of BLC (46).

The development of several cancers is also influenced by m^6A modification of ncRNA. In CRC, an oncogenic long non-coding RNA (lncRNA), the X inactive-specific transcript (*XIST*), has been shown to be highly methylated and degraded by YTHDF2 (47). Thus, m^6A-modification-mediated loss of *XIST* expression leads to the inhibition of cell proliferation, metastasis, and tumorigenesis (47).

Pseudouridine (ψ)

Pseudouridine (also known as 5-ribosyluracil or ψ) was first discovered in the early 1950s (48). Initially identified in tRNA and rRNA, pseudouridine has also been found in mRNA, lncRNA, and small nuclear RNA (snRNA) (49, 50). Pseudouridine is the most abundant RNA modification occurring in tRNAs and rRNAs (50). In tRNAs, pseudouridine is generally localized to the anticodon stem-loop in the D stem, and to the nucleotide position 55 in the T loop, and thus contributes to the stabilization of the tertiary structure of tRNA (51). Pseudouridines are generated post-transcriptionally via C5-ribosyl isomerization of one or a few target uridines catalyzed by pseudouridine synthases (PUSs) (52). PUSs employ two mechanisms of pseudouridine modification: guide RNA-dependent H/ACA box small nucleolar RNA (snoRNA) and guide RNA-independent pseudouridylation. In guide RNA-dependent pseudouridylation, the H/ACA box snoRNA forms a complex with dyskerin pseudouridine synthase 1 (DKC1), which recognizes specific sequences for pseudouridylation on target RNAs, including rRNA, snRNA, and snoRNA (53). In contrast, in guide RNA-independent pseudouridylation, modification of the target RNA is directly catalyzed by stand-alone PUSs (54). PUS enzymes are classified into six families: TruA, TruB, TruD, RluA, RsuA, and Pus10 (54). PUS1, which belongs to the TruA family, was originally thought to pseudouridylate tRNA alone; however, recent studies have identified that PUS1 also

pseudouridylates rRNA, snRNA, and mRNA (49, 50). In addition, PUS4 and PUS7 were found to target mRNAs for pseudouridylation in HEK293T cells (50).

Unlike other known RNA modifications, neither reader nor eraser proteins for pseudouridine have been identified to date. Moreover, the functional role of pseudouridylation in mRNA remains unclear.

Upregulation of pseudouridine has been shown to be associated with the progression of various cancers, including prostate cancer (PC), BrC, and HCC (55-58). The nucleolar protein DKC1 plays an important role in two separate cell proliferation pathways: the pseudouridylation of rRNAs, which is necessary for their processing, and the stabilization of the telomerase RNA component that is necessary for telomerase activity (55). Similarly, DKC1 expression is generally upregulated in PC (56, 59). The knockdown of DKC1 by siRNA has been shown to inhibit the proliferation of the e22Rv1, LNCaP, PC3, and Du145 in PC cell lines; however, the knockdown had no significant effects on apoptosis or senescence (56). Moreover, HCC patients with high DKC1 expression have been found to exhibit shorter overall survival rates when compared to those with low DKC1 expression (57). Moreover, elevated DKC1 expression has been shown to positively correlate with MYC oncogene expression, which triggers the expression of target genes to induce cell proliferation and cell survival. In addition, DKC1 expression was shown to induce MKI67 expression, which is considered a marker for cell proliferation (57, 58).

However, pseudouridine modification has also been shown to exert negative effects on the development of several types of cancer. Downregulated pseudouridine has been associated with BrC and HCC development (60, 61). The impairment of DKC1 protein can lead to the inactivation of p53, a well-known anti-tumor development factor that induces cell cycle arrest or apoptosis, due to abnormal *p53* mRNA translation (60). However, low expression of a snoRNA, *SNORA24*, has been associated with poor survival of patients with steatohepatitic

HCC (61). As *SNORA24* is known to mediate the psedouridylation of 18S rRNA, the depletion of *SNORA24* increases the rate of translational miscoding and stop codon read-through frequencies in human HCC (61).

N1-methyladenosine (m^1A)

N1-methyladenosine (m^1A) was first identified in yeast $tRNA^{Phe}$ and later found to be widespread in tRNAs (62, 63). In human cells, the methyltransferases TRMT10C, TRMT61B, and TRMT6/61A catalyze m^1A at positions 9 and 58 of mitochondrial and cytoplasmic tRNA, respectively (63). m^1A modification of tRNA plays a pivotal role in maintaining its proper structure, stability, and function (64-67). Although m^1A modification is abundant in tRNA, relatively low levels of modification, ranging from 0.015% to 0.054%, have been found in mammalian mRNA (68). The majority of m^1A modification sites in mRNA are located in the 5′ UTR near the translation start codon (68). TRMT6/61A has been found to localize in the cytoplasm, and contributes to m^1A modification in select mRNAs that harbor a tRNA-like motif, GUUCRA (63). As the TRMT6/61A complex seems to play a minor role in m^1A mRNA modification, other m^1A methyltransferases likely remain to be discovered (63).

Similar to m^6A modification, m^1A modification is reversible. Demethylation of m^1A modification is mediated by ALKBH1 and ALKBH3 (65, 66). Interestingly, the m^6A reader proteins, YTHDF2 and YTHDF3, have also been implicated in the recognition of m^1A mRNA modifications (16, 69). Reminiscent of m^6A modification, m^1A-modified mRNA undergoes rapid degradation upon binding with YTHDF2 or YTHDF3 (16, 69).

To date, several studies have reported a positive correlation between m^1A tRNA modification and cancer development. Elevated expression levels of both TRMT6/61A and initiator methionine tRNA ($tRNA_i^{Met}$) have been detected in highly aggressive GBM compared with grade II/III gliomas (67). Depletion of the TRMT6/61A complex suppresses proliferation and

promotes cell death in C6 glioma cells, which can be rescued in part by $tRNA_i^{Met}$ overexpression (67). Conversely, the ectopic expression of TRMT6/61A has been shown to upregulate the translation of oncogenic mRNAs, leading to increased colonization of C6 glioma cells (67). Similarly, ALKBH1-mediated demethylation of m^1A modified tRNA attenuates translation initiation and elongation in HeLa cells, thereby reducing cell proliferation (66). Intriguingly, another m^1A demethylase, ALKBH3, has been shown to induce an opposite effect to that of ALKBH1 in m^1A tRNA modification (70). ALKBH3-mediated m^1A tRNA demethylation increases the susceptibility of tRNA to angiogenin cleavage and generates tRNA-derived small RNAs in various cancer cells during cancer cell proliferation, migration, and invasion (70).

2'-*O*-methylation (Nm)

A rather unique RNA modification is mediated via 2′-*O*-methylation (also known as Nm or 2′-*O*-me). Nm can occur in any nucleotide via the addition of a methyl group to the 2′ hydroxyl of the ribose moiety (71). Nm is an abundant RNA modification that occurs in multiple RNA species, including tRNA, rRNA, snRNA, and mRNA (71-73). Nm modification sites in mRNA are mostly located within the coding sequence (CDS) regions (73). Interestingly, Nm modification is also found in approximately 16.2% of introns, suggesting that Nm modification occurs in the nucleus prior to splicing events (73). In higher eukaryotes, Nm modification occurs specifically in the first nucleotide [N1] and/or second nucleotide [N2] next to the m^7G-cap of mRNA (74). While N1 methylation occurs in almost all mRNA molecules, N2 methylation accounts for about half of the capped mRNA. Additional m^6A methylation following Nm leads to m^6Am formation. Two conserved Nm writer proteins have been discovered for N1 and N2 methylation. FTSJ2 (also known as hMTr1) is responsible for the methylation of N1, whereas FTSJ1 (also known as hMTr2) contributes to N2 methylation (75,

76). FTSJ2 also methylates mitochondrial 23S rRNA (77). Another Nm writer protein, fibrillarin (FBL), is an essential nucleolar protein that catalyzes the Nm of rRNA by interacting with a C/D box family member of U3 snoRNA that mediates the processing of precursor rRNA (74). Given that more than 70% of all Nm occurs in the CDS regions of mRNA and that known Nm writers have not been associated with CDS methylation, further studies are needed to identify additional Nm methyltransferases. Nm readers and erasers also remain unknown.

Many studies suggest that FBL expression is abnormally high in various cancers, including PC and BrC (78-80). One report suggested that a p53 mutation fails to suppress transcription of the *FBL* gene, resulting in the overexpression of FBL (80). Another report suggested that the MYC protein directly regulates the transcription of *FBL* by binding to the promoter region of this gene (78). The elevated level of FBL expression has been shown to be associated with a higher transcriptional activity of RNA polymerase I, thus increasing rRNA production (74, 80). As increased rRNA biogenesis has been observed in various cancer cells, it is not surprising that many cancers appear to be related to FBL-mediated rRNA production. Indeed, depletion of FBL has been shown to significantly inhibit tumorigenesis in PC and BrC (79, 80). In contrast, increased FBL expression not only induces high levels of rRNA synthesis, but also promotes rRNA Nm that regulates ribosomal quality (74, 80). Altered rRNA Nm patterns have been shown to be associated with a decrease in translational fidelity (*i.e.*, increased nonsense suppression, frame-shifts, and mis-incorporation) and increased internal ribosome entry site (IRES)-dependent translation initiation of key cancer genes (74, 80). In case of another Nm methyltransferase, only one report indicated that the increased expression of FTSJ2 was positively correlated with cell proliferation in lung cancer cell lines (77). To date, a negative correlation between Nm and cancer has not been reported. Furthermore, since reversible Nm has not been reported, it may be important for cells to maintain Nm levels below the cancer initiation threshold.

_N5_-methylcytosine (m^5C)

_N5_-methylcytosine was originally found in ncRNA, including tRNA and rRNA, and subsequently in most RNA species (81, 82). More than 10,000 m^5C modification sites have been discovered in human mRNA and ncRNA (81). m^5C modifications have been found to be enriched in the 5′ UTRs and 3′ UTRs of mRNA (81). Recently, a study reported that m^5C modifications are enriched in CG-rich regions and immediately downstream of translation initiation regions of mRNA in multiple mice tissues (82). In rRNA, m^5C modification sites have been shown to be fairly conserved, and both the small and large subunits of rRNA in bacteria and eukaryotes harbor m^5C methylation sites at similar positions (83). Although several m^5C locations have been identified in human 28S rRNA, the functional roles of these sites remain unclear (83). Several m^5C modification writer proteins have been identified, including members of the NOL1/NOP2/Sun (NSUN)-domain-containing family (from NSUN1 to NSUN7) and DNA methyltransferase 2 (DNMT2) families (84). Initially, NSUN2 was thought to be responsible for m^5C modification of tRNA, but was later found to also methylate many RNA species, including tRNA, mRNA, and miRNA (81, 82, 85). tRNA methyltransferase 1 (TRDMT1), a member of the DNMT2 family, is primarily responsible for the m^5C modification of tRNA and mRNA (86). tRNA m^5C methylation mediated by either TRDMT1 or NSUN2 increases tRNA stability and protein synthesis (87). Other members of the NSUN family have been associated with m^5C modification in enhancer RNA, rRNA, mitochondrial tRNA (mt-tRNA), or mt-rRNA (88). In the two protein families, NSUN2 and DNMT2 are known to be associated with malignant cancer. Although an m^5C eraser protein has not been identified, Aly/REF, an mRNA export adapter protein complex, has been found to act as a novel m^5C reader protein that methylates mRNA in CG-rich sequences and in translation initiation regions (82). A recent study identified Y-box-binding protein 1 (YBX1) as

a reader protein that recognizes m^5C modification and maintains the stability of the target mRNA (89).

A few studies have investigated the effects of m^5C on tumorigenesis. It has been identified that NSUN2 protein is a downstream target of MYC that methylates RNA polymerase III transcripts (85). Elevated expression of NSUN2 has been shown to mediate MYC-induced cell proliferation and growth in squamous cell carcinoma and BLC (85, 89). Moreover, *HDGF* mRNA has been reported to undergo m^5C modification in the 3' UTR and is stabilized by YBX1, increasing HDGF protein synthesis (89). This finding was consistent with those of another study showing that reduced survival of BLC patients was correlated with increased expression of NSUN2 and HDGF (89). Another m^5C methyltransferase, TRDMT1, is also correlated with cell proliferation and migration and gene ontology (GO) analyses have shown that the depletion of TRDMT1 leads to altered expression of genes associated with the regulation of the cell cycle, RNA transport, and RNA degradation (90).

While few studies have reported a correlation between m^5C modification and cancer, it will be informative to consider the effects of m^5C on the regulation of translation, given that m^5C modifications occur mainly in 5' or 3' UTRs.

Internal *N7*-methylguanosine (m^7G)

N7-methylguanosine (m^7G) is the best-known modification in the form of mRNA 5′ cap (91). Recently, an internal m^7G modification was also identified in rRNAs, tRNAs, miRNAs, and mRNAs (92-94). m^7G modification is found at the nucleotide position 46 in several tRNA variable loops known to stabilize the tRNA tertiary structure (95, 96). In mammalian cells, the internal m^7G/G ratio of mRNA ranges from 0.02% to 0.05% (94). Internal m^7G modification has been found to be preferentially enriched in either AG-rich or GA-rich regions in mRNA (94, 95) and is mediated by METTL1 and its co-factor, WD repeat domain 4 (WDR4) in mRNA,

miRNA, and tRNA (6, 96). Specifically, the knockdown of METTL1 has been shown to lead to a global decrease in internal m^7G modification by approximately 54% and 61% in HeLa cells and HepG2 cells, respectively (94). To date, no readers or erasers for m^7G modification have been identified.

A positive association between internal m^7G and cancer has yet to be identified. However, internal m^7G modification of primary miRNA transcripts has been shown to have negative effects on lung cancer and colon cancer development. Specifically, the *pri-let-7e* transcript has been found to be targeted by METTL1 during internal m^7G modification and generates a mature form of *let-7e* via efficient processing (93, 97). The increased expression of mature *let-7e* downregulates its target, the high-mobility group AT-hook 2 (*HMGA2*) mRNA, which is known to drive cancer metastasis (93, 97). The association with m^7G modification is unclear; however, the m^7G writer protein, METTL1, itself inhibits the PTEN signaling pathway in HCC to promote cell proliferation and migration, resulting in tumor growth and greater tumor vascular invasion (6).

DISCUSSION

Given the plethora of signaling pathways converging upon gene expression regulatory pathways to satisfy the increased anabolic demands in cancer, a better understanding of the gene regulatory mechanisms controlling tumorigenesis will facilitate their therapeutic exploitation (5, 6, 11). The field of epitranscriptomics has attracted the attention of various biological investigators in recent years; however, the molecular players and mechanisms underlying epitranscriptome regulation remain to be elucidated. In particular, several studies on the relationship between RNA modification and cancer have been published, many of which either lack data regarding the detailed mechanisms involved or often report contrasting results. For instance, pseudouridine, m^1A, Nm, and internal m^7G modifications have been found in

mRNAs across the gene body; however, their roles in mRNA metabolism and their effects on cancer progression remain unknown (49, 65, 94, 95). Instead, most published studies have merely investigated the effects of the enzymes catalyzing the modifications on cancers, without confirming the effects of RNA modification. With the exception of m^6A and m^1A, the reversibility of RNA modifications and their specific reader proteins also remain unclear. Moreover, the molecular functions and cellular consequences of m^6A or pseudouridine modification often differ across studies, depending on the degree of methylation in the specific target RNAs (Fig. 1).

In addition to the extensive interest in the roles of RNA modification in cancer, the development of potential drugs to treat cancer by modulating RNA modification has also attracted attention (98). However, there are currently no inhibitors or antagonists targeting writer and reader proteins, or the RNA modifications discussed in this review (98). Instead, several inhibitors of demethylases have been suggested. The ALKBH family and FTO share a common structure required for the binding of Fe^{2+} as a co-factor and 2-oxoglutarate (2OG) as a co-substrate (98). Therefore, most of the known compounds, including 2OG competitors such as N-oxalylglycine and its cell-penetrating derivative dimethyl oxalylglycine, succinate, fumarate, and 2-hydroxyglutarate,or metal chelators such as hydroxamic acids and flavonoids, were designed to target either Fe^{2+} or 2OG binding sites (98, 99). However, these compounds are still far from being used in anticancer drugs because they nonspecifically inhibit various demethylases. Some specific demethylase inhibitors have also been discovered, such as the ALKBH3 inhibitor, 1-(5-methyl-1H-benzimidazol-2-yl)-4-benzyl-3-methyl-1H-pyrazol-5-ol (HUHS015), and FTO inhibitor, Rhein. However, the efficacy of these drugs remains doubtful because of their nonspecificity for target RNAs (98, 99). More recently, clustered regularly interspaced short palindromic repeats (CRISPR) based RNA-editing technology has been suggested for modulating target mRNA specific modifications (100). The CRISPR-associated

nuclease Cas13 has been shown to cleave the targeted single-stranded RNA (100). A catalytically inactive mutant of Cas13 (dCas13) fused with m^6A methyltransferases METTL3 or METTL14 has been found to bind to the target mRNA specifically directed by the guide RNA, without the cleavage of the mRNA (100). Targeting of these fusion proteins has been shown to specifically methylate adenosine within a small range of target sites, regardless of the m^6A consensus sequence (100). Thus, CRISPR-based approaches can be applied not only in the modulation of m^6A modifications, but also to other types of RNA modifications. Given the abnormal up/downregulation of modified RNA transcripts and their regulatory proteins in cancer, the development of this technology will shed more light on the feasibility of controlling the modification of RNA targets for cancer treatment. Taken together, the analysis presented in this review highlights the need to further elucidate the mechanisms of RNA modification-mediated regulation of gene expression to improve cancer therapy.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

FIGURE LEGENDS

Figure 1. Role of RNA modification in cancer. Six representative cancer-associated RNA modifications are listed with their chemical structures, molecular functions, target RNA species, and binding proteins. Positive or negative regulation indicates the correlation between each

RNA modification and the corresponding cancer progression. Blue boxes indicate general information about each RNA modification regardless of cancer association, while the yellow boxes represent information about RNA modification dependent-cancer regulation. NR, not reported.

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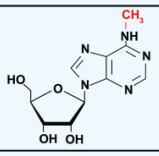
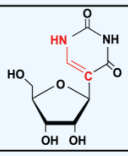
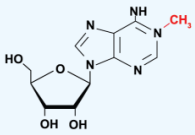
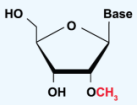
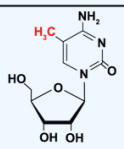
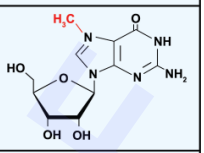
Modification		m^6A	Ψ	m^1A	Nm	m^5C	m^7G
Structure							
Writer		METTL3/METTL14	PUSs(1-10), DKC1	TRMT6/TRMT61A	FTSJ1, FTSJ2, FBL	NSUNs(1-7), TRDMT1	METTL1/WDR4
Eraser		FTO, ALKBH5	NR	ALKBH1, ALKBH3	NR	NR	NR
Reader		YTHDFs(1-3), YTHDCs(1, 2) IGF2BPs(1-3), hnRNPC hnRNPG, eIF3, METTL3	NR	YTHDF2, YTHDF3	NR	Aly/REF, YBX1	NR
Molecular function	mRNA	Splicing, Export, Translation, Stability	NR	Stability	NR	Export, Stability	NR
	ncRNA	Processing, Stability	Processing, Stability	Processing	Translation	Stability	Processing
RNA species		mRNAs (34-43, 45, 46), *miR221/222* miRNA (44), *LINC00958* lincRNA (16), *XIST* lncRNA (47)	18S rRNA (61)	$tRNA_i^{Met}$ (67), tRNA-derived small RNAs (70)	rRNAs (80)	*HDGF* mRNA (89)	*let-7e* miRNA (93, 97)
Positive regulation		HCC (16), Lung Cancer (34, 35), BLC (42, 44)	BrC (55), PC (56, 59), HCC (57)	GBM (67)	PC (78), BrC (79, 80)	Squamous-Cell Carcinoma (85), BLC (89)	HCC (6)
Negative regulation		CRC (45, 47), BLC (46)	BrC (60), HCC (61)	HCC, PC (70)	NR	NR	Lung Cancer (93), Colon Cancer (97)

Fig. 1.