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REVIEW ARTICLE

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Recent advances in noncoding RNA modifications of gastrointestinal cancer

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Abstract

Elucidating the mechanisms underlying cancer development and proliferation is important for the development of therapeutic methods for the complete cure of cancer. In particular, the identification of diagnostic markers for early detection and new therapeutic strategies for refractory gastrointestinal cancers are needed. Various abnormal phenomena occur in cancer cells, such as functional changes of proteins, led by genomic mutations, and changes in gene expression due to dysregulation of epigenetic regulation. This is no exception for noncoding RNA (ncRNA), which do not encode proteins. Recent reports have revealed that microRNA (miRNA), long noncoding RNA (lncRNA), and circular RNA (circRNA) are deeply involved in cancer progression. These ncRNAs have attracted attention as gene expression regulatory molecules. Recent advances in technology have made it possible not only to read DNA and RNA sequences but also to study the modification state of each base. In particular, comprehensive analysis of N6-methyladenosine (m^6A) has been performed by many research groups, with multiple studies reporting that m^6A modifications of specific genes are associated with cancer progression. Based on the above, this review examines how ncRNA modifications are related to cancer progression in gastrointestinal cancers such as colorectal and pancreatic cancer. We also discuss enzyme inhibitors that have been reported to have drug discovery potential targeting m^6A modifications. By utilizing the new perspective of

Abbreviations: 5FU, 5-fluorouracil; AML, acute myeloid leukemia; ceRNA, competing endogenous RNA; circRNA, circular RNA; CRC, colorectal cancer; EMT, epithelial-to-mesenchymal transition; GC, gastric cancer; GISTs, gastrointestinal stromal tumors; lncRNA, long noncoding RNA; m^1A, N1-methyladenine; m^5C, 5-methylcytosine; m^6A, N6-methyladenosine; MeRIP-seq, methylated RNA immunoprecipitation sequencing; miRNA, microRNA; NFIC, nuclear factor I-C; PC, pancreatic cancer; PGF, placental growth factor; SNPs, single-nucleotide polymorphisms; TAMs, tumor-associated macrophages.

[Correction added on 7 November 2024, after first online publication. The affiliation of the author Toru Kitagawa has been corrected in this version.]

ncRNA modification, we may be able to accumulate knowledge on the molecular biology of cancer and contribute to human health through diagnosis and treatment.

KEYWORDS
circRNA, gastrointestinal cancer, lncRNA, miRNA, RNA modifications

1 | BACKGROUND

Gastrointestinal cancers have become an important issue in medicine due to their high incidence estimated to affect 1 in 12 people worldwide and refractory nature.[1] In particular, colorectal cancers (CRCs) and pancreatic cancers (PCs) tend to be diagnosed late, and some cases are difficult to operate on, thus limiting treatment. Recently, it has become clear that noncoding RNA (ncRNA) plays an important role in understanding cancer pathology.[2] ncRNAs are classified into various classes, including microRNA (miRNA), long noncoding RNA (lncRNA), and circular RNA (circRNA) and function as the regulators of gene expression and cellular physiological processes. A variety of RNA modifications, particularly N6-methyladenosine (m^6A), have been shown to affect gene expression and are involved in cancer progression.[3,4] The m^6A modification is deposited onto RNAs cotranscriptionally via the writer complex whose main components involve methyltransferase-like 3 (METTL3), methyltransferase-like (METTL16), and Wilms' tumor 1-associating protein (WTAP), which together recognize and bind sites sitting at the consensus motif RR[m^6A]CH (R = A, G; H = A, T, C). These methylation marks are subsequently recognized and bound by protein families referred to as "readers," the most prominent being the highly conserved YTHDF family but also including the families YTHDC, IGF2BP, and HNRNP. Reader proteins confer m^6A function by leading to stabilization or degradation of m^6A-modified RNA and greatly affecting translation.[5] Additionally, m^6A is erased via demethylation by the erasers fat mass and obesity-related protein (FTO) and AlkB homolog 5 (ALKBH5).[6] Since METTL3 expression is estimated to be upregulated by MYC, a proto-oncogene, METTL3 has a greater impact on m^6A modification in cancer cells.[7] Another abundant mark which is less well characterized is the modification 5-methylcytosine (m^5C), which is enriched on tRNAs but can significantly influence mRNA stability and translation.[8] Deposition of m^5C to mRNA is facilitated by the NSUN protein family, particularly NOP2/SUN RNA methyltransferase 2 (NSUN2). Furthermore, in recent years it has become clear that ncRNAs are also affected by RNA modification, and their roles in diseases such as cancer are just emerging. In this review, we focus on modifications which occur on ncRNA and their functions in gastrointestinal cancers. In particular, we explore how these ncRNAs are involved in cancer progression, metastasis, and resistance and suggest new perspectives in therapeutic development. In addition, we discuss the impact of ncRNA modifications on the pathobiology of cancer. As the functions of various ncRNAs have been revealed in recent years, it has become essential to understand the functions of ncRNAs, including their modification states, in order to overcome the limitations of current therapies for gastrointestinal cancers and to seek more effective treatments. This review focuses on this point and summarizes recent reports on ncRNA modifications.

2 | MicroRNA MODIFICATION

MicroRNAs (miRNAs) are short ncRNAs consisting of approximately 22 bases and are involved in the regulation of gene expression by affecting mRNA translation and stability through binding to target mRNAs. Some miRNAs function as markers of cell types. In cancer cells, some miRNAs can function in a cancer growth-promoting manner, while others function in a cancer growth-suppressing manner, in particular by modulating the expression of oncogenes or tumor suppressors, and research and development of miRNAs as therapeutic and diagnostic markers is underway.[9] miRNAs function primarily in the cytoplasm but are expressed as precursors and undergo processing in the nucleus.[10] Interestingly, a number of them were found to undergo m^6A modification during the process. SNORD11B, one of the small nucleolar RNAs (snoRNAs), is highly expressed in CRC, and 2'-O-methylated (Nm) modification of pri-let-7a suppresses let-7a-5p expression and promotes cancer growth. SNORD11B was a diagnostic marker comparable to CEA and CA19-9.[11] The miRNA pri-miR-146b is m^6A modified by METTL3 and binds to HNRNPA2B1, and its knockdown was found to promote CRC growth. This knockdown enhanced PD-L1 expression through the p110β/PI3K/AKT pathway in tumor-associated macrophages (TAMs) and enhanced the efficacy of anti-PD-1 immunotherapy.[12] Another example is pri-miR-17, which is m^6A modified by METTL14 and its expression is repressed by binding of YTHDC2. METTL14 expression is repressed in CRC. When METTL14 expression is decreased, the m^6A of pri-miR-17 is reduced and the expression of pre-miR-17 and miR-17-5p is increased by suppressing YTHDC2 binding. Suppression of MFN2, a target gene of miR-17-5p, results in 5-fluorouracil (5FU) resistance by reducing mitochondrial fusion and promoting mitochondrial fission and mitophagy.[13] METTL3 is overexpressed in CRC, and knockdown of METTL3 suppresses CRC proliferation. METTL3 upregulates miR-196b expression by modifying pri-miR-196b with m^6A and promotes metastasis in cancer cells.[14] METTL3 is induced by *Fusobacterium nucleatum* in CRC, and m^6A modification of pri-miRNA-4717 increases miR-4717 expression, resulting in cell proliferation by repressing MAP2K4.[15] METTL3 modifies pri-miR-1246 with m^6A and upregulates miR-1246 expression, thereby repressing SPRED2, a target of miR-1246,

and activating the MAPK pathway, which promotes CRC invasion and EMT.[16] pre-miR-1184 is upregulated by m^{6}A modification by METTL3, and miR-1184 generated from it promotes gastric cancer (GC) growth by upregulating TRPM2 expression.[17] METTL3 modifies pri-miR-17-92 with m^{6}A and stabilizes it by binding the reader, DGCR8, resulting in increased miR-17-92 expression. miR-17-92 activates the AKT/mTOR pathway by repressing PTEN or TMEM127, promoting GC growth and metastasis.[18] miR-675 is highly expressed in gastrointestinal stromal tumors (GISTs) and has a poor prognosis. The m^{6}A modification of microRNA-675 by METTL3 stabilizes miR-675, which inhibits the tumor-initiating RhoA/NF2/YAP1 signal pathway by suppressing myosin phosphatase-targeting protein 1 (MYPT1), thereby promoting cancer growth.[19] Overexpression of METTL3 upregulates the expression of pre-miR-25, which in turn upregulates the expression of miR-25-3p, promoting the proliferation of GISTs.[20] miR-380-3p is upregulated in PC and is m^{6}A modified. Knockdown of METTL3 and METTL14 decreases miR-380-3p expression, indicating that miR-380-3p is stabilized by m^{6}A modification. Overexpression of miR-380-3p promotes cancer growth. miR-380-3p targets PTEN, suggesting that suppression of PTEN activates the AKT pathway and promotes PC growth.[21] Smoking causes hypomethylation of METTL3, promotes binding of the transcription factor NFIC, and increases METTL3 expression. The upregulation of miR-25-3p by m^{6}A modification of pri-miR-25 and the repression of its target PHLPP2 activates AKT-p70S6K signaling and promotes PC progression.[22] As described above, there are RNA-modified miRNA precursors that promote cancer progression mainly by MAPK, AKT, and mTOR signaling, and those that inhibit cancer progression by suppressing the PD-L1 pathway (Figure 1). The modifications of these miRNAs are summarized in Table 1. Thus, abnormal m^{6}A modifications of miRNA precursors affect cancer progression. Studies of miRNA modifications provide an important foundation for understanding the pathobiology of cancer and for the development of new therapeutic strategies, and it is expected that further progress will be made in their elucidation.

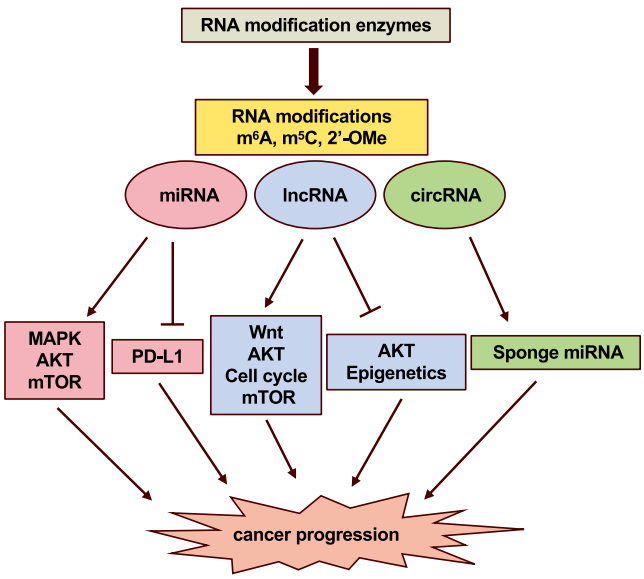

FIGURE 1 Functional overview of RNA modifications. Some modifications of microRNAs (miRNAs), long noncoding RNAs (lncRNAs), and circular RNAs (circRNAs) activate and others inactivate signals that promote cancer progression.

3 | LONG NONCODING RNA MODIFICATIONS

Long noncoding RNAs (lncRNAs) are transcripts longer than 200 bases and are functionally diverse RNA molecules involved in a variety of biological processes in the cell, including transcriptional regulation, chromosomal structural regulation, epigenetic modification, and protein function regulation.[23] Therefore, modifications of lncRNAs are presumed to have a significant impact on their biological functions and behavior within the cell. Recent studies have shown that lncRNA modifications are involved in cancer progression and resistance to therapy. Specific examples are noted in the following sections.

3.1 | m^{6}A-modified lncRNA in CRC

The lncRNA POU6F2-AS1 is highly expressed in CRC and is associated with CRC prognosis. POU6F2-AS1 is stabilized and upregulated by IGF2BP2 binding upon m^{6}A modification by METTL3. The transcriptional factor YBX1 was also shown to bind to the FASN promoter, and the interaction between YBX1 and POU6F2-AS1 promotes expression of FASN to promote CRC growth and lipogenesis.[24] lncRNA GHRLOS inhibits CRC growth by promoting KDM5D expression, but m^{6}A modification by ZCCH4, an m^{6}A methyltransferase, decreases lncRNA GHRLOS expression and promotes CRC growth.[25] In addition, linc00659 is highly expressed in CRC and knockdown of linc00659 suppresses cancer growth. Knockdown of linc00659 or IGF2BP1 suppresses FZD6 expression, suggesting that IGF2BP1 binds to the m^{6}A modification of linc00659 and promotes FZD6 expression and activation of the Wnt/β-catenin signaling pathway.[26] Small nucleolar RNA host gene 1 (SNHG1), a kind of lncRNA, is stabilized and upregulated by m^{6}A.[27] LINC02038 is a tumor suppressor gene whose overexpression suppresses cancer cell growth. By sponging miR-552-5p, LINC02038 increases FAM172A expression and suppresses the PI3K/AKT pathway. LINC02038 is m^{6}A modified by METTL3, and YTHDF2 binding to that site promotes its degradation.[28] HNF1A-AS1 sponges miR-93-5p and upregulates CCND1 expression. HNF1A-AS1 is stabilized by binding of IGF2BP2 upon m^{6}A modification by METTL3. HNF1A-AS1 activates the PI3K/AKT pathway and upregulates CCND1 expression by suppressing PDCD4 expression.[29] LINC01559 is poorly expressed in CRC, and its low expression is associated with a poor prognosis. LINC01559 is stabilized by METTL3-mediated m^{6}A modification and sponges miR-106-5p, which increases the expression of PTEN, a target of miR-106-5p, and promotes cancer growth.[30] ZFAS1 binds to NOP58 and promotes ribosomal RNA 2′-O-methylation by mediating the binding of NOP58

TABLE 1 Modifications of miRNA.

Tumor	Gene name	Writer	Reader	Modification	Expression	miRNA target	Modification effect on tumor	References
CRC	pri-let-7a	SNORD11B	DGCR8	2'-O-methylation	Down	N/A	Promote proliferation	11
CRC	pri-miR-146b	METTL3	HNRNPA2B1	m6A	Up	Suppress PD-L1 expression by the p110β/PI3K/AKT pathway in TAMs	Suppress proliferation	12
CRC	pri-miR-17	METTL14	YTHDC2	m6A	Down	MFN2 downregulation	Suppress 5FU resistance	13
CRC	pri-miR-196b	METTL3	N/A	m6A	Up	N/A	Promote metastasis	14
CRC	pri-miRNA-4717	METTL3	N/A	m6A	Up	MAP2K4 downregulation	Promote proliferation	15
CRC	pri-miR-1246	METTL3	N/A	m6A	Up	Activate the MAPK pathway by SPRED2 downregulation	Promotes invasion and EMT	16
GC	pre-miR-1184	METTL3	N/A	m6A	Up	TRPM2 upregulation	Promote proliferation and EMT	17
GC	pri-miR-17-92	METTL3	DGCR8	m6A	Up	Activate the AKT/mTOR pathway by PTEN or TMEM127 downregulation	Promote proliferation and metastasis	18
GISTs	miR-675	METTL3	N/A	m6A	Up	Activate the RhoA/NF2/YAP1 pathway by MYPT1 inhibition	Promote proliferation	19
GISTs	pre-miR-25	METTL3	N/A	m6A	Up	N/A	Promote proliferation	20
PC	pre-miR-380-3p	METTL3	N/A	m6A	Up	Activate the AKT pathway by PTEN downregulation	Promote proliferation	21
PC	pri-miR-25	METTL3	NKAP/DGCR8	m6A	Up	Activate the AKT-p70S6K pathway by PHLPP2 downregulation	Promote proliferation	22

Abbreviations: CRC, colorectal cancer; GC, gastric cancer; GISTs, gastrointestinal stromal tumors; miRNA, microRNA; PC, pancreatic cancer, TAMs, tumor-associated macrophages.

to SNORD12C and SNORD78 RNAs, and promotes cancer growth by increasing the translation of LAMC2 and EIF4A3.[31]

3.2 | m^{6}A-modified lncRNA in GC

Long noncoding RNA AGAP2-AS1 promotes WTAP/METTL3/METTL14 complex formation by binding to WTAP. STAT3 mRNA m^{6}A modification increases STAT3 expression, activates the interleukin 6 (IL6)/STAT3 pathway, and promotes GC growth.[32] LINC00355 is highly expressed in GC and promotes the expression of HNRNPA2B1. HNRNPA2B1 binds to m^{6}A of CDC42 and promotes CDC42 expression. LINC00355 promotes CDC42 transcription by binding to p300 histone acetyltransferase and activating the CDC42 promoter. Knockdown of LINC00355 suppresses tumor growth, suggesting that LINC00355 has a cancer growth-promoting function.[33] OIP5-AS1 is highly expressed in GC. IGF2BP3 binds to m^{6}A-modified OIP5-AS1 and binds to hnRNPA1, preventing binding of hnRNPA1 to TRIM21. This prevents hnRNPA1 degradation mediated by TRIM21 ubiquitination, and high expression of hnRNPA1 increases PKM2 expression promoting cancer growth. Knockdown of OIP5-AS1 suppressed tumor growth.[34] lncRNA SNHG3 is upregulated by m^{6}A modification by METTL3 and sponges miR-186-5p, promoting cyclinD2 expression.[35] High expression of DIAPH2-AS1 promotes migration, invasion, and neural invasion potential. DIAPH2-AS1 binds NSUN2 and enhances NTN1 mRNA stability via m^{5}C modification of NTN1 mRNA and induces neural invasion of GC.[36] The expression of lncRNA TP53TG1 is low in GC, and its low expression is associated with poor prognosis. TP53TG1 is a tumor suppressor gene that inhibits cancer growth and metastasis. TP53TG1 has an m^{6}A modification site. Demethylation by ALKBH5 reduces the stability of TP53TG1 and suppresses its expression. Knockdown of ALKBH5 increases TP53TG1 m^{6}A and TP53TG1 expression. TP53TG1 suppresses the PI3K/AKT pathway by binding to protein phosphatase 2A (CIP2A).[37] THAP7-AS1, stabilized and upregulated by binding of IGF2BP1 through m^{6}A modification by METTL3 in GC, is transcribed by the transcription factor SP1. THAP7-AS1 binds to CUL4B and importin α1 in the cytoplasm and promotes nuclear translocation of CUL4B. The complex of THAP7-AS1 and CUL4B binds to the promoter regions of miR-22-3p and miR-320a, thereby repressing their expression and promoting cancer growth by activating PI3K/AKT signaling.[38] High expression of LINC00958 promotes aerobic glycolysis and has a poor prognosis in GC. The m^{6}A methyltransferase KIAA1429 modifies LINC00958 with m^{6}A. m^{6}A-modified LINC00958 stabilizes GLUT1 mRNA and promotes cancer growth by increasing GLUT1 expression.[39] High expression of LINC01320 in GC has a poor prognosis. METTL14-mediated m^{6}A modification and upregulation of LINC01320 and sponging of miR-495-5p promote cancer growth by upregulating the expression of RAB19, a target of miR-495-5p.[40] NEAT1, an lncRNA, is repressed by m^{6}A modification, but when m^{6}A modification is repressed by demethylase, ALKBH5, its expression increases and contributes to cancer invasion and metastasis by promoting EZH2 expression.[41]

3.3 | m^{6}A-modified lncRNA in PC

SH3BP5-AS1 is upregulated in gemcitabine-resistant PC. SH3BP5-AS1 is stabilized by ALKBH5/IGF2BP1-mediated m^{6}A modification, and knockdown of SH3BP5-AS1 reduces gemcitabine resistance and suppresses cancer growth. SH3BP5-AS1 functions as a competing endogenous RNA (ceRNA) against miR-139-5p targeting CTBP1. The expression of SH3BP5-AS1 and CTBP1 are positively correlated, suggesting that their elevated expression activates the Wnt signaling pathway and promotes gemcitabine resistance.[42] DDIT4-AS1, a target of ALKBH5, is modified with m^{6}A and stabilized by HuR binding. DDIT4-AS1 is upregulated in PC and is associated with prognosis. Knockdown of DDIT4-AS1 reduces gemcitabine resistance and suppresses cancer growth. DDIT4-AS1 promotes UPF phosphorylation by inhibiting the binding of SMG5 and PP2A to UPF1. This leads to degradation of DDIT4 mRNA and activation of the mTOR pathway.[43] lncRNA ANRIL is spliced by m^{6}A modification through binding of serine/arginine-rich splicing factor 3 (SRSF3). In PC, SRSF3 expression is associated with anticancer drug resistance and poor prognosis. ANRIL enhances DNA homologous recombination repair capacity by forming a complex with Ring1b and EZH2.[44] LIFR-AS1 is upregulated in PC cell lines, and knockdown of LIFR-AS1 suppresses cell proliferation. LIFR-AS1 is stabilized by METTL3 through m^{6}A modification of the 3′ UTR and activates PI3K/Akt signaling by repressing miR-150-5p, which targets VEGFA and upregulates VEGFA expression.[45] KCNK15-AS1 is downregulated in PC, and when KCNK15-AS1 was overexpressed in a PC cell line, migration and invasion were inhibited. ALKBH5, an m^{6}A eraser, is also downregulated in PC and removes the m^{6}A modification of KCNK15-AS1, suggesting that removal of the m^{6}A modification of KCNK15-AS1 increases KCNK15-AS1 expression and suppresses migration and invasion.[46] Linc00662 is m^{6}A-modified in PC and is stabilized by binding of IGF2BP3. Linc00662 activates ITGA1 transcription by recruiting GTF2B to the promoter region of ITGA1 and activates the ITGA1-FAK-Erk pathway, leading to PC progression.[47] As described above, there are RNA-modified lncRNAs that promote cancer progression mainly by Wnt, AKT, cell cycle, or mTOR signaling, and those that inhibit cancer progression by suppressing AKT signaling or changes in epigenetics (Figure 1). These lncRNA modifications are summarized in Table 2. Thus, research on lncRNA modification is an important foundation for understanding the pathophysiology of cancer and developing new treatment methods, and it is expected that this understanding will be further deepened in the future.

4 | MODIFICATION OF circRNAs

Circular RNAs are ncRNAs with a circular structure, which are produced by back-splicing events in the genome and are known to act as sponges for miRNAs.[48] Due to their stability and functional diversity, circRNAs play important roles in cellular physiological and pathological processes. Modifications to circRNA have also been shown to have important biological functions,

TABLE 2 Modifications of lncRNA.

Tumor	Gene name	Writer	Reader	Eraser	Modification	Expression	lncRNA target	Modification effect on tumor	References
CRC	POU6F2-AS1	METTL3	IGF2BP2	N/A	m6A	Up	Activate FASN transcription by binding to YBX1	Promote proliferation and lipogenesis	24
CRC	GHRLOS	ZCCH4	N/A	N/A	m6A	Down	KDM5D upregulation	Promote proliferation	25
CRC	linc00659	N/A	IGF2BP1	N/A	m6A	Up	Activate the Wnt/β-catenin pathway by FZD6 upregulation	Promote proliferation	26
CRC	SNHG1	METTL3	N/A	N/A	m6A	Up	N/A	Promote proliferation	27
CRC	LINC02038	METTL3	YTHDF2	N/A	m6A	Down	Suppress the PI3K/AKT pathway by sponging miR-552-5p, which downregulates FAM172A	Suppress proliferation	28
CRC	HNF1A-AS1	METTL3	IGF2BP2	N/A	m6A	Up	CCND1 upregulation by sponging miR-93-5p and PDCD4 downregulation, which activates the PI3K/AKT pathway	Promote proliferation, migration, and angiogenesis	29
CRC	LINC01559	METTL3	N/A	N/A	m6A	Up	PTEN upregulation by sponging miR-106-5p	Promote proliferation	30
CRC	ZFAS1	N/A	N/A	N/A	N/A	N/A	LAMC2 and EIF4A3 upregulation by binding to NOP58 promoting rRNA 2'-O-methylation	Promote proliferation	31
GC	AGAP2-AS1	N/A	N/A	N/A	N/A	N/A	m6A modification of STAT3 mRNA by binding to WTAP/METTL3/METTL14, activating the IL6/STAT3 pathway by STAT3 upregulation	Promote proliferation	32
GC	LINC00355	N/A	N/A	N/A	N/A	N/A	CDC42 and HNRNPA2B1 upregulation	Promote proliferation	33
GC	OIP5-AS1	METTL3	IGF2BP3	N/A	m6A	Up	PKM2 upregulation by preventing hnRNPA1 degradation	Promote proliferation	34
GC	SNHG3	METTL3	N/A	N/A	m6A	Up	CyclinD2 upregulation by sponging miR-186-5p	Promote proliferation	35
GC	DIAPH2-AS1	N/A	N/A	N/A	N/A	N/A	NSUN2 upregulation resulting in NTN1 mRNA m5C modification by NSUN2	Promote migration, invasion, and neural invasion	36
GC	TP53TG1	N/A	N/A	ALKBH5	m6A	Up	Suppress the PI3K/AKT pathway by CIP2A downregulation	Suppress proliferation and metastasis	37
GC	THAP7-AS1	METTL3	IGF2BP1	N/A	m6A	Up	Activate the PI3K/AKT pathway by transcriptional suppression of miR-22-3p and miR-320a	Promote proliferation	38
GC	LINC00958	KIAA1429	N/A	N/A	m6A	Up	Activate aerobic glycolysis by GLUT1 upregulation	Promote proliferation	39
GC	LINC01320	METTL14	N/A	N/A	m6A	Up	RAB19 upregulation by sponging miR-495-5p	Promote proliferation	40
GC	NEAT1	N/A	N/A	ALKBH5	m6A	Down	EZH2 upregulation	Suppress invasion and metastasis	41
PC	SH3BP5-AS1	N/A	IGF2BP1	ALKBH5	m6A	Up	Activate the Wnt pathway by function as ceRNA against miR-139-5p targeting CTBP1	Enhance gemcitabine resistance	42
PC	DDIT4-AS1	N/A	HuR	ALKBH5	m6A	Up	Activate the mTOR pathway by DDIT4 mRNA degradation	Enhance gemcitabine resistance	43

TABLE 2 (Continued)

Tumor	Gene name	Writer	Reader	Eraser	Modification	Expression	lncRNA target	Modification effect on tumor	References
PC	ANRIL	METTL3	SRSF3	N/A	m6A	Up	Enhance DNA homologous recombination repair capacity by binding to Ring1b and EZH2	Enhance gemcitabine resistance	44
PC	LIFR-AS1	METTL3	N/A	N/A	m6A	Up	Activate the PI3K/AKT pathway by inhibiting miR-150- 5p targeting VEGFA	Promote proliferation	45
PC	KCNK15-AS1	N/A	N/A	ALKBH5	m6A	Down	Vimentin downregulation	Promote migration and invasion	46
PC	Linc00662	METTL3	IGF2BP3	N/A	m6A	Up	Activate the ITGA1-FAK-Erk pathway by recruiting GTF2B to ITGA1 promoter region	Promote proliferation	47

Abbreviations: ceRNA, competing endogenous RNA; CRC, colorectal cancer; GC, gastric cancer; lncRNA, long noncoding RNA; PC, pancreatic cancer.

intracellular dynamics, and roles in disease. In cancer, abnormal circRNA modifications have been found to contribute to cancer progression and the formation of therapeutic resistance. For example, circRNA_0102913 inhibits miR-571 by binding to miR-571 in CRC and contributes to cancer promotion by upregulating the expression of RAC2 mRNA, a target of miR-571. Expression of circRNA_0102913 is promoted by m5C modification by NOP2/Sun RNA methyl transferase 5.[49] circRNAs generated by back-splicing of YAP mRNA (circ-YAP) are expressed by the transcription factor YAP, and when modified by METTL3 with m^6A, binding of YTHDF3 recruits the translation initiation factor eIF4G2 to translate the 220 amino acid protein. Circ-YAP is involved in cancer progression, as the low-expression group of circ-YAP and its 220 amino acid have a better prognosis than the high-expression group in CRC.[50] circQSOX1 is stabilized by binding of IFG2BP2 when modified with m^6A by METTL3 and sponges miR-326 and miR-330-5p, thereby upregulating PGAM1 which is the target of miR-326 and miR-330-5p. Elevated PGAM1 expression enhances glycolysis, leading to lactate accumulation and Treg activity, which contributes to cancer proliferation. Knockdown of circQSOX1 suppresses cancer growth and even higher efficacy is obtained in combination with anti-CTLA4 antibody (D9D).[51] circRNA_0003215 is generated by back-splicing exons 7 and 8 of the MYO9B transcript. circRNA_0003215 is a tumor suppressor gene that is m^6A modified in CRC, and its degradation is promoted by the binding of its leader, YTHDF2. Overexpression of circRNA_0003215 suppresses cancer growth. circRNA_0003215 upregulates DLG4 expression by sponging miR-663b. DLG4 suppresses the pentose phosphate pathway (PPP) by K48-linked ubiquitination of glucose-6-phosphate dehydrogenase.[52] circALG1 is stabilized and upregulated by m^6A modification and YTHDF1 binding in CRC and sponges miR-342-5p, upregulating the expression of placental growth factor (PGF), a target of miR-342-5p, and promoting migration and invasion.[53] As described above, RNA-modified circRNAs promote cancer progression by sponging miRNAs with tumor-suppressive functions (Figure 1). The modifications of these circRNAs are summarized in Table 3. Thus, research on circRNA modification is expected to advance our understanding of the biology of these RNA molecules and provide a foundation for the development of new therapies. It is hoped that future research will further advance our understanding of this field and lead to clinical applications of circRNA modification.

5 | METHYLATED RNA MARKER GENES

A search for m^6A-modified marker genes is also underway (Table 4). When methylated RNA immunoprecipitation sequencing (MeRIP-seq) was performed on a CRC cell line, 6.35% of detected RNAs were noncoding regions, including 261 miRNAs, 201 lncRNAs, and 360 circRNAs. Of the circRNAs, 349 were exonic circRNAs and 11 were intronic circRNAs. circNBPF19, circNBPF20, and circNBPF10 were found to have m^6A in all tested CRC cell lines. The

TABLE 3 Modifications of circRNA.

Tumor	Gene name	Writer	Reader	Modification	Expression	circRNA target	Modification effect on tumor	References
CRC	circ_0102913	NOP2/Sun RNA methyl- transferase 5	N/A	m5C	Up	RAC2 upregulation by sponging miR-571	Promote proliferation	49
CRC	circ-YAP	METTL3	YTHDF3	m6A	Up	220 amino acids protein translation by recruiting eIF4G2	Promote migration and invasion	50
CRC	circQSOX1	METTL3	IFG2BP2	m6A	Up	PGAM1 upregulation by sponging miR-326 and miR-330-5p targeting PGAM1	Promote proliferation	51
CRC	circ_0003215	N/A	YTHDF2	m6A	Down	DLG4 upregulation by sponging miR-663b	Promote proliferation	52
CRC	circALG1	METTL3	YTHDF1	m6A	Up	PGF upregulation by sponging miR-342-5p	Promote migration and invasion	53

Abbreviations: circRNA, circular RNA; CRC, colorectal cancer.

proportion of circRNA containing m^6A modification was 4.7%.[54] In 5-FU-resistant CRC cell lines, lncRNA ADIRF-AS1 m^6A modification is decreased while lncRNA AL139035.1 m^6A modification is increased. As a result, these expression levels are reduced. In vitro experiments showed that knockdown of these two lncRNA suppressed cancer cell growth.[55] MeRIP-seq of lncRNA detected 6433 N1-methyladenine (m^1A) peaks in CRC, and HGGAGRA (H = A, T, C; R = A, G) and WGGGANGA (W = A, T; N = A, T, G, C) were common sequences.[56]

6 | CONSTRUCTION OF PROGNOSTIC MODELS BY USING METHYLATED RNA MARKER GENES

Noncoding RNAs have recently been shown to play an important role in predicting cancer prognosis due to their diverse functions and biological effects.[57] The utilization of ncRNA modifications is also being promoted to predict cancer progression and prognosis. Using TCGA data from PC, the expression of $m^6A/m^5C/m^1A$-associated lncRNAs were evaluated with the COX regression model, and 39 lncRNAs were identified as being associated with prognosis. Seven of these lncRNAs, SOCS2-AS1, LINC00941, UCA1, CASC8, TM4SF1-AS1, SNHG10, and DNMBP-AS1, were used to construct a risk model. The $m^6A/m^5C/m^1A$-associated lncRNAs were suggested to be biomarkers that can be used for early diagnosis.[58] The Cancer Genome Atlas (TCGA) and the International Cancer Genome Consortium (ICGC) PC dataset were searched for m^6A-related lncRNAs, and 262 m^6A-related lncRNAs were identified. Risk models were constructed with four of these m^6A-related lncRNAs (MIR4435-2HG, Lnc-BIN3-1, Lnc-C14orf37–1, and Lnc-MFSD4B-1).[59] The expression of m5C-related lncRNAs in PC patients were compared with healthy controls, and it was found that eight m5C-related lncRNAs (AC022098.1, AL031775.1, AC005332.6, AC096733.3, AC025165.1, CASC8, AC009974.1, and PAN3- AS1) were used to construct the risk model. PAN3-AS1 was upregulated and seven other m^5C-related lncRNAs were downregulated in PC.[60] The Pearson correlation test and univariate Cox analysis from RNA sequencing and clinical data of GC patients in the TCGA database identified eight m^6A-related lncRNAs (AL512506. 1, AP000873.4, AC005586.1, AL390961.2, AL590705.3, AL139147.1, AC022031.2, AL355574.1, and LINC00106), showing high microsatellite instability (MSI-H) and high efficacy of immune checkpoint inhibitors in the low-risk group.[61] By identifying modifications of ncRNAs that are related to prognosis, it is expected that these modifications can be used as biomarkers and be utilized for early diagnosis and treatment of cancer. The use of prognostic models is also expected to improve the accuracy of diagnosis, making it one of the most important areas of cancer research. It is hoped that future research will deepen our understanding of the biology of ncRNAs, further advance the accuracy of prognostic prediction models and their clinical application, and realize highly accurate personalized medicine.

TABLE 4 Methylated RNA marker genes.

Tumor	Modification	Marker ncRNAs	References
CRC	m6A	circNBPF19, circNBPF20, and circNBPF10	54
CRC	m6A	ADIRF-AS1 and AL139035.1	55
CRC	m1A	BC005081, FW340027, HP09053, RP11-936I5.1, RP11-156P1.3, BC062349, RP11-134L10.1, AK128252, CTD-2587H19.2, and RP5-1159O4.1	56
PC	m6A/m5C/m1A	SOCS2-AS1, LINC00941, UCA1, CASC8, TM4SF1-AS1, SNHG10, and DNMBP-AS1	58
PC	m6A	MIR4435-2HG, Lnc-BIN3-1, Lnc-C14orf37–1, and Lnc-MFSD4B-1	59
PC	m5C	AC022098.1, AL031775.1, AC005332.6, AC096733.3, AC025165.1, CASC8, AC009974.1, and PAN3-AS1	60
GC	m5C	AL512506. 1, AP000873.4, AC005586.1, AL390961.2, AL590705.3, AL139147.1, AC022031.2, AL355574.1, and LINC00106	61

Abbreviations: CRC, colorectal cancer; GC, gastric cancer; ncRNA, noncoding RNA; PC, pancreatic cancer.

Comprehensive analysis of ncRNA combinations and expression patterns and the construction of prognostic prediction models will enable more accurate evaluation of patient prognosis.

7 | DRUG DISCOVERY BY INHIBITION OF RNA MODIFICATION

As mentioned above, RNA modification is deeply involved in cancer progression and has recently attracted attention as a target for drug discovery. In particular, many m^6A modification inhibitors and methylation removal inhibitors have been developed, and their antitumor effects have been verified. In this section, we discuss METTL3 inhibitors, FTO inhibitors, and ALKBH5 inhibitors, which have been the focus of much research.

7.1 | METTL3 inhibitors

STM2457 has been developed as an inhibitor of METTL3, a key methyltransferase responsible for catalyzing deposition of m^6A to RNA sites. Treatment with STM2457 prolonged survival in a mouse model of acute myeloid leukemia (AML).[62] It was also reported that STM2457 suppressed the growth of PC cells.[63] STC-15 (WO2021111124A1) is in a phase 1 clinical trial by STORM Therapeutics starting in 2022 (ClinicalTrials.gov ID: NCT05584111). UZH2 is found as a METTL3 inhibitor with an IC50 of 5 nM.[64] It has been used as a proteolysis targeting chimera (PROTAC) linked to an E3 ubiquitin ligase-binding compound to promote the degradation of METTL3 by ubiquitination and has been shown to inhibit METTL3 in AML and prostate cancer cell lines.[65] Mutations in METTL3 have been found in cancer cell lines, and single-nucleotide polymorphisms (SNPs) have also been identified (depmap portal and NCBI database). However, whether these mutations are predisposing factors for cancer or targets for drug discovery is unknown. Further studies will be needed in this regard.

7.2 | FTO inhibitors

CS1 is an inhibitor of the m^6A demethylase FTO and has been shown to inhibit tumor growth in a mouse model in which the human colon cancer cell line HCT116 was transplanted. CS2 inhibited tumor growth in a mouse model transplanted with the human hepatocellular carcinoma cell line MHCC97L. FB23-2 prolonged survival in a mouse model transplanted with patient-derived AML. MO-I-500 inhibited the proliferation of SUM149 triple-negative inflammatory breast cancer cell line.[66] The FTO inhibitor 44/ZLD115, a compound derived from FB23, inhibited tumor growth in a mouse model transplanted with MV-4-11 cells of an AML cell line.[67] 11b, an HZ-MA hybrid combining HZ and MA, inhibited the growth of AML NOMO-1 cells.[68] FTO-43 inhibited the proliferation of human GC cell lines AGS and SNU16.[69] High-throughput screening using m^6A-sensitive ribonuclease, MazF, showed that xanthine derivatives such as lisofylline, pentoxifylline, and pentifylline had FTO-inhibiting activity.[70] Compound C6 inhibited the growth of esophageal cancer cell line EC109 cells, with cell viability of 0% at a concentration of 16 μM.[71] Compound 18097 was found to inhibit the enzymatic activity of FTO by DNA cleavage

activity assay using Dnp II and inhibited the growth of human breast cancer cell line MDA-MB-231 cells.[72]

7.3 | ALKBH5 inhibitors

ALKBH5 is one of the m^{6}A demethylases. DDO-2728 inhibited tumor growth in a mouse model transplanted with the MV4-11 cell line, an AML cell line.[73] Ena15 and Ena21 showed 0% viability against LN229 cells, a glioblastoma cell line, at a concentration of 80 μM.[74] Compound 3 (2-[(1-hydroxy-2-oxo-2-phenylethyl)sulfanyl] aceticacid) and compound 6 (4-{[(furan-2-yl)methyl]amino}-1,2-diazinane-3,6-dione) were found to be effective against the growth of leukemia cell lines (HL-60, CCRF-CEM, andK562).[75] Many other ALKBH5 inhibitors have been reported, including MV1035, NOG, succinate, 2,4-PDCA, and citrate.[76] These are expected to be used as anticancer agents.

7.4 | Future prospects for modified RNAs as potential targets for drug discovery

As we have discussed, modified ncRNAs are closely related to cancer progression. Thus, drug discovery targeting modified ncRNAs will provide new cancer treatment methods. We identified cancer-specific m^{6}A-modified RNAs by gene expression and MeRIP-seq analysis of cancer tissues, ductal tissues, and acinar cells of PC patients. Among them, m^{6}A-modified TCEAL8 was shown to be a new PC marker. In addition, m^{6}A levels of the ncRNAs PMDN2-AS1, LINC00396, LOC101928324, MIR4791, LOC100288842, and APCDD1L-AS1 were found to be elevated in cancer tissue.[77] Since the detailed function of these ncRNAs through m^{6}A modification has not yet been reported, further functional analysis may reveal new mechanisms in PC progression. Currently, METTL3 inhibitors, FTO inhibitors, and ALKBH5 inhibitors are only used to inhibit writer and eraser to comprehensively control m^{6}A modification and obtained antitumor effects. In the future, pinpoint targeting technology of m^{6}A-modified RNA may be needed to enhance antitumor effect. Molecules that perform position-specific methylation or demethylation are being developed using PUF protein that binds to specific RNA sequences. Specifically, a fusion protein conjugating PUF and METTL3/METTL14 performs position-specific methylation, while a fusion protein conjugating PUF and FTO performs position-specific demethylation, thereby controlling m^{6}A modification at the target position.[78] Further refinement of such techniques may enable highly precise control of position-specific m^{6}A modifications. In addition, since a SNP in the m^{6}A reader, IGF2BP1, rs9906944 C > T, has been reported to reduce the risk of GC, understanding the function of mutations in RNA modification-related genes may lead to new drug discovery methods.[79] Recent studies have reported that certain ncRNAs translate micropeptides. We recently reported data suggesting that RN7SL1, a signal recognition particle component RNA responsible for recognizing the translation of secreted proteins and membrane proteins and recruiting them to the endoplasmic reticulum, translates a micropeptide.[80] Since RN7SL1 contains four METTL3-binding sequences (RRACH: R = A, G; H = A, T, C), it is possible that the m^{6}A modification is related to the translation of RN7SL1 micropeptide, and further functional analysis is needed. The relationship between m^{6}A modifications of various ncRNAs and translation of micropeptides will also need to be further elucidated in the future.

Finally, it should also be mentioned that modifications on ncRNAs can be challenging to detect due to several factors. Many ncRNAs tend to have low expression levels and are not polyadenylated, resulting in low or zero capture rates when using common RNA-sequencing methods, such as the commonly employed MeRIP-seq for m^{6}A detection. Additionally, only a subset of transcripts overlapping a given m^{6}A site may be methylated in a sample. These factors combined mean that many significant m^{6}A modifications which could have potential value as prognostic markers in gastrointestinal cancer may go undetected. Therefore, it is envisaged that future technological advances will uncover a plethora of methylation events, including many that are dysregulated in various cancers, potentially boosting drug discovery.

8 | CONCLUSIONS

RNA modifications have a profound effect on gene expression. In cancer, it causes up- or downregulation of lncRNA expression, which is involved in cell proliferation and regulates downstream gene expression. Since m^{6}A modifications are involved in cancer growth, inhibitors of the m^{6}A modification molecules METTL3, the demethylase FTO, and ALKBH5 are expected to be developed as new anticancer agents. Since m^{6}A modification of circRNA regulates the translation of small protein, more and more research in this field will be conducted in the future. Further functional analysis of m^{6}A modifications of ncRNAs employing more powerful technologies for facilitating their detection and development of drug discovery technologies targeting them will become increasingly necessary.

AUTHOR CONTRIBUTIONS

Tomoaki Hara: Conceptualization; data curation; formal analysis; writing – original draft; writing – review and editing. **Sikun Meng:** Conceptualization; data curation; formal analysis; writing – original draft; writing – review and editing. **Yasuko Arao:** Conceptualization; investigation; validation; visualization; writing – original draft. **Yoshiko Saito:** Conceptualization; data curation; investigation; methodology; validation; visualization; writing – original draft. **Kana Inoue:** Conceptualization; data curation; validation; visualization; writing – original draft. **Sarah Rennie:** Conceptualization; data curation; writing – original draft. **Ken Ofusa:** Conceptualization; data curation; formal analysis; supervision; validation; visualization; writing – original draft. **Yuichiro**

Doki: Conceptualization; project administration; supervision. **Hidetoshi Eguchi:** Conceptualization; project administration; supervision. **Toru Kitagawa:** Project administration; supervision. **Hideshi Ishii:** Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; software; supervision; validation; visualization; writing – original draft; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

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ETHICS STATEMENT

Approval of the research protocol by an Institutional Reviewer Board: N/A.

Informed Consent: N/A.

Registry and the Registration No. of the study/trial: N/A.

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