Interaction between m6A and ncRNAs and Its Association with Diseases

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Abstract
Noncoding RNAs (ncRNA) are a kind of endogenous RNA that regulate many vital bioprocesses with limited ability to encode polypeptides. Most of them are involved in transcriptional and posttranscriptional regulations, thus showing some biological effects. N6-methyladenosine (m6A) RNA modification is a reversible modification that adjusts RNA’s functions and stability. The enzymes that regulate m6A can be divided into “writers,” “readers,” and “erasers.” Mechanically, m6A modification of microRNA is mainly identified by DGVR8, participating in the processing of primary microRNAs, while m6A modification on long noncoding RNA (lncRNA) can change its spatial structure and stability to regulate its RNA- or protein-binding ability. The m6A-modified lncRNA and circular RNA can act as competing endogenous RNAs, sponge downstream miRNA. Moreover, ncRNA can also regulate m6A level of downstream molecules. Here, we elaborate on recent advances about pathways and underlying molecular mechanisms of how the interaction between m6A and ncRNA is involved in the occurrence and development of various diseases, especially cancer.

Introduction
Noncoding RNA (ncRNA) refers to RNA molecules in the transcriptome that are not translated into proteins. Only 1–2% of the human genome encodes proteins, while a great part of the genome does not encode proteins, but is highly transcriptional and produces a wide range of ncRNAs with regulatory and structural functions [Poller et al., 2018]. ncRNAs are composed of rRNAs and other RNAs including long ncRNA (lncRNA) and short ncRNA [Chan and Tay, 2018]. Studies have shown that ncRNAs are involved in almost all cellular processes such as proliferation, senescence, immobilization, differentiation, apoptosis, stress, and immune response, and are closely associated with a variety of human diseases [Ibanez-Ventoso et al., 2006; Wingo et al., 2015; Fuschi et al., 2017; Huang et al., 2017].

At present, more than 100 types of RNA modifications have been identified in coding RNA and ncRNA, among which N6-methyladenosine (m6A) is the most abundant and important modification base in eukaryotic messenger RNA (mRNA) [Boccaletto et al., 2018]. m6A is the methylation of the sixth nitrogen atom on the adenylate...
of RNA molecules. m6A was first detected as a predominant chemical modification of mRNA from Novikoff hepatoma cells by Desrosiers et al. [1974]. Its functions include maintaining mRNA stability, mRNA precursor shear, promoting mRNA transport and translation initiation. It has been found that m6A is present in 0.1–0.4% adenosine in global cellular RNAs, accounting for 33–46% of the variability in methylation levels [Rottman et al., 1974; Cosgrove, 1998; Garcia-Campos et al., 2019]. m6A is a widespread modification with more than 25% of mRNAs containing at least one m6A. m6A is highly enriched in the vicinity of stop codons and in the 3′UTR [Meyer et al., 2012; Ries et al., 2019]. More and more evidence has shown that m6A modification plays an important role in gene expression, cell differentiation, inflammatory response, immune regulation, and carcinogenesis [Xu et al., 2017, 2021; Yu et al., 2019].

In addition to mRNA, m6A modifications have been found in some ncRNA, such as ribosomal RNAs (rRNAs), small nuclear RNAs (snRNAs), IncRNAs, miRNAs, and circular RNAs (circRNAs) in turn (Fig. 1) [Desrosiers et al., 1974; Meyer et al., 2012; Alarcón et al., 2015a; Yang Y et al., 2017]. Studies have found that the modification of m6A on these ncRNAs can affect their metabolism and function, and is also closely related to some metabolic diseases, neurological diseases, cancer, and other diseases, thus providing new targets for the treatment of these diseases [Rowles et al., 2012; Yang et al., 2020; Xu et al., 2021]. The correlation between the interaction of ncRNA and m6A modifications and various diseases is shown in Figure 2.

Although still in its infancy, the current research has shown great potential for disease prevention and treatment. In this paper, we focus on the molecular mechanisms of how m6A-modified ncRNAs are involved in the occurrence and development of various diseases, especially cancer. Meanwhile, we also describe the potential prediction, prognosis, and therapeutic function of ncRNA or its m6A site.

**m6A-Related Enzymes**

The m6A modification is a process in which methyltransferases catalyze the methylation of RNA adenine at the 6N position [Huang et al., 2020]. In 1994, Bokar et al. [1994] first discovered the m6A methyltransferase complex. The dynamic process of m6A modification includes methylation, demethylation, and recognition, which are mediated by m6A writers, erasers, and readers, respectively.

**Writers**

The m6A writers usually refer to the m6A writer complex consisting of methyltransferase-like 3 (METTL3), methyltransferase-like 14 (METTL14), Wilm’s tumor-associated protein (WTAP), RNA-binding motif protein 15 (RBM15) and its paralog RBM15B, and vir-like m6A methyltransferase associated (VIRMA) [Zhu et al., 2021]. METTL3 was identified with a methyltransferase role at first [Bokar et al., 1994, 1997]. Other methyltransferases such as METTL14 [Liu et al., 2014], KIAA1429 (VIRMA) [Schwartz et al., 2014], WTAP [Ping et al., 2014], ZC3H13 [Wen et al., 2018], and RBM15/15B [Patil et al., 2016] were gradually discovered. The complex formed by them can promote m6A modification of RNA. The methyltransferase structural domain of METTL3 is the catalytic core. METTL14 acts as an RNA binding site to enhance methyltransferase activity by forming a heterodimer with METTL3 [Wang et al., 2016]. WTAP is thought to be a key bridging protein for stabilizing the METTL3-METTL14 complex [Schölzer et al., 2018]. The core components of the m6A writer complex include METTL3, METTL14, and WTAP [Liu et al., 2014]. In addition, other proteins, such as VIRMA [Schwartz et al., 2014], RNA-binding motif protein ZC3H13 [Wen et al., 2018], and RBM15/15B [Patil et al., 2016] were found to be components of the m6A writer complex and also play an important role in m6A methylation modification. A recent study found that VIRMA recruits catalytic core components (METTL3/METTL14/WTAP) to direct regioselec-
Inactive m6A methylation [Yue et al., 2018]. ZC3H13 binds RBM15/15B and links them to WTAP [Knuckles et al., 2018]. Both WTAP and ZC3H13 are important for nuclear localization of the m6A writer complex [Wen et al., 2018]. One study showed that RBM15 and RBM15B interact with METTL3 in a WTAP-dependent manner [Patil et al., 2016]. In addition, RBM15/15B contains an RNA-binding structural domain and thus can facilitate the recruitment of the m6A writer complex to specific sites in the mRNA. With time delay, more and more new methylation enzymes were discovered, such as METTL16, METTL5, and ZCCHC4.
Erasers

Just as the name implies, the demethylases can eliminate the m6A modification of RNA, so they are also named “erasers.” The first and second discovered m6A erasers are fat mass and obesity-associated protein (FTO) [Jia et al., 2011] and α-ketoglutarate-dependent dioxygenase homolog 5 (ALKBH5) [Zheng et al., 2013]. Both of them belong to the α-ketoglutarate-dependent dioxygenase family, which catalyze the demethylation with ferrous iron as cofactor and α-ketoglutarate as cosubstrate [Fedeles et al., 2015]. FTO mediates the demethylation of internal m6A and N6,2′-O-dimethyladenosine (m6Am). FTO preferentially targets nuclear m6A, cytoplasmic m6A, and m6Am mRNA [Wei et al., 2018]. FTO-mediated demethylation of m6A in mRNA affects the transcription level of target mRNA.

ALKBH5 is an endogenous m6A demethylase, but it has no activity towards m6Am [Mauer et al., 2017]. In addition, ALKBH5 mainly acts on mRNA, and it can also act on ncRNA. ALKBH5 increases the export of mRNA to the cytoplasm mainly through its demethylation activity [Zheng et al., 2013].

Readers

“Readers” recognize and bind m6A sites of target RNA, leading them to different destinies. The common readers are YTHDF (YTH N6-methyladenosine RNA binding protein), YTHDC (YTH domain containing), and eIF3. YTHDFs include YTHDF1, YTHDF2, and YTHDF3. YTHDF1 binds to the m6A site and improves the translation efficiency of target RNAs in mammals by facilitating translation initiation. The association between YTHDF1 and the translation initiation mechanism may depend on the eIF4G-mediated loop structure and the interaction of YTHDF1 with eIF3 [Wang et al., 2015]. YTHDF2 can accelerate the degradation of m6A-modified transcripts by directly recruiting the CCR4–NOT adenylosome complex [Du et al., 2016]. YTHDF1 and YTHDF2 each have their own set of target mRNAs, and they also share a large number of common target mRNAs. For shared mRNA targets, YTHDF1 binds RNA earlier in the mRNA life cycle than YTHDF2. This may imply that during cell differentiation or development, YTHDF1 may activate translation of methylated mRNAs to achieve sufficient protein production, while YTHDF2 acts to limit the lifespan of these mRNAs for proper cell development or differentiation. YTHDF3 synergizes with YTHDF1 to promote translation of methylated RNAs and also accelerates mRNA degradation by directly interacting with YTHDF2 [Wang et al., 2015]. The three intracellular YTHDF proteins have coordinated functional interactions. YTHDF3 affects translation and decay of methylated mRNAs through cooperation with YTHDF1 and YTHDF2 [Shi et al., 2017].

YTHDCs include YTHDC1 and YTHDC2. YTHDC1 acts on pre-mRNA splicing and mediates the transport of methylated mRNA from the nucleus to the cytoplasm [Alarcón et al., 2015a; Roundtree et al., 2017]. YTHDC2 enhances the translation of target RNA and reduces the abundance of target RNA [Hsu et al., 2017].

eIF3 is a large multiprotein complex comprising 13 subunits [des Georges et al., 2015]. Translation initiation in eukaryotes begins with the binding of the eIF3, eIF1, eIF1A, and eIF2–GTP/Met-tRNA, Met tery complexes (TC) to the 40S subunit to form the 43S pre-initiation complex [Jackson et al., 2010]. After unraveling the secondary structure by eIF4A, eIF4B, and eIF4F, the 43S complex attaches to the proximal region of the mRNA and extends downstream to the initiation codon where it forms the 48S initiation complex by codon-anticodon base pairing. In addition, eIF5 and eIF5B facilitate the binding of the 60S subunit to the 48S complex, producing an 80S ribosome with extension capability [des Georges et al., 2015]. It has been demonstrated that eIF3a binds to the m6A site in the 5′UTR to promote mRNA translation [Jackson et al., 2010; Meyer et al., 2015].

Regulation of ncRNA by m6A

miRNA

miRNAs are noncoding small RNAs with a length of 18–24 nucleotides. We used to think the most common function of it is to complement with the 3′UTR of the corresponding mRNA, and then endogenously regulate gene expression by inhibiting translation or inducing mRNA degradation [Bartel, 2009]. However, it is interesting that studies have suggested miRNA can promote mRNA translation by binding to the 5′UTR [Fabian et al., 2010].

The biogenesis of miRNAs is complex, it begins in the nucleus and ends in the cytoplasm. In general, the maturation of primary microRNAs (pri-miRNAs) is mediated by a microprocessor complex composed of RNA-binding proteins, DiGeorge syndrome critical region 8 (DGCR8), and a kind of RNase DROSHA. DGCR8 is responsible for recognizing the junction between the stem and the flanking strand of pri-miRNA hairclips and then recruits DROSHA to cleave the double-stranded RNA to produce the pre-miRNA [Han et al., 2006] (Fig. 3a).
m6A Affects miRNAs That Act as Recognition Sites

Present studies have shown that m6A modification plays an important role in the biogenesis of miRNAs. The m6A modification can promote the specific recognition of pri-miRNA by DGCR8 and initiate a series of processing processes from pri-miRNA to pre-miRNA and mature miRNA. The m6A-associated proteins play an important role in various pathological and physiological processes by regulating miRNA levels. Among them, in addition to working with METTL14 to mediate the m6A modification of pri-miRNA, METTL3 also works with hnRNPA2B1 to recruit DGCR8 [Alarcón et al., 2015a, b]. This mechanism has been demonstrated in studies of various diseases (Table 1).

METTL3, as one of the most studied m6A enzymes, has been proved to affect the occurrence and development of various diseases including cancer through the above-mentioned pathways in multiple studies. Numerous research results provide us with new thinking directions for understanding related diseases. In cancers with multiple systems, the relevant mechanisms have been demonstrated in different literatures: METTL3 promotes pri-miR-34a to decrease silent information regulator 1 (SIRT1) expression and induce the formation of abdominal aortic aneurysm [Zhong et al., 2020]. Cigarette smoke condensation induces the overexpression of METTL3, significantly increases the formation of METTL3-mediated m6A in pri-miR-25, promoting the recognition of pri-miR-25 by DGCR8 and the maturation of miR-25-3p, which leads to the occurrence and development of pancreatic duct adenocarcinoma [Zhang J et al., 2019]. Deoxycholic acid, a bile acid, was found to bind and dissociate METTL3 from the METTL3-METTL14-WTAP complex, reducing the m6A modification of miR-92b-3p and inhibiting its maturation, thus inhibiting gallbladder cancer by increasing the protein level of the phosphatase and tensin homolog [Lin et al., 2020]. METTL3 promotes the maturation of miR-143-3p, which can promote the brain
metastasis of lung cancer through VASH1-mediated re-programming of angiogenesis and microtubule protein deaggregation [Wang H et al., 2019]. METTL3 may participate in the maturation of pri-miR-221/222 and play a carcinogenic role in bladder cancer [Han et al., 2019]. And a similar mechanism has also been found in breast cancer [Pan et al., 2021]. METTL3 promotes the maturation of pri-miR-1246 and inhibits the expression of tumor suppressor gene SPRED2, which inactivates the Raf/MEK/ERK pathway to enhance the metastasis ability of colorectal cancer [Peng et al., 2019]. METTL3- and METTL14-mediated m6A modification promotes the maturation of miR-126, then activates the PI3K/AKT/mTOR pathway and promotes the formation of pulmonary fibers after carbon black exposure [Han et al., 2020]. m6A modification affects the content changes of multiple miRNAs (miR-106b, miR-18a/b, miR-3607, miR-423, miR-30a, miR-320b/d/e), affecting the carcinogenesis induced by arsenite [Gu et al., 2018]. In addition to cancer, these mechanisms have been sporadically reported in other diseases. METTL3 positively regulates the pruning of pri-miR-365-3p in an m6A-dependent manner, which promotes the Complete Freund’s Adjuvant (CFA)-induced pain behavior and spinal cord neuron sensitization [Zhang et al., 2020a]. METTL3-mediated m6A methylation mediates miR-7212-5p maturation and inhibits frac-

| Table 1. m6A protein, miRNAs, and the role they play in diseases |
|-----------------------------|----------------|------------------|-----------------|------------------|----------------|
| m6A component | Related miRNA | Disease or physiological processes | Function | Role in disease | Regulation | Reference |
|----------------|----------------|---------------------------------|----------|----------------|------------|----------|
| METTL14 | miR-146a-5p | Breast cancer | Writer | Promotion | Upregulation | Yi et al., 2020 |
| METTL14 | miR-19a | Atherosclerosis | Writer | Promotion | Upregulation | Zhang BY et al., 2020 |
| METTL14 | miR-126 | Hepatocellular carcinoma | Writer | Inhibition | Downregulation | Ma et al., 2017 |
| METTL14 | miR-375 | Colorectal cancer | Writer | Inhibition | Downregulation | Chen X et al., 2020 |
| METTL3 | miR-25 | Pancreatic duct adenocarcinoma | Writer | Promotion | Upregulation | Zhang J et al., 2019 |
| METTL3 | miR-34a | Abdominal aortic aneurysm | Writer | Promotion | Upregulation | Zhong et al., 2020 |
| METTL3 | miR-92b-3p | Gallbladder cancer | Writer | Promotion | Upregulation | Lin et al., 2020 |
| METTL3 | miR-143-3p | Lung cancer | Writer | Promotion | Upregulation | Wang H et al., 2019 |
| METTL3 | miR-221/222 | Bladder cancer | Writer | Promotion | Upregulation | Han et al., 2019 |
| METTL3 | miR-1246 | Breast cancer | Writer | Promotion | Upregulation | Pan et al., 2021 |
| METTL3 | miR-126 | Straight colon cancer | Writer | Promotion | Upregulation | Peng et al., 2019 |
| METTL3 | miR-106b, miR-18a/b, miR-3607, miR-423, miR-30a, miR-320b/d/e | Arsenite-induced carcinogenesis | Writer | Promotion | Upregulation | Gu et al., 2018 |
| METTL3 | miR-365-3p | Pulmonary fibrosis | Writer | Promotion | Upregulation | Han et al., 2020 |
| METTL3 | miR-7212-5p | Inflammatory pain | Writer | Promotion | Upregulation | Mi et al., 2020 |
| METTL3 | miR-134, miR-146 | Osteoblast differentiation and fracture healing | Writer | Promotion | Upregulation | Jimenez-Mateos et al., 2012 |
| METTL3 | miR-320 | Osteoporosis | Writer | Inhibition | Downregulation | Yan G et al., 2020 |
| METTL3 | miR-335 | Acute ischemic stroke | Writer | Inhibition | Downregulation | Si et al., 2020 |
| METTL3 | miR-873-5p | Oxidative stress and apoptosis induced by colistin | Writer | Inhibition | Downregulation | Wang J et al., 2019 |
| METTL3 | miR-25-3p | Diabetic retinopathy | Writer | Inhibition | Downregulation | Zha et al., 2020 |
| ALKBH5 | miR-181b-1 | Osteosarcoma | Eraser | Inhibition | Downregulation | Yuan et al., 2021 |
| ALKBH5 | miR-675 | / | Promotion | Upregulation | Hao et al., 2020 |
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...tural healing [Mi et al., 2020]. Some scholars believe that the potential m6A modification sites of miR-134 and miR-146a may affect the maturation process of corresponding primary miRNAs, which promote the occurrence and development of epilepsy [Jimenez-Mateos et al., 2012]. METTL3-mediated m6A methylation increases the maturation of miR-335, promotes the formation of stress granules and reduces the apoptosis level of damaged neurons and cells [Si et al., 2020]. METTL3 promotes the maturation of miR-873-5p, which regulates the KEAP1-NRF2 pathway and fights against the oxidative stress and apoptosis induced by colistin [Wang J et al., 2019]. METTL3 positively regulates the maturation of miR-25-3p in retinal pigment epithelium (RPE) cells, and this mechanism plays a protective role in the death of RPE cells induced by high glucose and alleviates diabetic retinopathy [Zha et al., 2020]. m6A modification can regulate the expression of H19 and miR-675 and induce cell apoptosis [Hao et al., 2020].

Although the number of relevant studies is not as high as for METTL3, a considerable number of literatures also indicate that METTL14 plays a similar role in this mechanism. METTL14-mediated m6A methylation facilitates the maturation of miR-126 and inhibits hepatocellular carcinoma metastasis [Ma et al., 2017]. METTL14-mediated m6A methylation also suppresses colorectal cancer (CRC) cell growth via the miR-375/Yes-associated protein 1 (YAP1) pathway, as well as inhibits CRC cell migration and invasion through the miR-375/SP1 pathway [Chen X et al., 2020]. Overexpressed METTL14 leads to the increase of miR-146a-5p, which promotes the migration and invasion of the breast cancer cells [Yi et al., 2020]. In atherosclerosis, METTL14 increases the maturation of miR-19a to promote the proliferation and invasion of atherosclerotic vascular endothelial cells [Zhang BY et al., 2020].

Similarly, it is not difficult to infer that eraser plays the opposite role in the above pathway because of its opposite role to writer. ALKBH5 reduces the m6A methylation of pre-miR-181b-1, making it unable to be recognized by m6A binding protein YTHDF2, reducing RNA degradation, thus increasing the inhibitory effect on YAP and inhibiting the progression of osteosarcoma [Yuan et al., 2021].

But it is worth noting that not all m6A methylation modifications act on pri-miRNA to promote its shear, m6A also acts on pre-miRNA to inhibit its maturation. A study has shown that METTL3 inhibits the production of miR-320 by increasing the m6A modification of pre-miR-320, enhancing the effect of Runx-related transcription factor 2 (RUNX2), a key transcription factor for osteoblast differentiation and bone formation, and inhibiting the occurrence of osteoporosis [Yan G et al., 2020]. Comparatively, there are few literatures on this mechanism for reference, but it still suggests that m6A modification seems to have completely opposite regulatory effects in different stages of miRNA maturation. There may be a deeper regulatory mechanism in the regulation between m6A and miRNA waiting for exploration.

**lncRNA**

lncRNA is a kind of ncRNA which is more than 200 nucleotides long. With our better understanding of the genome, more and more evidence supports the role of lncRNA in cell proliferation, differentiation, and the occurrence of human cancer. As early as 2018, Kopp and Mendell [2018] provided a systematic review of the basic functions of lncRNA. lncRNA can be divided into cis-lncRNA and trans-lncRNA. The cis-lncRNA regulates the expression and/or chromatin state of nearby genes, while trans-lncRNA can be transported into the cytoplasm. The biological function of trans-lncRNA can be summarized in 3 parts: (1) it can regulate chromatin states and gene expression at regions farther from transcription than cis-lncRNA; (2) some trans-lncRNAs influence nuclear structure and organization; (3) it can bind with other RNA or protein molecules and regulate their stability or function. The lncRNAs that can sponge target miRNA and restrain its function are also called competing endogenous RNAs (ceRNAs) [Tay et al., 2014]. The adsorbed miRNA could not bind to target mRNA, thus enhancing the translation level of target mRNA. The lncRNA-miRNA-mRNA axis has been shown to exert oncogenic effects in a variety of cancers [Jin et al., 2019; Shang et al., 2019; Zheng et al., 2019; Chen S et al., 2020; Wang et al., 2020; Zuo et al., 2020] (Table 2).

**Function of m6A Modification in lncRNA**

Research in recent years has found that the m6A modification is common in lncRNAs. A deep learning model called TDm6A was developed to predict RNA m6A modifications in human cells. The results showed that m6A modifications might be enriched in the middle region of lncRNA sequences, and lncRNAs have been prioritized for high-level m6A modifications [Wang and Wang, 2020]. In CRC tissue, a total of 8,332 m6A peaks were detected within 6,690 lncRNAs. Comparatively, 7,064 m6A peaks were also detected within 5,513 lncRNAs in paired normal tissue, suggesting that the m6A level of lncRNA is different between the cancerous tissues and the normal tissues [Zuo et al., 2020]. The role of m6A modification in lncRNAs has not been fully elucidated. Some studies...
found that the modification of m6A on lncRNAs can affect its binding ability to RNA-binding proteins (RBPs), mainly m6A “reader,” and regulate lncRNAs degradation [Ni et al., 2019; Zhu et al., 2019; Chen S et al., 2020; Liu H et al., 2020]. In 2015, Liu et al. reported that the 2,577-m6A residue of MALAT-1 by METTL3 destabilizes the stacking properties of the region centred around the U residue, which increases the U-tract accessibility and enhances heterogeneous nuclear ribonucleoprotein C (HNRNPC) binding by 3–4 times [Liu et al., 2015] (Fig. 4a). It is the first report about the effect of m6A modification on the spatial structure of lncRNAs and their binding ability to RBPs. However, there was not sufficient evidence to prove m6A modification as a general mechanism to regulate the spatial structure of lncRNAs.

Upregulation of m6A-Modified lncRNA Can Act as ceRNA and Sponge Target miRNA

In various cancer cells, m6A modification can stabilize lncRNA. Those lncRNAs can act as ceRNA and sponge target miRNA to promote downstream mRNA translation (Fig. 4b). The lncRNA-miRNA-mRNA axis is a basic mechanism in cancer initiation and development, and one of the most typical examples is non-small cell lung cancer (NSCLC). METTL3 is highly expressed in various cancer cells which can modify lncRNA through m6A mechanism. In lung cancer tissues, high expression of METTL3 increases the level of m6A of MALAT1 and its stability. As a ceRNA, MALAT1 sponges miR-1914-3p and further improves the expression of YAP, finally inducing NSCLC drug resistance and metastasis [Jin et al.,

| Proteins | Cancer | Role of IncRNA | lncRNA | miRNA | mRNA | Reference |
|----------|--------|----------------|--------|-------|------|-----------|
| METTL3   | NSCLC  | Oncogenic      | MALAT1*| miR1914-3p | YAP | Jin et al., 2019 |
|          | GC     | –              | LINC00470| –      | miR-145 | FAK | Liu P et al., 2020 |
|          | EOC    | Oncogenic      | RHPN1-AS1*| miR-596 | FAK | Wang et al., 2020 |
|          | CRC    | Oncogenic      | RP11* | –      | Zeb1 | Wu et al., 2019 |
|          | GC     | Oncogenic      | ARHGAS-AS1 | –      | ARHGAP5* | Zhu et al., 2019 |
| Myelocytic leukemia | Oncogenic | Olfr29-psl* | miR-214-3p | –      | MyD88 | Shang et al., 2019 |
| HCC      | Oncogenic | LINC00958* | miR-3691-5p | –      | HDGF | Zuo et al., 2020 |
| NPC      | Oncogenic | FAM225A* | miR-590-3p | –      | FAK | Zheng et al., 2019 |
| CC       | Oncogenic | ZFAS1* | miR-647 | –      | – | Yang et al., 2020 |
| METTL3, METTL14 | HNSCC | Oncogenic | LNCAROD* | –      | YBX1 | Ban et al., 2020 |
| METTL14  | BC     | Oncogenic      | LNC942* | –      | CXCR4*, CY1P1B1* | Sun et al., 2020 |
| VIRMA    | PCa    | Oncogenic      | CATT1/2* | –      | – | Barros-Silva et al., 2020 |
| KIAA1429 | HCC    | Oncogenic      | GATA3-AS | –      | GATA3* | Lan et al., 2019 |
| ALKBH5   | OS     | Oncogenic      | PVT1* | miR-195 | BCL2, CCND1, FASN | Chen S et al., 2020 |
|          | Glioblastoma | Oncogenic | FOXM1-AS | –      | FOXM1* | Zhang et al., 2017 |
|          | CRC    | Suppressor     | GASS* | –      | YAP | Ni et al., 2019 |
|          | Oncogenic | NEAT1* | –      | – | Guo et al., 2020 |
| METTL14, YTHDF1 | AML  | Oncogenic      | MALAT1 | –      | PML-RARα* | Chen ZH et al., 2020 |

The asterisks (*) indicate m6A modifications. NSCLC, non-small cell lung cancer; GC, gastric cancer; EOC, epithelial ovarian cancer; CRC, colorectal cancer; HCC, hepatocellular carcinoma; NPC, nasopharyngeal carcinoma; CC, cervical cancer; HNSCC, head and neck squamous cell carcinoma; BC, breast cancer; PCa, prostate cancer; OS, osteosarcoma; AML, acute myeloid leukemia.
In renal fibrosis patients with obstructive nephropathy, the m6A modification on MALAT1 by METTL3 increases its stability and induces epithelial-mesenchymal transition (EMT). The MALAT1/miR-145/FAK (focal adhesion kinase) pathway plays an important role in TGF-β1-induced renal fibrosis [Liu P et al., 2020]. FAK promotes tumor progression and metastasis through effects on cancer cells as well as stromal cells of the tumor microenvironment [Sulzmaier et al., 2014], and EMT is a crucial step of the transformation of normal cells into tumor cells [Lamouille et al., 2014], providing extra evidence of MALAT-1’s regulatory functions.

Similar lncRNA-miRNA-mRNA axis has also been reported in other cancer. In CRC tissues, m6A-induced lncRNA RP11 by METTL3 can trigger the dissemination of CRC cells [Wu et al., 2019]. In myeloid-derived suppressor cells (MDSCs), IL6-mediated m6A modification of lncRNA Olfr19-ps1 not only enhances itself, but also facilitates interaction between Olfr29-ps1 and miR-214-3p, promoting the differentiation and development of MDSCs through Olfr29-ps1/miR-214-3p/MyD88 regulatory network [Shang et al., 2019]. In hepatocellular carcinoma (HCC) cells, METTL3-mediated m6A modification led to LINC00958 up-regulation through stabilizing its RNA transcript. The LINC00958 sponged miR-3619-5p to upregulate hepato-ma-derived growth factor expression, finally facilitating HCC progression [Zuo et al., 2020]. High m6A level of lncRNA FAM225A induced by METTL3 promotes NPC cells proliferation, migration, and invasion by acting as a ceRNA that sponges miR-590-3p and miR-1275, leading to enhanced integrin β3 (ITGB3) expression and activation of the FAK/P13K/Akt pathway [Zheng et al., 2019].

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Overexpression of ALKBH5 inhibits degradation of lncRNA PVT1 via decreasing its m6A level, which contributes to the tumorigenesis of osteosarcoma [Chen S et al., 2020].

circRNA

circRNAs are a class of noncoding RNA molecules without a 5' end and a 3' end poly(A) tail, which form a circular structure by covalent bonding. They are generated by a reverse splicing mechanism with a covalent closed-loop structure. Unlike conventional splicing, circRNAs are usually formed by ligation of the upstream 3' acceptor and the downstream 5' donor, which is called "reverse splicing" [Chen and Yang, 2015]. circRNAs play many roles in several cellular physiological processes, such as mRNA sponges or decoys, protein sponges or decoys, protein function enhancers, protein scaffolds, protein recruiters, and translation templates [Kristensen et al., 2019]. Numerous studies have shown that circRNAs are widely expressed in all human tissues, and circRNAs have been found to be involved in the regulation of neuronal function, cell proliferation and transformation, and innate immunity through different molecular mechanisms [Chen, 2020]. In addition, dysregulation of circRNA expression is closely associated with human diseases such as cancer and cardiovascular diseases, neurodegenerative diseases and eye diseases [Zhang et al., 2020b].

The m6A modification is the most common type of eukaryotic methylation [Zhang H et al., 2020]. Although there have been several studies on the effect of m6A modification on circRNA, further studies are needed on the effect of circRNA related to methylation modifications. In this part, we summarize the effects of circRNAs' m6A modification in respect of molecular function. The introduction of the m6A modification on circRNA will be carried out from 4 aspects. Firstly, it has been widely demonstrated that circRNAs can act as binding sites (miRNA sponges) and thus regulate the activity of miRNAs on other target genes. Secondly, circRNA can be translated and act as protein-encoding [Du WW et al., 2017]. Thirdly, m6A affects circRNAs that act as protein sponges or form protein scaffolds. Finally, there are other/unclear mechanisms for the effect of m6A on circRNA.

m6A Affects circRNAs That Act as miRNA Sponge

The most classical pathway for circRNAs to perform specific functions is by acting as ceRNAs. circRNAs with miRNA response elements (MREs) can bind specific miRNAs to negatively regulate their activity. Specifically, circRNA affect miRNA activity through sequestration, thereby indirectly mediating the posttranscriptional up-regulation of miRNA target mRNAs [Thomson and Dinger, 2016]. CDR1as/ciRS-7, containing more than 70 MREs of miR-7, was the first circRNA identified as a miRNA sponge [Hansen et al., 2013]. Several studies have found reduced levels of m6A in circRNAs in hypoxia-mediated pulmonary hypertension. m6A affects circRNA-miRNA-mRNA co-expression network under hypoxic conditions. At the same time, m6A circXpo6 and m6A circTmtc3 were first identified to be downregulated [Su et al., 2020]. In hepatitis B virus-associated HCC, high expression of METTL3 increases the m6A modification of circ-ARL3, thus making circ-ARL3 upregulated. Circ-ARL3 functions as a sponge and antagonizes the repressive effect of miR-1305 on the target oncogene population, thereby promoting the development of hepatitis B virus-associated HCC through the circ-ARL3/miR-1305 axis [Rao et al., 2021]. In sorafenib-resistant HCC cells, the m6A modification of specific adenosines on circRNA-SORE increases the RNA stability, leading to increased levels of circRNA-SORE in cells. circRNA-SORE binds miR-103a-2-5p and miR-660-3p by acting as a miRNA sponge, thereby competitively activating the Wnt/β-catenin pathway and inducing sorafenib resistance [Xu J et al., 2020].

m6A Affects circRNAs That Act as Translation Templates

Some circRNAs have protein-coding potential and the translation process can be driven by m6A modification [Pamudurti et al., 2017; Yang Y et al., 2017]. Typically, translation of RNA in eukaryotic cells requires the eukaryotic translation initiation factor 4F (eIF4F) complex, which consists of the 3 initiation factors eIF4A (helicase protein), eIF4E (m7G reader), and eIF4G (scaffolding protein) [Pelletier and Sonenberg, 2019]. On mRNA, these transcription initiation elements are located on the 5' end cap structure, so it is defined as a cap-dependent pathway. However, in the absence of a dissociative 5' end, this traditional cap-dependent pathway does not work in closed loop transcripts. Thus, circRNA translation relies on cap-independent mechanisms of translation initiation, such as the internal ribosomal entry site (IRES)-dependent pathway and the m6A-dependent pathway [Zhang L et al., 2020].

Some sequences were found to function as an IRES to drive circRNAs translation [Coots et al., 2017]. In addition, circRNAs contain a large number of m6A modifications that are sufficient to drive protein translation in a cap-independent manner mediated by m6A reader YTHDF3 and the translation initiation factors eIF4G2.
m6A Affects circRNAs That Act as Protein Sponges or Form Protein Scaffolds

It has been determined that dynamic tertiary structures of circRNAs enable them to interact with various proteins [Du et al., 2016]. The m6A-modified circRNA circNSUN2 is upregulated in CRC patients with liver metastasis. By forming circNSUN2/IGF2BP2/HMGA2 RNA-protein ternary complexes in the cytoplasm, circNSUN2 enhances the stability of HMGA2 mRNA and thus promotes the CRC metastatic process [Chen et al., 2019]. In addition, circNDUFB2 is downregulated in NSCLC tissues, and reduced circNDUFB2 promotes NSCLC cell growth and metastasis. m6A modification of circNDUFB2 enhances this effect. circNDUFB2 enhances TRIM25 and IGF2BPs (positive regulators of tumor progression and metastasis) by forming a TRIM25/circNDUFB2/IGF2BPs ternary complex to promote the ubiquitination and degradation of IGF2BPs [Li et al., 2021].

snRNA

snARNAs are a rich set of noncoding, nonpolyadenylated transcripts that perform their functions in the nucleoplasm [Matera and Wang, 2014]. snARNAs are the main component of RNA spliceosomes during post-transcriptional processing in eukaryotes and are involved in the processing of mRNA precursors. The spliceosome is composed of 5 small nuclear ribonucleoprotein particles (snRNPs) and an additional group of non-snRNP protein splicing factors [Lamond, 1993; Valadkhan, 2005; Didychuk et al., 2018]. snRNPs consisting of snRNAs (U1, U2, U4, U5, and U6) and protein are the key components of the spliceosome and are absolutely required for the removal of introns from nuclear pre-mRNA. All 5 spliceosomal snRNAs are extensively posttranscriptionally modified [Karijolich and Yu, 2010]. m6A modifications have been identified on U6snRNA, which may have critical impact in mRNA splicing and biogenesis.

snRNA m6A Modification Affects the Splicing of mRNA

Human U6 snRNA is required for splicing of pre-mRNAs. It has been confirmed that U6 snRNA carries a single m6A at position 43 [Shimba et al., 1995]. Recently, it was found that methylation on U6 snRNA is mediated by the m6A methyltransferase METTL16 and its eukaryotic homologue [Pendleton et al., 2017; Warda et al., 2017]. U6-m6A43 modifications are introduced in the early stages of U6 snRNP biogenesis, occurring simultaneously with the 5’ end capping [Warda et al., 2017]. The modified sequence is paired with the base at the 5’ splicing site of the pre-mRNA. Mutations at this modified site have been found to block snRNA-mRNA interaction, which implies that U6-m6A43 may play an important role in mRNA splicing and biogenesis by fine-tuning snRNA-pre-mRNA interactions to regulate 5’ splicing site recognition or splicing body assembly [Warda et al., 2017; Doxtader et al., 2018].

rRNA

rRNA is an important component of ribosomes which are necessary for the translation of cellular proteins. Ribosomes consist of 4 rRNAs and 79 ribosomal proteins arranged into a large subunit and a small subunit in eukaryotes [Henras et al., 2015; Sloan et al., 2017]. The small subunit (40S) is composed of 18S rRNA and 33 ribosomal proteins, whereas the large subunit (60S) is composed of 5S, 5.8S, and 25S (yeast)/28S rRNAs (human) [Henras et al., 2015]. There are 3 main modifications on rRNA: the conversion of uridine to pseudouridine, the methylation of ribose 2’-hydroxyl, and nucleotide base methylation [Polikanov et al., 2015]. These modifications affect the structure and function of rRNA, thus ensuring the stability and accuracy of translation [Decatur and Fournier, 2002; Henras et al., 2015; Polikanov et al., 2015]. So far, 2 m6A methylation modifications have been found in rRNA, which are modified by 2 different enzymes: the CCHC zinc finger-containing protein ZCCHC4 and the methyltransferase METTL5.

m6A Methylation Modifications Affect the rRNA

At present, research has identified 2 types of m6A modifications on rRNA, one at position 4220 of 28S rRNA and the other at 1832 of 18S rRNA, which are mediated by ZCCHC4 and METTL5, respectively [Ma et al.,
The modification of m6A induced by METTL5 and ZCCHC4 is highly specific and both act on rRNA exclusively [Ignatova et al., 2020].

ZCCHC4 mainly methylates human 28S rRNA and also interacts with a portion of mRNA. ZCCHC4 has no substantial effect on the processing of pre-rRNA and rRNA maturation [Ma et al., 2019; Ignatova et al., 2020]. ZCCHC4 is localized in the nucleoli and interacts with a portion of mRNA [Ma et al., 2019; Pinto et al., 2020]. The absence of 28S rRNA m6A modification would reduce the strict control of mRNA translation, suggesting that ZCCHC4 may affect global translation through its methylation activity. Recent studies have found that ZCCHC4 knockout cells significantly reduced tumor size in xenograft mouse models and also found ZCCHC4 protein overexpression in HCC tissues compared with paracancer tissues in HCC patients, underscoring the important role of rRNA m6A modification in cancer cell growth and tumor progression [Ma et al., 2019].

METTL5 has also been identified as the writer of rRNA m6A modification, responsible for the m6A1832 methylation of human 18S rRNA [Ma et al., 2019; van Tran et al., 2019]. METTL5 must form a heterodimer complex with the multifunctional methyltransferase subunit TRM112-like protein (TRMT112) in order to be activated [van Tran et al., 2019; Ignatova et al., 2020]. As ZCCHC4, METTL5 is not essential for mature rRNA production and the deposited modifications [van Tran et al., 2019]. Although there has been less relevant research in human physiology or pathology, there have been some relevant animal experiments that could provide direction for future research. It has been found that METTL5-related m6A modification on rRNA is important for pluripotent type and correct differentiation of mouse embryonic stem cells. After knockout of METTL5, the translation rate and the number of S-stage cells decreases, early apoptotic cells increase, the differentiation potential decreases, and behavioral abnormalities occur [Ignatova et al., 2020]. Richard et al. [2019] found that METTL5 protein is enriched in the nucleus and synapse of human hippocampal neurons and proved that METTL5 variation is the basis of autosomal recessive intellectual disability and microcephaly in humans. To reveal its molecular mechanism, Leismann et al. [2020] found that drosophila CG9666 protein, a homolog to human METTL5, is responsible for m6A deposition on 18S rRNA, affecting the flight behavior of drosophila, and its deletion can cause drosophila neurological defects, which may provide a potential molecular mechanism for human intellectual disability.

**Regulation of m6A by ncRNA**

**miRNA**

Like the mRNAs of other genes regulated by miRNAs, miRNAs regulate the m6A modification level in vivo in most ways by complementing the 3'UTR binding of mRNAs of m6A-related enzymes, inhibiting the translation (Fig. 3a). Experiments have proved that miRNAs can regulate the m6A level in vivo through the above mechanism, which can greatly affect the physiological process of the body and the occurrence and development of diseases.

Numerous studies have shown that miRNA affects writers through the above mechanism. miR-33a targets the 3'UTR of METTL3 mRNA and reduces the expression of METTL3 to weaken the proliferation of NSCLC cells [Du et al., 2017]. miR-338-5p inhibits the expression of METTL3 in INA549 and H520 cells, enhancing the invasion and metastasis of lung cancer [Wu et al., 2021]. miR-600 inhibits lung cancer via downregulating the expression of METTL3 [Wei et al., 2019]. miR-4429 inhibits gastric cancer proliferation and enhances apoptosis by reducing the expression of METTL3 [He et al., 2019]. Metformin can inhibit breast cancer cell proliferation through the miR-483-3p/METTL3/m6A/p21 pathway [Cheng et al., 2021]. Mono-(2-ethylhexyl)phthalate (MEHP) up-regulates expression of miRNAs (miR16-1-3p, miR101a-3p, miR362-5p, miR501-5p, miR532-3p, miR542-3p) to inhibit the expression of METTL14, affecting cholesterol efflux and atherosclerosis [Park et al., 2020].

Similarly, there are also reports showing that miRNA has a similar mechanism for readers. The has-miR-346 reduces the expression of YTHDF1 mRNA, improving the prognosis of glioma patients and reducing the probability of recurrence [Xu C et al., 2020]. miR-145 targets the 3'UTR of YTHDF2 mRNA and decreases the expression of YTHDF2, promoting the degradation of m6A-modified mRNAs and inhibiting the proliferation of HCC cells [Yang Z et al., 2017]. The overexpression of miR-493-3p inhibits the translation of YTHDF2 by targeting the 3'UTR, which promotes the degradation of m6A-modified mRNAs and finally terminates the progression of prostate cancer [Li et al., 2018].

It is not difficult to see that in a considerable number of disease processes, not only m6A modification can regulate the miRNA level to a certain extent, but miRNA also regulates m6A modification in turn.

**Feedback Composed of m6A and miRNA**

Interestingly, in the course of some diseases, m6A modifications regulated by miRNA can regulate some
molecules which affect the miRNA to form a feedback. For example, in breast cancer, HBXIP regulates METTL3 by inhibiting miRNA let-7g, which downregulates the expression of METTL3 by targeting the 3′ UTR of METTL3, and METTL3 promotes the expression of HBXIP by modifying m6A. So, HBXIP upregulates METTL3 by inhibiting let-7g, and the expression of HBXIP is upregulated by METTL3 to form a positive feedback loop of HBXIP/LET-7g/METTL3/HBXIP, leading to accelerated proliferation of breast cancer cells [Cai et al., 2018]. In addition, studies have shown that miR-103-3p can negatively regulate the expression of METTL14 and the level of m6A in osteoblasts, thereby inhibiting bone formation and increasing the probability of fracture and osteoporosis. METTL14, in turn, can affect miR-103-3p processing by inhibiting DGCR8, exerting a negative feedback effect [Sun et al., 2021]. These studies suggest that there may be more complex regulatory networks involved in miRNA and m6A modifications, which may enable us to more clearly elucidate the molecular mechanisms of related diseases.

**IncRNA**

IncRNA can act as scaffold and regulate downstream molecular stability. The IncRNA scaffold with specific binding domain can recruit m6A writer to enhance the downstream mRNA’s m6A level (Fig. 4c). The expression of target mRNA depends on the RBP that recognizes it. In gastric cancer cells, IncRNA LINC00470 is highly expressed and suppresses PTEN mRNA stability by recruiting METTL3; then YTHDF2 identifies and mediates the degradation of m6A-modified PTEN mRNA [Yan J et al., 2020]. SQSTM1 is an autophagy receptor and can directly interact with IncRNA ARHGAP5-AS1, which is the IncRNA upregulated in chemoresistant gastric cancer cells, and transport it to the autophagosome for degradation. In chemoresistant gastric cancer cells, autophagy is inhibited, causing the upregulation of ARHGAP5-AS1, which activates the transcription of ARHGAP5 in the nucleus and stimulates m6A modification of ARHGAP5 mRNA to stabilize it in the cytoplasm by recruiting METTL3, further promoting chemoresistance [Zhu et al., 2019]. LNC942 recruits METTL14 via specific binding domain (+176 to +265) and stabilizing its downstream targets CXCR4 and CYP1B1, promoting breast cancer cell proliferation [Sun et al., 2020]. In glioblastoma stem-like cells (GSCs), antisense IncRNA FOXM1-AS promotes the interaction between the m6A “eraser” ALKBH5 and FOXM1, leading to the upregulation of FOXM1. Depleting ALKBH5 or FOXM1-AS disrupted GSC tumorigenesis through the FOXM1 axis in a mouse model [Zhang et al., 2017].

IncRNA GAS5 directly interacts with WW domain of YAP to facilitate YAP translocation to the cytoplasm and promotes degradation of YAP to inhibit CRC progression in vitro and in vivo. The m6A “reader” YTHDF3 is a key player in YAP signaling by facilitating METTL14-induced m6A-modified IncRNA GAS5 degradation [Ni et al., 2019]. In blood malignancies, MALAT1 regulates chimeric mRNAs’ export in an m6A-dependent manner and thus controls hematopoietic cell differentiation. MALAT1 and fusion protein complexes facilitate the interaction of chimeric mRNA and m6A “writer” METTL14, then the m6A “reader” YTHDC1 recognizes and exports the mRNA, promoting the expression of tumor fusion protein PML-RARA [Chen ZH et al., 2020].

In addition, antisense transcript-derived IncRNAs participate in various biological processes, such as heritable cell-specific alternative splicing of the parental pre-mRNAs by cis regulation [Gonzalez et al., 2015]. KIAA1429 is a kind of m6A “writer” that is highly expressed in HCC tissue. The IncRNA GATA3-AS acts as a cis-acting element for targeted m6A regulation of KIAA1429 on GATA3 pre-mRNA’s 3′UTR. Research showed that GATA3-AS knockdown markedly suppresses the malignant phenotypes of hepatoma cells [Lan et al., 2019].

**Conclusion**

In recent years, m6A modification and ncRNAs have been confirmed to be related to a variety of diseases, and an increasing number of studies have shown that the internal relationship between the two may play an important role in explaining the occurrence and development of many diseases. m6A modification can affect the biogenesis, structural stability, and regulatory networks of ncRNAs. It is even closely related to some cap-independent translation initiation mechanisms, which enable some ncRNAs to translate polypeptide structures. At the same time, some ncRNAs can also affect the process of m6A modification; for example, there is a proven feedback regulatory network between miRNA and ncRNAs. The interaction between m6A methylation and ncRNA can affect diverse life activities, including proliferation, invasion, and metastasis of cancer cells, the occurrence and development of inflammation, metabolic diseases, neurological and psychiatric diseases, drug resistance to disease, and differentiation, maturation, senescence, and...
apoptosis of cells. Since the antibody-based sequencing method came out in 2012 [Dominissini et al., 2012], the rapid development of relevant technology enabled a deeper understanding of the interaction between m6A and ncRNAs. However, at present, relevant studies still remain at the level of clarifying the mechanism, and the correlative clinical applications are mostly limited to the prediction of disease risk, the realization of early diagnosis, and the evaluation of prognosis effect, etc., so there is still a long way to go in clinical treatment and new drug development.

In conclusion, expounding the interaction between m6A modification and ncRNAs can help us better understand the development and aging of organisms and the occurrence and development of diseases, thus providing new insights and ideas for the diagnosis and treatment of various diseases.

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Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Shuangyi Chen, Jianxiang Dong, Xinjie Luo, and Zixiong Nie are responsible for the idea and writing of manuscript. Shanshan Lu and Hanqi Liu are responsible for the revision of manuscript. Junwen Liu is responsible for the total design and quality of manuscript.

Data Availability Statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.
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