

Novel insight into the functions of N⁶-methyladenosine modified lncRNAs in cancers (Review)

YINGJIE HE^{1,2*}, XUEZHI DU^{1,2*}, MING CHEN²,
LEI HAN¹ and JINJIN SUN²

¹Key Laboratory of Post-Neuroinjury Neuro-Repair and Regeneration in Central Nervous System, Ministry of Education and Tianjin City, Tianjin Neurological Institute, Tianjin Medical University General Hospital, Tianjin 300052; ²Department of Hepatopancreatobiliary Surgery, The Second Hospital of Tianjin Medical University, Tianjin 300211, P.R. China

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Abstract. Emerging evidence has suggested that N⁶-methyladenosine (m6A) modification, a typical RNA methylation modification, controls the fate of modified transcripts and is involved in the pathogenesis of various human diseases, such as metabolic disorders, nephropathy, osteoarthritis and malignant tumours. Long noncoding RNAs (lncRNAs), transcripts of >200 nt in length, have also been indicated to be involved in various diseases by participating in processes such as epigenetic modifications, transcriptional alternations and posttranslational regulation. Recent studies revealed that lncRNAs were widely modified by m6A, which has a critical role in various cellular processes that

are associated with numerous disorders, particularly human cancers. The present review first examined functions of m6A modification of lncRNAs, including changing the lncRNA structure, mediating transcriptional regulation, affecting mRNA precursor splicing, and regulating lncRNA stability and translation. Furthermore, the regulatory mechanisms of m6A-modified lncRNAs in cancers were summarized and the up-to-date detection methods and prediction tools for identifying m6A sites on lncRNAs were presented. In addition, viewpoints on potential future directions in the field were discussed, including more accurate detection methods, roles of lncRNAs-encoded micropeptides in cancers, the relationship

Correspondence to: Dr Jinjin Sun, Department of Hepatopancreatobiliary Surgery, The Second Hospital of Tianjin Medical University, 23 Pingjiang Road, Hexi, Tianjin 300211, P.R. China
E-mail: jsun02@tmu.edu.cn

Dr Lei Han, Key Laboratory of Post-Neuroinjury Neuro-Repair and Regeneration in Central Nervous System, Ministry of Education and Tianjin City, Tianjin Neurological Institute, Tianjin Medical University General Hospital, 154 Anshan Road, Heping, Tianjin 300052, P.R. China
E-mail: superhanlei@tmu.edu.cn

*Contributed equally

Abbreviations: m6A, N⁶-methyladenosine; lncRNA, long noncoding RNA; m6Am, N^{6,2'}-O-dimethyladenosine; m1A, N¹-methyladenosine; meRIP-Seq, methylated RNA immunoprecipitation and sequencing; SAM, S-adenosylmethionine; METTL3, methyltransferase-like 3; WTAP, Wilms' tumor 1-associating protein; VIRMA, vir-like m6A methyltransferase associated; ZC3H13, zinc finger CCCH-type containing 13; CBLL1, casitas B-lineage lymphoma-transforming sequence-like protein 1; RBM15/15B, RNA-binding motif protein 15/15B; MTC, methyltransferase complex; FTO, fat mass and obesity-associated protein; ALKBH5, AlkB homologue 5; α -KG, α -ketoglutarate; hm6A, N⁶-hydroxymethyladenosine; f6A,

N⁶-formyladenosine; A, adenosine; YTH, YT521-B homology; HNRNP, heterogeneous nuclear ribonucleoprotein; IGF2BP, insulin-like growth factor 2 mRNA binding protein; eIF3, eukaryotic initiation factor 3; miRNA/miR, microRNA; RBPs, RNA binding proteins; NSCLC, non-small-cell lung cancer; ORF, open reading frame; sORF, short ORF; smORF, small ORF; ESCC, esophageal squamous cell carcinoma; OS, osteosarcoma; NPC, nasopharyngeal carcinoma; ceRNA, competing endogenous RNA; ITGB3, integrin β 3; EC, esophageal cancer; YY1, yin yang 1; YY1BM, YY1-blocking micropeptide; GC, gastric cancer; EZH2, enhancer of zeste homologue 2; GLUT1, glucose transporter-1; CRC, colorectal cancer; YAP, Yes-associated protein; HCC, hepatocellular carcinoma; OATP1B1, organic anion transporting polypeptide 1 B1; PC, pancreatic cancer; EMT, epithelial to mesenchymal transition; LC, lung cancer; LUAD, lung adenocarcinoma; FUBP1, far upstream element binding protein 1; KC, kidney cancer; ccRCC, clear-cell renal cell carcinoma; GSK3 β , glycogen synthase kinase 3 β ; PCa, prostate cancer; BRCA, breast cancer; HMGA2, high-mobility-group A2; GCs, gynaecological cancers; EEC, endometrioid endometrial carcinoma; EOC, epithelial ovarian cancer; LETM1, leucine zipper/EF hand-containing transmembrane-1; CC, cervical cancer; CDKN1A, cyclin-dependent kinase inhibitor 1A; DLBCL, diffuse large B-cell lymphoma; HNSCC, head and neck squamous cell carcinoma; IDH, isocitrate dehydrogenase; TC, thyroid cancer

Key words: m6A, lncRNA, aberrant expression, cancer, biomarker

between m6A-modified lncRNAs and the tumour microenvironment, and m6A-modified lncRNAs as potential biomarkers and therapeutic targets in human cancer.

Contents

1. Introduction
2. Regulators of m⁶A modification
3. Functions of lncRNAs after m6A modification
4. Roles of m6A-modified lncRNAs in cancers
5. Detection methods and tools to predict m6A sites on lncRNAs
6. Conclusion and future perspectives

1. Introduction

In parallel to DNA and protein, there are >170 types of biochemical modifications that occur in RNA (1,2). The most remarkable RNA modification is methylation. Numerous studies have indicated that methylation modifications are able to alter RNA structure, stability and function. These modifications include 7-methylguanine, 5-methylcytosine, N^{6,2'}-O-dimethyladenosine (m6Am), N¹-methyladenosine (m1A), 5-hydroxymethylcytosine and N⁶-methyladenosine (m6A) (3-5). Since 1974, m6A has been gradually identified as the most abundant modification in human RNAs (6) and has been proven to affect numerous functional processes of RNAs, such as alternative splicing (7-9), decay (10), subcellular distribution (11) and translation (12).

Mostly transcribed from DNAs, long noncoding RNAs (lncRNAs) are transcripts of >200 nt in length (13). To date, numerous studies have indicated that lncRNAs exert various functions in epigenetic regulation (14), mRNA transcription or degradation, alternative splicing and translation (15). Undoubtedly, lncRNAs have roles in the pathogenesis, development, diagnosis and treatment of various disorders, including numerous cancers (16), neurological diseases (17) and autoimmune dysregulation (18).

The interplay between lncRNAs with m6A modification has been reported to have important roles in cancers (19). With the development of next-generation sequencing, studies have indicated that lncRNAs are modified in this way using methylated RNA immunoprecipitation and sequencing analysis (20). Given the important roles of m6A-modified ncRNAs in carcinogenesis, accumulating evidence has focused on m6A-modified lncRNAs (21,22). The present review summarized how m6A affects lncRNAs and the functions of m6A-modified lncRNAs in cancers.

2. Regulators of m6A modification

M6A modification is defined as methylation or demethylation and recognition of the methyl group on the sixth nitrogen atom of adenine (A) in RNAs (6), which is reversible due to the interplay among three m6A regulators, writers, erasers and readers (23). The writers attach the methyl group to the m6A sites, whereas the erasers remove it. Readers recognize m6A marks and link RNAs with diverse regulatory mechanisms (Fig. 1) (24,25).

Writers. Writers are methyltransferases that utilize S-adenosylmethionine (SAM) to provide methyl groups for the methylation of RNAs (26). The first m6A writer was identified as a complex, which contains the following proteinic subunits: methyltransferase-like 3 (METTL3), METTL14 and their cofactors: Wilms' tumour 1-associating protein (WTAP), vir-like m6A methyltransferase associated (VIRMA), zinc finger CCH-type containing 13, casitas B-lineage lymphoma-transforming sequence-like protein 1 and RNA-binding motif protein 15/15B (22,27-29). METTL3 is highly conserved and is able to transfer the methyl group of SAM to the targeted A (30). METTL14 acts as a platform for RNA binding (31) and may be used to construct a steady heterodimer with METTL3 to effect m6A modification (32). In addition, WTAP is another subunit of the m6A methyltransferase complex (MTC). It was reported that the interplay between WTAP and METTL3/METTL14 is required for the chemical activity of m6A writers *in vivo* (33). METTL16 is a newly identified writer that catalyses the m6A methylation of ncRNAs (34). Similar to METTL3, METTL16 contains the Rossmann-like fold of class I methyltransferases and uses SAM as the methyl donor but appears to have additional regulatory and RNA binding domains (35). Consistent with studies that focus on dysregulated m6A writers, abnormal lncRNAs interacting with writers have been identified in various cancers (36,37).

Erasers. M6A modification is reversible and erasers have important roles in its reversion. Two essential erasers, namely fat mass and obesity-associated protein (FTO) and AlkB homologue 5 (ALKBH5) (23,38), belong to the α -ketoglutarate (α -KG)-dependent ALKB dioxygenase family and clear m6A methylation in an Fe(II)- and α -KG-dependent manner (39). First, they oxidize m6A to form N⁶-hydroxymethyladenosine (hm6A). Next, they catalyse the conversion of hm6A to N⁶-formyladenosine (f6A). Finally, f6A is converted to A and demethylation is completed (40).

FTO. The first m6A demethylase discovered on mRNA was FTO (38). In the nucleus, FTO demethylates m6A-modified poly(A) RNA, m6A- or m6Am-modified small nuclear RNA and m1A-modified transfer (t)RNA. In the cytoplasm, it may demethylate m6A- or m6Am-modified poly(A) RNA and m1A-modified tRNA (41).

ALKBH5. ALKBH5 is another RNA demethylase. It has been indicated that demethylation induced by ALKBH5 contributes to the dysregulation of certain oncogenic lncRNAs in tumours. Numerous clinical trials based on novel drugs targeting ALKBH5 are in progress. Of note, ALKBH5 may become a considerable target for cancer diagnosis, therapy and prognosis (23).

Readers. M6A readers, including the YT521-B homology domain (YTHD) family, heterogeneous nuclear ribonucleoprotein (HNRNP) family and insulin-like growth factor 2 mRNA binding protein (IGF2BP) family, may function as binding proteins to recognize m6A on RNAs (42). Different readers have distinct functions on methylated RNA.

YTH domain family. Among diverse readers, the YTH domain family, including YTHDF1, YTHDF2, YTHDF3, YTHDC1 and YTHDC2, has been indicated to interact with m6A modification on RNAs (10). YTHDF1 promotes

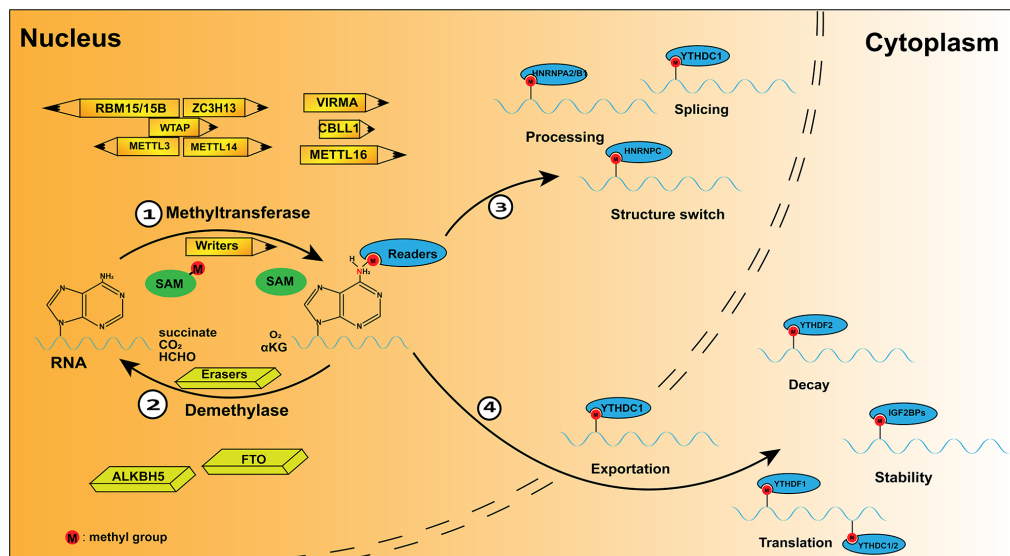


Figure 1. Process of m6A modifications on RNAs. ① M6A methylation may be induced by writers ('pencil'), including METTL3, METTL14 and WTAP. ② The reversal of this process may be induced by demethylases ('erasers'), including ALKBH5 and FTO. ③ In the nucleus, m6A modifications may be recognized by readers, including the YTH domain family, HNRNP family and IGF2BP family, and mediate RNAs processing, splicing and structure switch. ④ In the cytoplasm, different readers may recognize m6A sites on RNAs and mediate RNA exportation, decay, stability and translation. m6A, N⁶-methyladenosine; METTL3, methyltransferase-like 3; WTAP, Wilms' tumour 1-associating protein; VIRMA, vir-like m6A methyltransferase associated; ZC3H13, zinc finger CCH-type containing 13; CBLL1, casitas B-lineage lymphoma-transforming sequence-like protein 1; RBM15/15B, RNA-binding motif protein 15/15B; ALKBH5, AlkB homologue 5; α -KG, α -ketoglutarate; hm6A, N⁶-hydroxymethyladenosine; f6A, N⁶-formyladenosine; A, adenosine; YTH, YT521-B homology; HNRNP, heterogeneous nuclear ribonucleoprotein; IGF2BP, insulin-like growth factor 2 mRNA binding protein; FTO, fat mass and obesity-associated protein; SAM, S-adenosylmethionine.

the initiation of translation by interacting with eukaryotic initiation factor 3 (eIF3, which interacts with mRNA in 48S complexes and participates in the progression of translation) (43). YTHDF2 recognizes m6A sites on mRNAs and promotes their decay (44). YTHDF3 is associated with lncRNA decay (45) and recruits effectors involved in translation (46). YTHDC1 participates in m6A-modified lncRNA-mediated gene silencing (22) and modulates the nuclear transportation of mRNA after being m6A modified (46). YTHDC2 binds to circular RNAs and interferes with their sponging of targeted microRNAs (miRNAs/miRs) (47), preferentially functioning as an m6A reader to enhance translation efficiency or decrease mRNA abundance (48).

HNRNP family. HNRNPs are a type of RNA binding proteins (RBPs), some of which may act as m6A readers (41). HNRNPA2/B1 binds to m6A sites to enhance the progression of primary miRNAs in an m6A-dependent manner. Furthermore, HNRNPA2/B1 may combine with certain binding sites indirectly via a mechanism called the 'm6A switch' (49), which means that reversible m6A modification within the RNA duplex changes the local RNA structures and accessibility for binding proteins such as HNRNPA2/B1 and HNRNPC. The function of HNRNPA2/B1 in m6A-dependent biological processes still requires further study. HNRNPC also binds to m6A-modified RNAs through an 'm6A switch' mechanism (21). Liu *et al* (50) reported HNRNPG as a novel m6A reader, as it utilizes the low-complexity region for the recognition of m6A modification and participates in the alteration of splicing diversity.

IGF2BP family. IGF2BPs recognize and bind RNAs with m6A modifications. Subsequently, the stability and translation of targeted RNAs may be promoted by IGF2BPs

in an m6A-dependent manner, which may strongly affect gene expression levels. IGF2BP1/2/3 are newly discovered m6A readers that may protect mRNAs with m6A modifications from degradation (51). In addition, IGF2BPs stabilize the mRNAs of certain oncogenes with m6A modifications, promoting the progression of certain cancers (52).

To date, attempts have been made to illustrate the regulatory mechanisms of lncRNAs in cancers. In addition, m6A modification appears to be an upstream mechanism that regulates lncRNAs (36,37). Through the joint efforts of numerous researchers, additional m6A writers, erasers, readers and lncRNAs regulated by m6A will be discovered, which will certainly broaden our horizon regarding the functional features of m6A-modified lncRNAs in cancers.

3. Functions of lncRNAs after m6A modification

Changing lncRNA structure and accessibility for proteins. Parallel analysis of RNA structure indicated that the structure of RRACH (R=A or G; H=A, U or C) motifs that contain m6A differs from those that lack m6A modification. *In vitro* studies indicated that whether m6A leads to RNA secondary structure destabilization or stabilization relies upon its position within or at the edge of duplexes (53). Due to disrupting base complementary pairing between A and U, m6A modification within duplexes makes the opposite strand more single-stranded and more accessible (21). While m6A sites on lncRNAs may bind readers, localized complementary chains may also expose a U-containing binding motif for RBPs (54). In addition, reversible m6A modification changes the local structure of lncRNAs, altering the RBPs attached to lncRNAs, referred to as the 'm6A switch' (Fig. 2A). For instance, m6A methylation of lncRNA

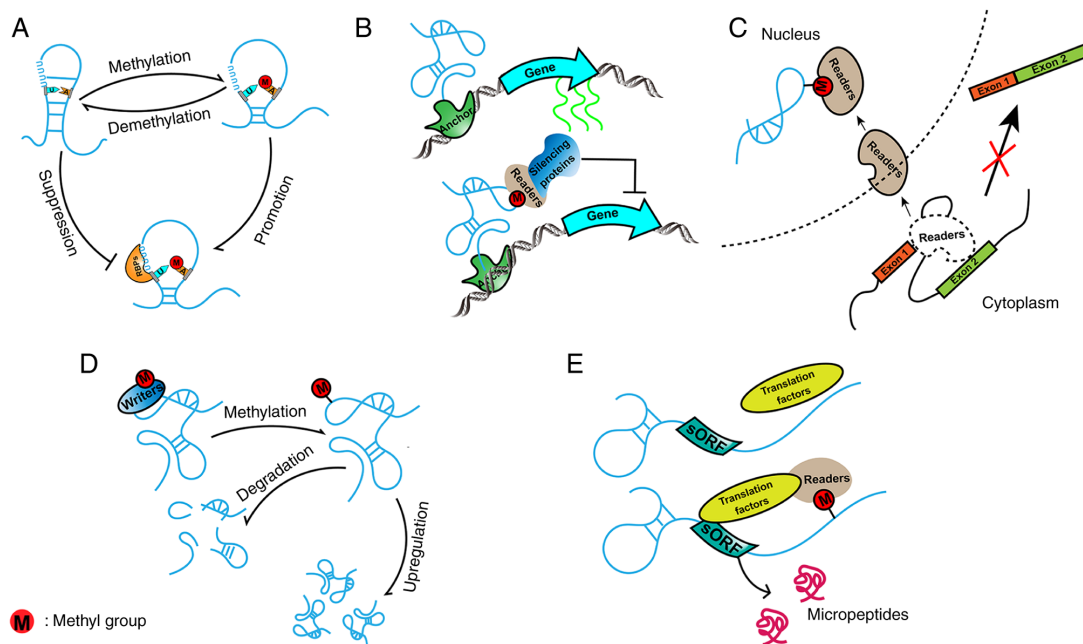


Figure 2. Functions of lncRNAs in response to m6A modification. (A) M6A modifications on lncRNAs within the duplex disturb base complementary pairing between A and U, providing new sites for RNA binding proteins. (B) Readers recognize m6A residues on lncRNAs and recruit protein complexes, resulting in transcriptional alterations. (C) Readers involved in alternative splicing are recruited to the nucleus, decreasing mRNA precursor splicing in the cytoplasm. (D) M6A modification of lncRNAs affects their stability and abundance, although the mechanisms remain elusive. (E) Readers recognize m6A modification on lncRNAs that are near lncRNA sORFs and recruit translation factors to produce micropeptides. m6A, N⁶-methyladenosine; sORF, short open reading frame; lncRNA, long non-coding RNA.

metastasis associated lung adenocarcinoma transcript 1 (MALAT1) affects its duplex structure and regulates the interaction with HNRNPC/HNRNPG, which suggests that m6A modification participates in structural switching of lncRNAs and contributes to the binding strength of proteins (21,50,54).

Mediating gene transcriptional regulation. M6A-modified lncRNAs are recognized by readers. Readers may also bind to partners that have distinct roles in transcriptional regulation, resulting in the assembly of transcriptional ribonucleoprotein complexes. Targeting activated transcriptional regions, m6A-modified lncRNAs with transcriptional ribonucleoprotein complexes may lead to alterations in gene transcription (Fig. 2B). This mechanism requires that m6A-modified lncRNAs may be recognized by readers and assist readers to recruit transcriptional ribonucleoprotein complexes. It is different from epigenetic regulation via methylation at promoters, whose regulation depends on methyltransferases directly binding to promoters other than m6A-modified lncRNAs.

In this regard, Patil *et al* (22) provided the first example. They confirmed that m6A modification on lncRNA X-inactive specific transcript (XIST) is recognized by YTHDC1. Subsequently, the reader YTHDC1 binds several proteins, including SMRT and HDAC associated repressor protein, lamin B receptor, HNRNPU and HNRNPK, each of which participates in transcriptional silencing. Finally, the X-linked genes *glipican 4* and *α thalassemia/mental retardation syndrome X-linked* are silenced by m6A-modified XIST binding to YTHDC1 and transcriptionally repressive proteins (22).

Affecting mRNA precursor splicing. Splicing has a vital role in regulating gene expression by removing introns and joining

exons together. During the process of splicing, diverse exons from the same encoded gene may be alternatively selected. This mechanism leads to the diversity of mRNA variants and protein products (55). It has been reported that m6A modifications affect alternative splicing (56). Furthermore, Ninomiya *et al* (57) demonstrated that during the process of thermal stress recovery, m6A-modified lncRNA highly repetitive satellite III (HSATIII) is recognized by YTHDC1, resulting in decreased YTHDC1-induced mRNA alternative splicing in the cytoplasm (Fig. 2C). In addition, m6A-modified HSATIII binding to YTHDC1 was surrounded by nuclear stress bodies, providing a reaction crucible for CDC-like kinase 1 to phosphorylate Ser/Arg-rich splicing factors, which were previously indicated to have an impact on pre-mRNA splicing during thermal stress (57).

Regulating lncRNA stability. Several studies have indicated that m6A-modified lncRNAs exhibit differential stability and abundance compared to those without m6A modification in different types of cancer (Fig. 2D). For instance, Xue *et al* (58) determined that METTL3-mediated methylation stabilizes lncRNA abhydrolase domain containing 11-antisense RNA 1, exerting its function in non-small cell lung cancer (NSCLC), while Guo *et al* (59) investigated whether ALKBH5 was able to increase the abundance of lncRNA nuclear enriched abundant transcript 1 (NEAT1) in colon cancer tissues and associated with poor prognosis.

However, the upstream mechanisms by which m6A modification changes lncRNA stability and abundance are still unclear. On the one hand, m6A modification itself may change the intermolecular forces or spatial structures of local lncRNAs to affect their stability or degradation. Furthermore,

m6A regulators may interfere with RNA degradosome recognition or binding to special sites on lncRNAs, thereby regulating the process of lncRNA degradation. Specifically, writers may interfere with degrading factors binding to key sites on lncRNAs, erasers may eliminate the methyl group on lncRNAs and remove special signals recognized by degrading complexes for starting the process of RNA decomposition, and m6A readers likely recruit other partners to form complexes that interfere with the degradation of m6A-modified lncRNAs.

Regulating lncRNA translation. Due to the lack of canonical open reading frames (ORFs >100 aa), lncRNAs are considered untranslatable (60,61). Of note, a study identified hundreds of short or small ORFs (sORFs or smORFs) in the sequence of lncRNAs that actually encode peptides (62). Mechanistically, certain m6A motifs on lncRNAs may be close to crucial sequences of sORFs or smORFs. M6A readers not only recognize m6A modification on lncRNAs but also recruit translation factors at the same time. As a result, micropeptides encoded by lncRNAs are produced in an m6A-dependent manner (Fig. 2E). Wu *et al* (63) indicated that the YTHDF1 reader, which interacts with the translation machinery, promotes protein translation and recognizes m6A modification on lncRNA LINC00278. This m6A motif is near the stop codon of a LINC00278-encoded micropeptide, which is termed yin-yang 1 (YY1)-blocking micropeptide (YY1BM). Subsequently, YY1BM is translated in an m6A-dependent manner, ultimately resulting in apoptosis of esophageal squamous cell carcinoma (ESCC).

Despite these findings, numerous points remain to be elucidated, e.g. how m6A influences lncRNA translation. Recently, a genome-wide study identified ~100 lncRNA-encoded micropeptides that regulate induced pluripotent stem cell growth (64). However, whether m6A modification participates in lncRNA translation and micropeptide roles in cancerous cells still requires extensive validation. In addition, m6A modification has been indicated to promote mRNA export from the nucleus and it has been suggested that m6A modification may affect the subcellular diffusion of lncRNAs. For instance, Wu *et al* (65) reported that upregulated METTL3 subsequently localizes lncRNA RP11 primarily in the nucleus. However, how m6A modification alters the subcellular distribution of lncRNA remains elusive and requires further study.

4. Roles of m6A-modified lncRNAs in cancers

In recent decades, emerging evidence has emphasized the essential role of m6A-modified lncRNAs in cancers, which has laid a solid foundation for future clinical applications targeting m6A-modified lncRNAs, even if their mechanisms of pathogenesis differ. Below, a brief review of relevant research in this area is provided (Fig. 3).

Musculoskeletal system

Osteosarcoma (OS). Originating from mesenchymal cells, OS is one of the most aggressive tumour types in the musculoskeletal system (66). Multiple studies have revealed that dysregulated m6A-modified lncRNAs may participate in multiple phenotypes in OS. Chen *et al* (67) indicated that lncRNA PVT1, an oncogenic lncRNA verified in different

tumour types, is upregulated in patients with OS. The m6A demethylase ALKBH5 'erased' the m6A mark of PVT1, subsequently decreasing the binding of PVT1 and YTHDF2, which is a common reader that promotes the decay of m6A-modified RNAs. ALKBH5-induced PVT1 upregulation promoted OS development both *in vitro* and *in vivo*. The study's conclusion suggests that ALKBH5-PVT1 may be a potential biomarker for OS (67). Evidence is emerging that m6A abundance and lncRNA abnormalities are involved in OS (68,69), indicating that m6A-modified lncRNAs may be better biomarkers for cancer. This provides a future direction for OS therapy that warrants further investigation.

Digestive system

Esophageal cancer (EC). EC is regarded as a globally common invasive tumour type in the digestive tract with a 5-year overall survival rate of ~20-30% (70). With the development of detection techniques, the role of m6A-modified lncRNAs in EC has been gradually revealed. Wu *et al* (63) indicated that smoking leads to ALKBH5 upregulation and removes m6A marks on the Y-linked lncRNA LINC00278. The classical m6A modification motif of LINC00278 that interacts with YTHDF1 is near the stop codon of YY1BM and the neighbouring YTHDF1 facilitates translation of the micropeptide YY1BM. YY1BM suppresses the transcription of eukaryotic elongation factor 2 kinase by blocking contact between YY1 and androgen receptor, resulting in apoptosis of ESCC. Mechanistically, smoking inhibits YY1BM translation, correspondingly inducing male ESCC progression (63). It is foreseeable that a deeper understanding of the complexity of m6A-modified lncRNAs involved in EC will offer novel and promising applications for EC management in the future.

Gastric cancer (GC). GC is the third most common cause of cancer-associated mortality worldwide (71). Previous studies on the dysregulation of m6A-modified lncRNAs revealed their function in GC. Zhang *et al* (72) indicated that in response to ALKBH5 demethylation, lncRNA NEAT1 regulates the expression of enhancer of zeste homologue 2 (EZH2), a subunit of the polycomb repressive complex, by decreasing NEAT1 function as a scaffold to bind with EZH2, affecting GC invasion and metastasis. Yan *et al* (44) confirmed that the association of lncRNA LINC00470 and METTL3 is recognized by the YTHDF2 reader and methylated LINC00470 binding YTHDF2 decreases the stability of phosphatase and tensin homolog (PTEN) mRNA, which encodes PTEN protein and acts as a classical tumour suppressor. M6A-modified LINC00470 ultimately promotes GC growth. Similarly, Yang *et al* (73) discovered that KIAA1429 recognizes the m6A site of LINC00958 and represses the decay of LINC00958, subsequently enhancing the cooperation of LINC00958 and glucose transporter-1 (GLUT1) mRNA to enhance its mRNA stability. Functionally, KIAA1429-mediated LINC00958 participates in GC tumour progression by upregulating GLUT1, which promotes aerobic glycolysis in GC. Hu and Ji (74) revealed that METTL14-mediated methylation upregulates levels of lncRNA LINC01320, which subsequently sponges miR-495-5p and affects RAB19 expression, ultimately regulating GC progression. As research moves forward, increasing knowledge will undoubtedly shed new light on improving the diagnosis and prognosis of this frequent disease.

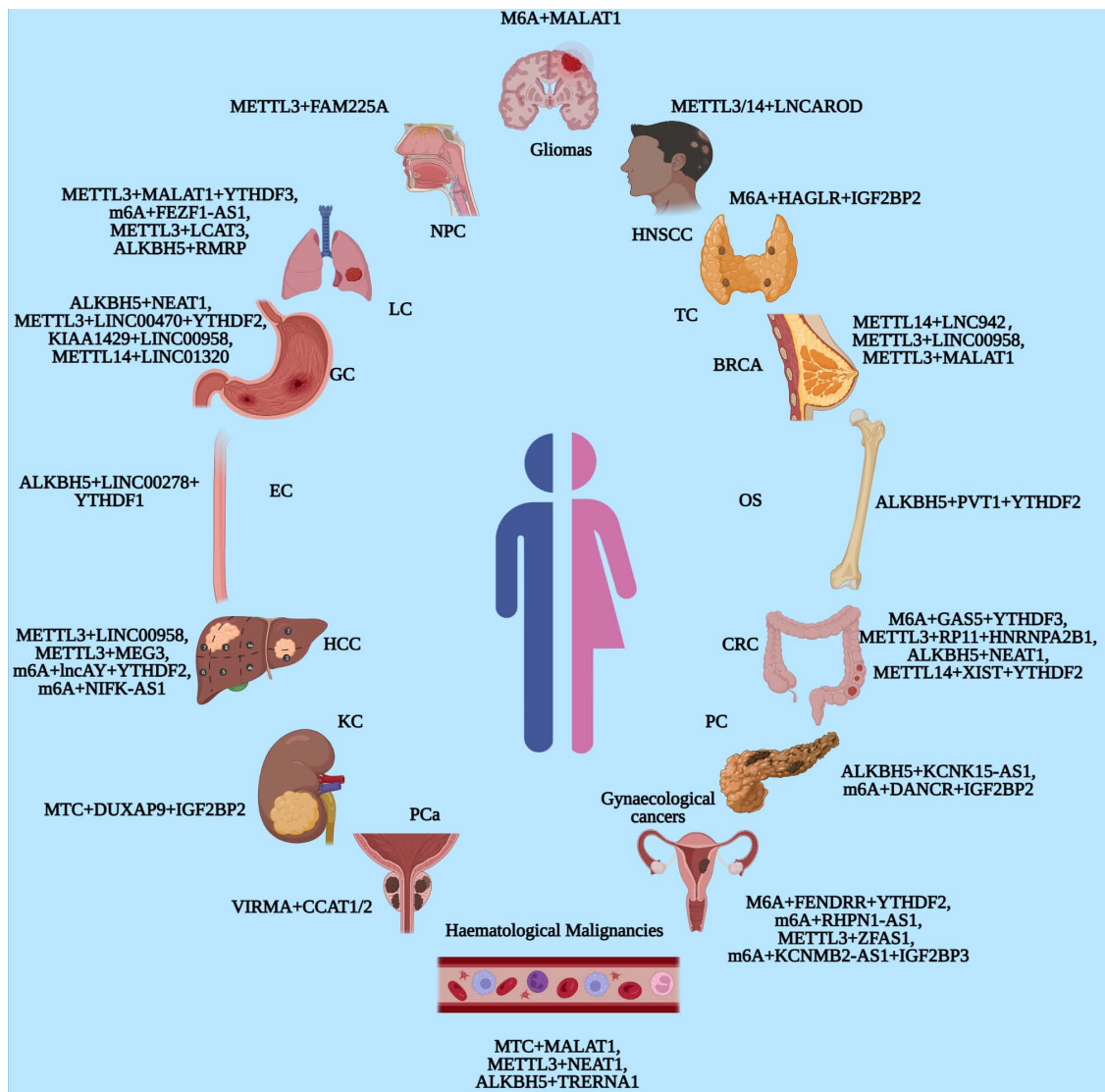


Figure 3. M6A-modified lncRNAs in different cancer types. In glioma, m6A-modified MALAT1 activated the NF- κ B pathway, leading to IDH-wildtype glioma cell proliferation and migration. In HNSCC, METTL3 and METTL14-mediated methylation increased LNCAROD stability, contributing to HNSCC cell proliferation and mobility. In TC, m6A-modified HAGLR may be recognized by IGF2BP2, inhibiting TC cell apoptosis and cell cycle arrest. In BRCA, METTL14 methylated LNC942, leading to BRCA epithelial to mesenchymal transition (EMT) and chemoresistance; METTL3-mediated m6A modification increased LINC00958 stability, finally leading to therapy resistance; METTL3 modified MALAT1, facilitating BRCA cell stemness. In OS, ALKBH5 demethylated PVT1 and decreased YTHDF2 recognition of the m6A mark on PVT1, contributing to OS growth. In CRC, m6A-modified GAS5 may be recognized and degraded by YTHDF3, contributing to CRC growth; METTL3-modified RP11 may be recognized by HNRNPA2B1, promoting CRC metastasis; ALKBH5-induced demethylation increased NEAT1 expression, correlating with poor prognosis of CRC. METTL14-modified XIST may be recognized and degraded by YTHDF2, inhibiting CRC invasion. In PC, ALKBH5-mediated demethylation upregulated KCN15-AS1 expression, resulting in EMT of PC; m6A-modified DANCR may be recognized and stabilized by IGF2BP2, leading to PC stemness-like properties. In gynaecological cancers, m6A-modified FENDRR may be recognized and degraded by YTHDF2, resulting in endometrioid endometrial carcinoma cell proliferation; m6A modification increased RHPN1-AS1 stability, contributing to epithelial ovarian cancer growth; METTL3 methylates ZFAS1, thus enhancing cervical cancer metastasis; m6A-modified KCNMB2-AS1 may be recognized and stabilized by IGF2BP3, promoting cervical cancer growth. In haematological malignancies, m6A-modified MALAT1 promoted chimeric mRNA exportation, contributing to myeloid progenitor cell differentiation in malignant haematopoiesis; METTL3 elevated NEAT1 stability, finally releasing chronic myelocytic leukaemia cells from cell cycle arrest; decreased m6A methylation of TRERNA1 regulated by ALKBH5 was indicated to regulate diffuse large B-cell lymphoma cell proliferation. In PCa, VIRMA is associated with CCAT1 and CCAT2, indicating poor prognosis of PCa. In KC, IGF2BP2 recognized MTC-mediated m6A modification on DUXAP9 and stabilized it, ultimately triggering EMT of clear cell renal cell carcinoma. In HCC, METTL3 mediated LINC00958 upregulation, promoting HCC lipogenesis and development; METTL3 induced decay of MEG3, resulting in HCC growth; m6A-modified lncAY may be recognized and degraded by YTHDF2, ultimately suppressing HCC cell proliferation and migration; m6A modification increased NIFK-AS1 expression, mediating HCC metastasis and therapeutic resistance. In EC, ALKBH5 removed m6A marks of LINC00278, finally inhibiting esophageal squamous cell carcinoma cell apoptosis. In GC, ALKBH5 demethylated NEAT1, affecting GC invasion and metastasis; METTL3 methylated LINC00470 may be recognized by YTHDF2, ultimately promoting GC growth; KIAA1429 repressed the decay of LINC00958, finally regulating GC metabolism reprogramming; METTL14 mediated LINC01320 upregulation, contributing to an aggressive phenotype of GC. In LC, METTL3-modified MALAT1 may be recognized by YTHDF3, resulting in poor prognosis of NSCLC; m6A modification upregulated FEZF1-AS1 expression, leading to NSCLC cell proliferation and invasion; METTL3 elevated LCAT3 stability, contributing to LUAD oncogenesis; ALKBH5 upregulated RMRP expression, inhibiting LUAD cell apoptosis. In NPC, METTL3-mediated modification elevated FAM225A stability, ultimately promoting metastasis of NPC. m6A, N⁶-methyladenosine; METTL3, methyltransferase-like 3; MTC, m6A methyltransferase complex; VIRMA, vir-like m6A methyltransferase associated; ALKBH5, AlkB homologue 5; YTHD, YT521-B homology domain; HNRNP, heterogeneous nuclear ribonucleoprotein; IGF2BP, insulin-like growth factor 2 mRNA binding protein; HNSCC, head and neck squamous cell carcinoma; TC, thyroid cancer; BRCA, breast cancer; OS, osteosarcoma; CRC, colorectal cancer; PC, pancreatic cancer; PCa, prostate cancer; KC, kidney cancer; HCC, hepatocellular carcinoma; EC, esophageal cancer; GC, gastric cancer; LC, lung cancer; NSCLC, non-small cell lung cancer; LUAD, lung adenocarcinoma; NPC, nasopharyngeal carcinoma.

Colorectal cancer (CRC). CRC remains one of the most common neoplasms in the lower digestive tract globally. In China, CRC is also a major cause of tumour-associated mortality (75). Researchers have identified numerous m6A-modified lncRNAs related to the tumorigenesis and metastasis of CRC. Ni *et al* (76) revealed that lncRNA GAS5 binds to the WW domain of yes-associated protein (YAP) directly and subsequently leads to phosphorylation and ubiquitin-mediated decomposition of YAP, inhibiting CRC progression. However, the m6A reader YTHDF3 facilitates m6A-modified GAS5 degradation and reverses YAP-mediated inhibition of tumour progression. Wu *et al* (65) discovered that METTL3-mediated methylation of lncRNA RP11 is recognized by HNRNPA2B1. After binding with HNRNPA2B1, m6A-modified RP11 facilitates the decay of seven in absentia homolog 1 (SIAH1) and F-box only protein 45 (FBXO45) mRNA, possibly through HNRNPA2B1 recruiting factors that participate in the mRNA degradation process, such as P bodies. The mRNA of SIAH1 and FBXO45 encodes proteins that function as ubiquitin E3 ligases and further mediate zinc finger E-box binding homeobox 1 protein degradation via the ubiquitin-proteasome pathway. In this way, m6A-modified RP11 and accelerated mRNA degradation trigger the dissemination of CRC. Guo *et al* (59) indicated that ALKBH5 increased lncRNA NEAT1 expression in colon cancer tissues and was associated with poor prognosis. Yang *et al* (77) reported that m6A-modified XIST, an oncogenic lncRNA, is degraded after recognition by the m6A reader YTHDF2. Furthermore, METTL4 suppresses the proliferation and invasion of CRC cells by degrading XIST in an m6A-dependent manner. Further exploration of m6A-related lncRNAs in CRC is necessary to lay a solid foundation for their future use in CRC diagnosis and treatment.

Hepatocellular carcinoma (HCC). HCC remains the most common liver cancer with high morbidity globally (75). Different underlying cellular mechanisms of hepatocarcinogenesis that are governed by m6A-modified lncRNAs have been unveiled through the joint efforts of scientists. Zuo *et al* (36) revealed that METTL3-mediated m6A modification induces increased lipogenesis-related lncRNA LINC00958. M6A-modified LINC00958 sponges miR-3619-5p to upregulate hepatoma-derived growth factor expression, thereby facilitating HCC lipogenesis and progression. By contrast, Wu *et al* (78) discovered that METTL3-mediated m6A modification induced the decay of lncRNA MEG3, which affected HCC progression through the miR-544b/B-cell translocation gene 2 axis. Chen *et al* (79) reported a decrease in lncRNA lncAY after binding the m6A reader YTHDF2. M6A-modified lncAY downregulates B cell-specific Moloney murine leukemia virus integration site 1 (BMI1), a protein that modulates stem cell maintenance in diverse human epithelial cancers and has considerable roles in the initiation and progression of numerous cancer types. Decreasing the expression levels of BMI1 inhibited the activation of Wnt/ β -catenin signalling, subsequently suppressing cell proliferation and migration of HCC. Another study suggested that m6A methylation upregulates the levels of lncRNA NIFK-AS1 in HCC (80). Furthermore, m6A-modified NIFK-AS1 functions as a competing endogenous RNA (ceRNA) to sponge miR-637, increasing AKT1 expression, which resulted in the upregulation

of MMP7 and MMP9, both of which are essential mediators of metastasis and are positively associated with NIFK-AS1. It was also confirmed that by suppressing the drug transporters organic anion transporting polypeptide 1 B1 (OATP1B1) and OATP1B3, upregulated NIFK-AS1 decreased the sensitivity of HCC cells towards sorafenib, which is a multikinase inhibitor and the first targeted drug for advanced HCC approved by the Food and Drug Administration. A rapid upsurge of research publications in recent decades has occurred; exploring novel pathogenesis mechanisms and identifying new biomarkers of HCC requires further efforts for the improvement of diagnosis and treatment.

Pancreatic cancer (PC). PC is one of the most malignant tumour types and lacks specific symptoms and effective treatment (81). In recent decades, the morbidity of PC has increased 6-fold in China (82). Recent studies indicated that m6A-modified lncRNAs have critical roles in PC. He *et al* (83) found that lncRNA KCNK15-AS1 levels were decreased by m6A methylation and functioned as an antitumor lncRNA by inhibiting pancreatic cancer cell migration, invasion and epithelial to mesenchymal transition (EMT). As an m6A eraser, ALKBH5 mediates the increase in KCNK15-AS1 via demethylation. Owing to the higher levels of KCNK15-AS1, the antitumor effects were recovered in PC. Hu *et al* (84) demonstrated that the stability of lncRNA DANCR, which was reported to promote cancer stemness-like properties, was strengthened by the interaction of IGF2BP2 with DANCR at the A664 site in PC. As a result, m6A-mediated upregulation of DANCR promoted cell proliferation, stemness-like properties and tumorigenesis of PC cells. In general, research in this field is progressing rapidly. With continued research on PC diagnosis, treatment and prognosis, profound changes will occur in the near future.

Respiratory system. Along with changes in the environment and lifestyle, lung cancer (LC) has gradually become the most frequently occurring cancer type (11.6% of total cases) and the major cause of cancer-associated mortality (18.4% of all cancer-associated deaths) (71,85). Studies have indicated that m6A-modified lncRNAs may serve as biomarkers for the dual purpose of timely screening and targeted therapy of lung cancer. Jin *et al* (37) demonstrated that METTL3-induced methylation upregulated the levels of lncRNA MALAT1 and the stability of MALAT1 was increased by the METTL3/YTHDF3 complex in NSCLC. Upon binding to METTL3-modified MALAT1, YTHDF1/3 recruits eIF3b to the translation initiation complex, enhancing translation of YAP, which is associated with the development, progression and poor prognosis of NSCLC. Furthermore, MALAT1 functions as a ceRNA to sponge miR-1914-3p and inhibit its binding to the 3'-untranslated region (3'-UTR) of YAP mRNA to decrease YAP expression. Finally, m6A-modified MALAT1 facilitates malignant phenotypes of NSCLC cells. Similarly, Song *et al* (86) discovered that the m6A-modified lncRNA FEZF1-AS1 facilitates NSCLC progression via the miR-516b-5p/ITGA11 axis. Qian *et al* (87) reported that m6A modification mediated by METTL3 contributes to the increasing level and enhanced stability of lncRNA LCAT3 in lung adenocarcinoma (LUAD). LCAT3 interacts with far upstream element binding protein 1 (FUBP1), resulting in

activation of c-Myc expression, which is a typical oncogenic transcriptional factor. Thus, LCAT3 promotes malignant phenotypes of LUAD cells via the FUBP1/c-Myc axis. Yu and Zhang (88) observed that ALKBH5 upregulated lncRNA RMRP expression via demethylation in both patients with LUAD and cell lines, conveying a poorer prognosis of LUAD. Despite numerous challenges, it is thought that m6A-modified lncRNAs have the potential to be applied in the diagnosis and therapy of LC.

Urinary system. Kidney cancer (KC) is the 14th most common malignancy worldwide (89). Aberrant m6A modification-related lncRNAs that may be involved in the progression of KC remain to be elucidated. Tan *et al* (90) revealed that by binding with IGF2BP2, the lncRNA DUXAP9 becomes stabilized in clear-cell renal cell carcinoma (ccRCC) in an m6A-dependent manner. Subsequently, DUXAP9 activates PI3K to induce Akt/mTOR signalling, which is common in the regulation of cell growth. Meanwhile, Akt activation phosphorylates and thereby inhibits glycogen synthase kinase 3 β (GSK3 β) function, which phosphorylates Snail and mediates Snail nuclear export and degradation. As a result, DUXAP9 was demonstrated to trigger EMT in ccRCC cells via the Akt/GSK3 β /Snail axis. In summary, m6A-modified DUXAP9 promotes proliferation and EMT in renal cancer cells.

Reproductive system

Prostate cancer (PCa). PCa is the most commonly new diagnosed cancer in males and the 5-year survival rate of patients with PCa with distant metastasis is as low as ~30% (91). Currently, a comprehensive analysis of how m6A-modified lncRNAs function in the clinicopathological characteristics, malignant progression, and prognosis of PCa is lacking. Barros-Silva *et al* (92) demonstrated that VIRMA, a subunit of MTC, is the most common writer in PCa, and m6A methylation may stabilize lncRNA CCAT1 and lncRNA CCAT2, which participate in the alteration of the proto-oncogene MYC. Regarding clinical relevance, VIRMA and upregulation of both lncRNAs (CCAT1 and CCAT2) predicts worse disease-free survival. Although their roles are not entirely understood, m6A-modified lncRNAs in PCa are thought to be very attractive biomarkers for early diagnosis, targeted therapy and improved prognosis in the future.

Breast cancer (BRCA). According to GLOBOCAN 2020, BRCA is the most commonly diagnosed cancer and the leading cause of cancer-associated death in females (71). Recently, solid evidence has led to research on the role of m6A-modified lncRNAs in BRCA. Sun *et al* (93) found that METTL14 recognizes and binds lncRNA LNC942 at a specific motif. Thereafter, METTL14-methylated LNC942 promotes the mRNA stability and expression of CXCR4 and CYP1B1, which induce EMT and drug resistance, respectively. Finally, m6A-modified LNC942 mediates BRCA cell vitality and EMT. Rong *et al* (94) revealed that METTL3-mediated m6A modification promotes the stability of LINC00958. Moreover, m6A-modified LINC00958 acts as a ceRNA to sponge miR-378a-3p and positively regulate YY1, which is involved specifically in metastasis, chemoresistance and EMT. Thus, m6A-modified LINC00958 promotes the tumour progression of BRCA cells via the miR-378a-3p/YY1 axis.

Likewise, Zhao *et al* (95) found that the METTL3-modified lncRNA MALAT1 facilitates the pathogenesis of BRCA through the miR-26b/high-mobility-group A2 (HMGA2) axis. HMGA2 is a transcriptional regulator that is known to promote stemness, aggression and tumour heterogeneity. The differential expression of m6A-related lncRNAs in BRCA makes them exciting candidates for diagnostic markers or therapeutic targets for BRCA.

Gynaecological cancers. Gynaecological cancers originate from and affect women's reproductive organs, while endometrial, ovarian and cervical cancers are the most frequent and major gynaecological cancers in females (96). Studies have indicated that m6A-modified lncRNAs have pivotal roles in the tumorigenesis and metastasis of gynaecological cancers. Shen *et al* (97) observed that abundant m6A modification increases the degradation of lncRNA FENDRR by recruiting the m6A reader YTHDF2 in endometrioid endometrial carcinoma (EEC). Subsequently, downregulation of lncRNA FENDRR results in the accumulation of SOX4 protein, which is reported to promote the proliferation of EEC cell lines via epigenetic repression of miR-129-2. Thus, m6A-modified FENDRR boosts the proliferation of EEC cells and further aggravates the pathological process of EEC. Wang *et al* (98) discovered that m6A upregulates the levels of lncRNA RHPN1-AS1 by decreasing RNA decay in epithelial ovarian cancer (EOC). Subsequently, RHPN1-AS1 acts as a ceRNA to sponge miR-596, consequently increasing leucine zipper/EF hand-containing transmembrane-1 (LETM1) expression, which has an oncogenic role as an activator of the focal adhesion kinase (FAK)/PI3K/Akt pathway in EOC. Therefore, RHPN1-AS1 facilitates EOC cell growth and metastasis via the miR-596/LETM1 axis. Likewise, Yang *et al* (99) revealed that METTL3 methylation is required for lncRNA ZFAS1 to sequester miR-647 by acting as a ceRNA, which was previously reported to be involved in tumour progression, ultimately resulting in enhanced cervical cancer (CC) proliferation and metastasis. Sun *et al* (100) also revealed that lncRNA KCNMB2-AS1 is stabilized by m6A modification and promotes CC growth by acting as a ceRNA to sponge miR-130b-5p and miR-4294. Despite these studies having contributed to the understanding of the disease pathophysiology, challenges in gynaecological cancers still require to be addressed by comprehensive research to accelerate the advancement of prognostic, diagnostic and therapeutic approaches.

Haematological system. Neoplasms of the haematopoietic and lymphoid tissues are closely linked to the circulatory and immune systems, accounting for ~9% of all cancers (101). Due to their roles in the regulation of blood cell turnover, m6A-modified lncRNAs are likely to participate in the tumorigenesis of haematopoietic malignancies. Focusing on tumorigenesis fusion proteins produced by aberrant chromosomal translocations, Chen *et al* (102) reported that lncRNA MALAT1 binds to these fusion proteins and is localized in nuclear speckles after m6A modification. Then, m6A-modified MALAT1 and fusion proteins serve as a functional platform for METTL14 to methylate chimeric mRNA. After binding YTHDC1, m6A-modified chimeric mRNA is exported to the cytoplasm, leading to upregulation of the fusion protein, which is involved

in the tumorigenesis of leukaemia. Yao *et al* (103) discovered that METTL3-mediated methylation increases the stability of lncRNA NEAT1. Subsequently, m6A-modified NEAT1 acts as a ceRNA to sponge miR-766-5p and subsequently increases levels of cyclin-dependent kinase inhibitor 1A (CDKN1A), which is the target gene of miR-766-5p and serves as a negative cell cycle regulator. Finally, m6A-modified NEAT1 suppresses the progression of chronic myelocytic leukaemia by regulating the miR-766-5p/CDKN1A axis. Song *et al* (104) reported that ALKBH5 decreases the m6A modification of lncRNA TRERNA1, resulting in increased TRERNA1 in diffuse large B-cell lymphoma (DLBCL). TRERNA1 then recruits EZH2 to the p21 promoter region, leading to the repression of this cyclin-dependent inhibitor. Finally, TRERNA1 regulates cell proliferation and is associated with poor prognosis in patients with DLBCL. The development of sensitive detection methods and targeted inhibitors for m6A-modified lncRNAs may have potential in the clinical management of haematological malignancies, particularly when combined with existing options.

Head and neck cancers

Head and neck squamous cell carcinoma (HNSCC). HNSCC is the sixth most frequently occurring neoplasm globally, claiming ~450,000 lives every year (91). To date, the complex molecular mechanisms of m6A-modified lncRNAs and their influence on the tumorigenesis and development of HNSCC still require to be explored. Ban *et al* (105) discovered that MTC-induced m6A modification increases the stability of the lncRNA LNCAROD. As a scaffold for the interaction between Y-box binding protein 1 (YBX1) and heat-shock 70-kDa protein 1A (HSPA1A), m6A-modified LNCAROD shields YBX1 from proteasomal degradation by promoting the YBX1-HSPA1A protein-protein interaction, leading to HNSCC cell growth by stabilizing the YBX1 protein. It is still necessary to verify the vital role of m6A-modified lncRNAs in HNSCC, understand the mechanisms of tumorigenesis and progression in depth and identify more effective therapeutic approaches.

Nasopharyngeal carcinoma (NPC). Due to early invasion and distant metastasis, the 5-year survival rate of NPC remains <20% (106). Accumulating studies suggest that dysregulation of m6A-modified lncRNAs has a pivotal role in the occurrence and metastasis of NPC. Zheng *et al* (107) identified that m6A modification improves the stability of lncRNA FAM225A. Consequently, upregulation of FAM225A facilitates its function as a ceRNA to sponge miR-590-3p and miR-1275, resulting in increased integrin $\beta 3$ (ITGB3) expression, which is one of the activators in the FAK/PI3K/Akt pathway. Functionally, FAM225A has been confirmed to promote NPC tumorigenesis.

Nervous system. Characterized by high tumour heterogeneity and poor survival, gliomas appear to be one of the deadliest intracranial cancer types (108). Recently, scientists demonstrated that abnormal m6A-modified lncRNAs are associated with the occurrence and growth of gliomas. Chang *et al* (109) indicated that METTL3 was elevated in isocitrate dehydrogenase (IDH) wild-type glioma, and with the assistance of HuR, upregulated levels of m6A methylation increase the stability of lncRNA MALAT1. M6A-methylated MALAT1

then activates the NF- κ B signalling pathway to promote the malignant progression of IDH wild-type gliomas (109). The association between m6A-modified lncRNAs and oncology is being increasingly explored, while studies in this field have the potential to lay a solid foundation for elucidating the mechanisms of brain tumours.

Endocrine system. Thyroid cancer (TC) is the most frequently occurring cancer type arising from the endocrine system and has exhibited an increasing trend in recent decades (91). However, its morbidity is significantly heterogeneous in terms of different regional factors, ethnic backgrounds and genders (110). To date, various m6A-modified lncRNAs have been studied. Dong *et al* (111) reported that the m6A reader IGF2BP2 recognizes m6A modification of lncRNA HAGLR and upregulates the expression and stability of HAGLR. Subsequently, elevated HAGLR markedly promotes TC cell proliferation, migration and invasion (111). The features of m6A-modified lncRNAs may be applied to the clinical management of TC and have the potential to expand the current knowledge regarding TC tumorigenesis.

Brief summary. Emerging evidence has revealed that m6A-modified lncRNAs participate in tumour cell proliferation, invasion and migration primarily through two different mechanisms: regulating lncRNA stability (58,59) and translation (63). However, fewer studies on cancers are relevant to the other three functions of m6A-modified lncRNAs, such as changing accessibility to binding proteins (50,54), mediating gene transcriptional silencing (22) and affecting mRNA precursor alternative splicing (57), which still require to be explored in depth. Furthermore, novel bioinformatics analyses based on sequencing databases focused on m6A-modified lncRNA roles in the tumour immune microenvironment, such as immunocyte infiltration and responses to immune checkpoint inhibitors (112,113), require in-depth studies to resolve challenges that are highly relevant to clinical application.

5. Detection methods and tools to predict m6A sites on lncRNAs

To explore the biological functions of m6A modification, it is urgent to clarify where m6A modifications occur and how many of these modifications exist in RNA molecules. To date, various novel detection technologies for m6A modification have been developed that benefit from mass spectrometric techniques and high-throughput sequencing (Table I). These detection technologies include antibody-based methods, such as methylated RNA immunoprecipitation sequencing (meRIP-seq), photo-cross-linking-assisted m6A sequencing strategy, m6A individual-nucleotide resolution UV crosslinking and immunoprecipitation, m6A-level isoform-characterization sequencing, digestion-based methods named m6A-sensitive RNA-endoribonuclease-facilitated sequencing or MAZTER-seq, a gene-editing based method named deamination adjacent to RNA modification targets-sequencing, and metabolism-based methods including m6A-label-seq and FTO-assisted m6A selective chemical labeling method sequencing. Among of them, meRIP-seq adopts specific antibodies of methylated RNAs to capture methylated RNA

Table I. Novel detection methods for m6A on long non-coding RNAs.

Classification/method	Author, year	Description	(Refs.)
Antibody-based			
MeRIP-seq	Meyer, 2012	MeRIP-seq is based on immunoprecipitation. Methylated RNAs may be recognized by specific antibodies and detected by RNA-seq after isolation and purification.	(20)
PA-m6A-seq	Chen, 2015	In PA-m6A-seq, 4SU is integrated into RNA by metabolism. In certain conditions, 4SU may interact with aromatic amino acid residues of m6A antibody. This tool vastly improves the accuracy of m6A detection. However, the application of PA-m6A-seq is limited due to the requirement of 4SU metabolism.	(123)
miCLIP	Linder, 2015	In miCLIP, RNAs with m6A modifications may bind with m6A antibody after 254 nm irradiation and induce nucleotide mismatches or truncation signatures near m6A sites in the process of reverse transcription.	(124)
m6A-LAIC-seq	Dominissini, 2012	m6A-LAIC-Seq is similar to meRIP-seq, but the spike-in RNA is introduced into the m6A sequence as an internal reference, and then the methylation level of each gene segment in the complete transcriptome is calculated. This method is performed directly with full-length transcripts for RIP as opposed to library construction without RNA fragmentation.	(56)
Digestion-based: m6A-REF-seq/MAZTER-seq	Zhang, 2019	m6A-REF-seq is a novel high-throughput approach to detect m6A. The PCR database may be constructed based on the RNA particles treated with RNA endonuclease MazF. Subsequently, sequencing and analysis were performed to confirm the m6A modification site.	(122)
Gene editing-based: DART-seq	Meyer, 2019	In DART-seq, certain technologies are used to make cells express apolipoprotein B mRNA-editing enzyme complex 1-YTH fusion protein. This protein may replace C of m6A with U. The m6A modification site may be confirmed by identifying the site where C-to-U editing of genomic information has taken place.	(125)
Metabolism-based			
M6A-label-seq	Shu, 2020	m6A-label-seq is able to generate a6A at the sites of m6A modifications. These a6A modifications may be recognized by antibodies and may be cyclized. Mis-incorporation induced by this cyclized a6A in reverse transcription may provide information on m6A sites at single-base resolution.	(126)
M6A-SEAL-seq	Wang, 2020	In order to detect m6A modification sites, hm6A, which is an oxidation product of m6A, is generated through a thiol addition reaction. Hm6A may form stable dm6A via interacting with DTT. Subsequently, dm6A may be enriched and subjected to sequencing.	(127)
MeRIP-seq, methylated RNA immunoprecipitation sequencing; m6A, N ⁶ -methyladenosine; PA-m6A-seq, photo-cross-linking-assisted m6A sequencing strategy; 4SU, 4-thiouridine; miCLIP, m6A individual-nucleotide resolution UV crosslinking and immunoprecipitation; m6A-LAIC-seq, m6A-level isoform-characterization sequencing; m6A-REF-seq/MAZTER-seq, m6A-sensitive RNA-endoribonuclease-facilitated sequencing; DART-seq, deamination adjacent to RNA modification targets-sequencing; a6A, N6-allyladenine; dm6A, N6-dithiolisitolmethyladenosine; hm6a, N6-hydroxymethyladenosine; DTT, dithiothreitol; YTH, YT521-B homology; 4SU, 4-thiouridine.			

Table II. Updated prediction tools for m6A on lncRNAs.

Method	Author, year	Species	Strength	Weblink	Motif restriction	(Refs.)
SRAMP	Zhou, 2016	Mammals	The m6A modification sites may be recognized by SRAMP, which may supply rational predicted results.	http://www.cuilab.cn/sramp	DRACH	(128)
RNA-MethylPred	Jia, 2016	Human, mouse	RNA-MethylPred is capable of distinguishing m6A sites that cannot be readily detected by iRNA-Methyl and pRNA-m-PC.	-	-	(129)
Targetm6A	Li, 2016	-	The Targetm6A method outperforms the above methods for predicting m6A sites and markedly improved the prediction performance.	http://esbio.njust.edu.cn/bioinf/TargetM6A	GAC	(130)
RMBase V2.0	Xuan, 2018	Human, mouse, rhesus monkey, chimpanzee, rat, pig, zebrafish, fly, <i>S. cerevisiae</i> , <i>A. thaliana</i> , <i>S. pombe</i> , <i>E. coli</i> , <i>P. aetuginosa</i>	The RMBase V2.0 database comprises >100 RNA modification forms, includes comprehensive and complex information on post-transcriptional modifications of RNA, and provides various interfaces and graphic visualizations to promote the analysis of different modification sites in normal tissues and cancer cells.	http://rna.sysu.edu.cn/rmbase	RACH	(131)
M6AVar	Zheng, 2018	Human, mouse	M6AVar is a user-friendly and specific database to explore the function of variations with m6A modification. It may provide detailed explanations and genomic locations of m6A modification sites. This tool may also perform analyses of RBP-binding regions, miRNA targets and splicing sites. M6AVar may also identify the functional disease-related variations.	http://m6avar.renlab.org	DRACH	(132)
DEEPM6ASeq	Zhang and Hamada, 2018	Human, mouse, zebrafish	Based on a deep learning framework to predict m6A-containing sequences and characterize biological features around m6A sites, DEEPM6ASeq exhibited an improved performance as compared with others, and it is competitive at predicting m6A-containing sequences. This model may recognize the position preference of sequences harboring m6A sites, function of motif detectors and saliency maps.	https://github.com/rreybeyb/DeepM6ASeq	DRACH	(133)
CVm6A	Han, 2019	Human, mouse	CVm6A collects available MeRIP-Seq and m6A-CLIP-Seq datasets in human and mouse cell lines and provides a visualized m6A database to benefit functional studies on m6A in cell lines.	http://gb.whu.edu.cn:8080/CV_m6A	RRACH	(134)

Table II. Continued.

Method	Author, year	Species	Strength	Weblink	Motif restriction	(Refs.)
im6A-TS-CNN	Liu, 2020	Human, mouse, rat	Based on the convolutional neural network, im6A-TS-CNN universality performs better than or comparable to existing methods	https://github.com/liukeweiaway/DeepM6A_cnn	-	(135)
M6A2Target	Deng, 2021	Human, mouse	M6A2Target is the first specific resource for m6A WER target genes	http://m6a2target.canceromics.org	-	(136)
m6A, N ⁶ -methyladenosine; lncRNA, long non-coding RNA; miRNA, microRNA; RBP, RNA binding protein; WER, writers, erasers, readers.						

segments for sequencing (20), which is widely used in investigating methylated RNAs (114,115).

In addition, bioinformatics is a powerful tool that is able to identify likely RNA methylation sites based on statistical evidence (Table II). Among these public databases for prediction, SRAMP and m6A2Target appear to be applied more widely (116,117). Of note, LITHOPHONE is a computational framework specifically designed for predicting m6A sites of lncRNAs (118). In addition, the use of public databases may allow researchers to reduce the cost of preliminary investigations and facilitate experimental design.

Although m6A detection and prediction methods are continuously improving, it is urgent to develop more accurate methods to identify or predict m6A sites on lncRNAs. In addition, public databases to explore m6A-modified lncRNAs must be established to broaden the horizon regarding their association with biological features of human cancers.

6. Conclusion and future perspectives

In conclusion, m6A modification of lncRNAs has pivotal roles in gene expression through several mechanisms, including the adaptation in structure and binding proteins of lncRNAs, direct and/or indirect regulation of transcription, alteration of mRNA precursor splicing and change of lncRNA stability and translation. Undoubtedly, lncRNAs modified by m6A writers, erasers or readers are involved in the development and progression of various human malignancies. However, further investigations are required in the following aspects.

Previous studies have indicated that certain lncRNAs encode micropeptides (63). However, the role of m6A in this process remains to be elucidated and the mechanisms involved remain to be explored. More importantly, whether m6A modification of lncRNAs represents a marker for predicting the coding potential of lncRNAs requires to be investigated in the future (119). In addition, the role of these micropeptides encoded by m6A-modified lncRNAs in tumour development should be examined (120).

The tumour microenvironment remains a hot spot in cancer research and a number of studies have identified lncRNAs as key regulators in the tumour microenvironment (121). However, only a small number of investigations have focused on the function of m6A-modified lncRNAs in the tumour microenvironment. It is possible that single-cell sequencing technology is used to scrutinize the m6A-modified lncRNAs in different cell types of the tumour microenvironment.

Current detection procedures of m6A-modified lncRNAs are complex and lack single molecule resolution. The technologies to quantify m6A-modified lncRNAs are also limited (122). For instance, transcriptome-wide m6A-seq methods frequently require a large amount of RNA, so it is difficult to apply in clinical practice due to the limitation of patient-derived samples. It is necessary to establish new detection approaches, which allow the detection of m6A-modified lncRNAs at a low concentration such as in tumour tissues, circulating tumour cells, exosomes and faeces, and a more comprehensive public database to collect information of lncRNAs modified by m6A, which will facilitate the investigation of m6A-modified lncRNAs as regulators, biomarkers and potential therapeutic targets in human cancer.

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Authors' contributions

YH and XD drafted the manuscript and completed the visualization. MC performed the literature search and selection, and participated in reviewing the paper. LH and JS performed revision of the manuscript, and conducted project administration and funding acquisition. All authors read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

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Competing interests

The authors declare that they have no competing interests.

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