


Progress and application of epitranscriptomic m⁶A modification in gastric cancer

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ABSTRACT

The relationship between epitranscriptomics and malignant tumours has become a popular research topic in recent years. N⁶-methyladenosine (m⁶A), the most common post-transcriptional modification in mammals, is involved in various physiological processes in different cancer types, including gastric cancer (GC). The incidence and mortality of GC have been increasing annually, especially in developing countries. Insights into the epitranscriptomic mechanisms of gastric carcinogenesis could provide potential strategies for the prevention, diagnosis, and treatment of GC. In this review, we describe the mechanisms of RNA m⁶A modification; the functions of m⁶A regulators in GC; the functional crosstalk among m⁶A, messenger RNA, and noncoding RNA; and the promising application of m⁶A in the diagnosis and treatment of GC.

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1. Background

Epigenetic modifications occur in various macromolecules *in vivo*, such as DNA, nucleosomes, and histones. These modifications change the molecular phenotype while ensuring the invariance of molecular genotypes, resulting in changes in molecular gene functions. Epigenetics is the science that recognizes and explains this phenomenon [1]. The study of epigenetic modification of RNA molecules is known as epitranscriptomics. There are more than 160 post-transcriptional chemical modifications of RNA [2], which affect the metabolic function and interaction of RNA with other molecules without changing the RNA sequence. The term ‘epitranscriptome’ was first used by Saletore et al. [3] in 2012. In the last decade, epitranscriptomics has developed vigorously in various fields owing to the development and application of high-resolution mass spectrometry and whole-transcriptome sequencing technology.

The N⁶-methyladenosine (m⁶A) modification is the most common post-transcriptional modification in mammals. Approximately 0.1–0.4% of adenosines in RNA are m⁶A, accounting for approximately 50% of all methylated ribonucleosides [4]. m⁶A modifications are most common in messenger RNA (mRNA), accounting for up to 80% of RNA methylation. These methyl marks occur at the consensus sequence RRACH (R = A or G and H = A, C, or U) close to the transcript termination codons and the 3′ untranslated regions (3′ UTRs) [5,6].

The initial challenge in mechanistic studies of m⁶A was the lack of methods to determine the stoichiometry of m⁶A modifications; therefore, the role of epitranscriptomics in regulating the fate and function of mRNA transcripts was not completely accepted for many years. Although

liquid chromatography-tandem mass spectrometry (LC-MS/MS) and colorimetry, which are common methods for micro-molecular quantification, can detect the overall m⁶A level of RNA, neither can distinguish differential m⁶A levels in individual RNA regions, rendering them incapable of analysing m⁶A changes in specific locations [7]. The development of methylated RNA immunoprecipitation sequencing (meRIP-seq) in 2012 partly overcame this problem [5,8]. MeRIP-seq first uses an anti-m⁶A antibody to recognize and bind to RNA fragments for RNA immunoprecipitation (RIP). Then, the enriched m⁶A-positive RNA fragments are eluted and sequenced by RNA-seq. Finally, bioinformatic analysis is performed using a previously obtained library and another library as background controls to identify areas of m⁶A enrichment. Recently, other m⁶A mapping and measuring techniques have been developed, such as LAIC-seq, which utilizes differences in tandem alternative polyadenylation sites bound to methylated and non-methylated transcripts to quantify m⁶A levels on a transcriptome-wide scale [9]; miCLIP-seq, which maps m⁶A residues at single-nucleotide resolution and distinguishes m⁶A from N^{6,2}-O-dimethyladenosine (m⁶A_m) by creating antibody-RNA crosslinks through UV light [10]; and MAZTER-seq, which relies on the bacterial RNase MazF to cleave RNA and identify m⁶A sites without using anti-m⁶A antibodies [11].

Owing to the development of high-throughput sequencing techniques, m⁶A has also been found in many types of non-coding RNAs (ncRNAs), including long ncRNAs (lncRNAs), microRNAs (miRNAs), circular RNAs (circRNAs), and ribosomal RNAs (rRNAs) [2]. Moreover, three types of key regulators involved in the regulation of m⁶A have been confirmed: ‘writers’ (methyltransferases [MTases] or MTase

complexes), ‘readers’ (m⁶A RNA binding proteins), and ‘erasers’ (demethylases) [12]. Writers and erasers regulate m⁶A levels, whereas readers regulate the target RNA after m⁶A modification. In particular, the ability of m⁶A erasers to demethylate m⁶A in RNA confirms that m⁶A methylation is a dynamic and reversible process [13,14], which was another important factor in recognizing the field of epitranscriptomics, in addition to the development of mapping and measuring techniques.

Gastric cancer (GC) is one of the most common malignant tumours. In 2020, GC was the fifth (5.6%) most common tumour and the fourth (7.7%) deadliest among 36 types of tumours [15], indicating that GC is a serious threat to global human health. Identifying the underlying molecular mechanisms of GC will promote the advancement of effective detection methods and novel therapeutic strategies for GC patients with poor prognoses. The dysregulation of RNA m⁶A modification has been demonstrated to play an important role in development, metastasis, and other biological processes in various malignant tumours [16]. Therefore, this review describes the biological mechanisms and functions of RNA m⁶A modification in GC and the application of m⁶A modification in the diagnosis and treatment of GC, aiming to better understand the research status of epitranscriptomics in GC and forecast future research directions.

2. m⁶A modification system

2.1. m⁶A writers

Intracellular post-transcriptional modification of m⁶A methylation in eukaryotes, including humans, is catalysed by a multi-component m⁶A MTase complex in the nucleus. The MTase complex was initially isolated into three proteins approximately 30 kDa, 200 kDa, and 875 kDa in size, although only two had MTase activity [17,18]. It is now known that the ~200 kDa complex contains methyltransferase like 3 (METTL3) and methyltransferase like 14 (METTL14), whereas the ~875 kDa complex appears to facilitate the recruitment of METTL3 to RNA. Bokar et al [19] purified the 70-kDa subunit MT-A70 from the ~200 kDa portion, now known as METTL3, in 1997 using conventional chromatography and electrophoresis. They found that METTL3 contains sites that bind to S-adenosyl methionine, the methyl donor, and proved its high conservation in eukaryotes from yeast to humans. Later, Bujnicki et al [20] identified METTL14, the homolog of METTL3, through phylogenetic analysis. METTL14 shares 43% identity with METTL3. In 2014, Liu et al [21] revealed that METTL3 and METTL14 form a complex at a stoichiometric ratio of 1:1 by analysing the co-expression of these two proteins through 2D native SDS-PAGE analysis and confirmed the stability of the heterodimer. METTL3 and METTL14 co-localize in nuclear speckles in mammalian cells, where their heterodimers methylate adenines in nuclear RNA [21]. Functionally, METTL3 is primarily responsible for catalysing the transfer of the methyl group, whereas METTL14 assists in the localization of the MTase complex by recognizing histone H3 Lys36 trimethylation in a cotranscriptional manner and

provides a structural scaffold that enhances the catalytic activity of METTL3 [21–23]. Wilms tumour 1-associated protein (WTAP), a regulator involved in mammalian pre-mRNA splicing, is also a component of the m⁶A MTase complex [24–26]. WTAP helps the METTL3-METTL14 dimer localize to the nuclear speckle, thereby promoting adenine methylation in mRNA [27]. Although METTL14 and WTAP are catalytically inactive, their presence is essential for promoting the catalytic activity of the MTase complex and regulating m⁶A levels. If METTL3, METTL14, or WTAP is knocked down, m⁶A levels in eukaryotic cells are decreased to varying degrees, and the effects of METTL14 and WTAP knockdown on m⁶A levels is significantly greater than that of METTL3 knockdown [21]. Therefore, METTL3/METTL14/WTAP is recognized as the core component of the m⁶A writer complex.

Other components of the m⁶A writer complex, such as VIRMA, RBM15/RBM15b, and ZC3H13, appear to regulate RNA methylation through a close association with WTAP. VIRMA-mediated adenosine methylation occurs preferentially in the vicinity of the 3' UTR and stop codon and influences variable polyadenylation via polyadenylation cleavage factor and the m⁶A writer complex [28]. The RNA-binding protein RBM15 and its homolog RBM15b interact with METTL3 in a WTAP-dependent manner, and RBM15 is a key component of the m⁶A writer complex in *Drosophila* [29]. Recently, the zinc finger protein ZC3H13 was shown to be a bridging component between WTAP and RBM15 in the m⁶A writer complex in *Drosophila*, promoting efficient functional adenosine methylation and successful localization of the complex in the nucleus [30]. Other proteins or enzymes, such as CBL1 and PRMT1, interact directly or indirectly with the core catalytic component METTL3/METTL14/WTAP [31,32].

Recent studies indicate that although the METTL3/METTL14/WTAP writer complex is mainly responsible for the vast majority of m⁶A modifications in mRNAs, a significant portion of m⁶A modifications in ncRNAs are methylated by other m⁶A writers [33]. For example, methyltransferase like 16 (METTL16), which is homologous to the *Escherichia coli* YbiN protein, is responsible for the N6-methylation of A1618 in the 23S rRNA [34], and has been shown to methylate methionine adenosyl transferase 2A mRNA and the small nuclear RNA (snRNA) U6 [35,36]. Recently, Dai et al [37] reported that METTL16 could downregulate the lncRNA RAB11B-AS1 in an m⁶A-dependent manner, promoting hepatocellular carcinoma progression. In addition, unlike METTL3/METTL14/WTAP-mediated m⁶A modification, which occurs close to the stop codon, most METTL16 binding sites are in introns, suggesting that the mechanism of target recognition by METTL16 may be different [36]. rRNA is the most abundant RNA in human cells and contains the majority of m⁶A. A single m⁶A modification in human 28S rRNA is generated by ZCCHC4, whereas a single m⁶A modification in 18S rRNA is generated by the METTL5-TRMT112 complex, in which METTL5 acts as a catalytic subunit and TRMT112 acts as an allosteric adapter [38,39]. These atypical m⁶A MTase complexes may provide novel insights into the mechanism of m⁶A modification.

2.2. m⁶A readers

m⁶A-binding proteins read the m⁶A RNA modification to exert m⁶A-mediated biological activity. Dozens of readers have been discovered, some of which have similar amino acid sequences, such as the YT521-B homology (YTH) domain family proteins YTHDC1, YTHDC2, YTHDF1, YTHDF2, and YTHDF3, and proteins without the YTH domain, including heterogeneous nuclear ribonucleoprotein (hnRNP) family proteins hnRNPA2B1, hnRNPC, and hnRNPG and insulin-like growth factor 2 mRNA-binding proteins (IGF2BPs) IGF2BP1, IGF2BP2, and IGF2BP3. Other unique types of readers include eukaryotic initiation factor (eIF) 3 and fragile X retardation protein [5,12].

Readers have different downstream effects based on their subcellular localization (nucleus or cytoplasm) and recognition mechanisms (direct or indirect). In the cytoplasm, YTHDF2 accelerates the decay of m⁶A-modified transcripts and reduces their stability by directly recruiting the CCR4-NOT adenylate complex [40], whereas YTHDF1 interacts with eIFs and ribosomes to improve the translation efficiency of target m⁶A-modified mRNAs [41]. Intracellular YTHDF3 not only cooperates with YTHDF1 to promote the translation of m⁶A-modified mRNA but also directly interacts with YTHDF2 to accelerate the degradation of mRNA [42,43]. In addition, YTHDF3 and eIF4G2 participate in the initiation of m⁶A-modified circRNA translation [44]. However, a recent study suggests that all DF paralogs have similar functions. Through structural analysis, motif analysis, CLIP studies, and immunofluorescence, Zaccara et al [45] demonstrated that all DF proteins have highly similar sequences, functional domains, protein binding partners, and intracellular localizations. Therefore, like YTHDF2, the major effect of YTHDF1 and YTHDF3 is to promote mRNA degradation rather than translation. Hence, there is controversy remains over the function of YTHDF proteins, which needs to be verified by more studies in the future.

The functions of YTHDC1 in the nucleus include regulation of the exportation of m⁶A-modified mRNA, participation in mRNA splicing by recruiting the pre-mRNA splicing factor SRSF3 and inhibiting the binding of SRSF10 to mRNA, and priority identification of m⁶A residues on the lncRNA *XIST*, which mediates its silencing [46–48]. hnRNPC and hnPNPG in the nucleus bind to pre-mRNAs adjacent to the m⁶A modification and indirectly affect their splicing [49,50].

The functions and mechanisms of some m⁶A readers are not clear. They exist in both the nucleus and cytoplasm, and their affinity for m⁶A is weaker than that of other readers. They directly or indirectly affect cellular activities through their effect on m⁶A-modified RNAs. IGF2BPs and YTHDC2 are representative of these readers. The former enhances the stability of m⁶A-modified mRNA, whereas the latter is associated with mRNA degradation and regulation of translation initiation [51,52]. m⁶A readers are essential for the biological functions of m⁶A and the best entry point to study how m⁶A regulates the occurrence, development, and prognosis of diseases at the molecular level. The

discovery of more potential m⁶A readers and clarification of their related mechanisms will be helpful for the prevention and treatment of diseases in clinical practice.

2.3. m⁶A erasers

m⁶A erasers catalyse demethylation in a dynamic, rapid, signal-dependent manner, which is critical for the functional regulation of m⁶A modifications [53]. Fat mass and obesity-associated protein (FTO) was the first confirmed m⁶A eraser and is involved in the oxidative demethylation of 3-methylthymidine and 3-methyluracil in single-stranded DNA and RNA [13,54]. In mammals, FTO oxidizes m⁶A to N6-hydroxymethyladenosine (hm⁶A) as an intermediate modification and further oxidizes hm⁶A to N6-formyladenosine. These relatively stable intermediates eliminate the possibility of re-methylation mediated by the m⁶A writer complex at the same site and can be used as another modification to recruit different readers, which differentially regulate subsequent RNA-related pathways. Moreover, they can be used as markers for newly transcribed RNA by exploiting their intrinsic degradation kinetic [55].

FTO belongs to the α -ketoglutarate-dependent dioxygenase homolog (ALKBH) family, and several other family members are also m⁶A erasers. ALKBH5 was the second m⁶A eraser discovered in mammals. ALKBH5 catalyzes the demethylation of m⁶A in an Fe(II)- and α -ketoglutarate-dependent manner [14]. ALKBH3 mediates the demethylation of m⁶A-modified tRNA, which significantly improves the translation efficiency of certain mRNAs, a process closely related to tumour cell proliferation [56]. ALKBH1, an m⁶A demethylase, is upregulated in lung cancer cells and reduces the m⁶A level, thereby promoting the metastasis and invasion of lung cancer cells [57].

There are still many questions regarding how m⁶A erasers regulate post-transcriptional m⁶A demethylation and select demethylation sites in target RNAs. snRNAs may have a stronger affinity for m⁶A erasers than mRNAs, and erasers may be more closely associated with post-transcriptional epigenetic modifications other than m⁶A. For example, Mauer et al. [58] suggested that the catalytic efficiency of FTO was approximately 100-fold higher towards m⁶A_m than m⁶A based on the results of in vitro biochemical assays, which confirmed that m⁶A_m was the preferred cellular substrate for FTO in vitro. However, another study showed that m⁶A_m and m⁶A in the same RNA sequence had the same affinity for FTO, suggesting that the interaction of residues in the catalytic pocket with the nucleobase determines the activity and substrate specificity of erasers [59]. Moreover, the catalytic activity of FTO can be influenced by the sequence and tertiary structure of the RNA. Recent studies have demonstrated that some potential eraser-interacting proteins can influence the selection of target RNAs by erasers. Song et al. [60] reported that FTO interacts with the RNA-binding protein splicing factor, proline- and glutamine-rich (SFPQ) via its C-terminal domain and mediates the demethylation of SFPQ-

bound RNA. More potential eraser regulators and demethylation mechanisms need to be investigated in the future.

2.4. m⁶A dysregulation in GC

Many studies have shown epitranscriptomic dysregulation of m⁶A in GC tissues, with most showing increased m⁶A compared to that in paired adjacent normal tissues (Table 1 highlights the results of some of these studies). However, the role of m⁶A in carcinogenesis and the development of GC is unclear. Multiple studies have also validated abnormal expression of some m⁶A regulators, which is associated with the malignant biological properties of GC. These findings can help us understand the potential mechanism of m⁶A in the occurrence, development, and metastasis of GC [61].

Writers are currently the most widely studied m⁶A regulators in GC. Yue et al. [62] found that GC patients with high METTL3 expression had a shorter median survival and more advanced TNM stage and were more likely to have diffuse-type disease compared to those with low METTL3 expression, and this corresponded to elevated m⁶A levels. By analysing big datasets acquired from The Cancer Genome Atlas (TCGA) and exploring METTL3 expression in GC cell lines and tumour tissues, Liu et al. [63] also reached the conclusion that METTL3 was significantly increased in GC tissues and correlated with poor prognosis. Many other studies suggest that METTL3 is an oncogene in GC, and transcription factors such as P300 and HBXIP might be involved in inducing METTL3 transcription [64,65]. Two other components of the m⁶A writer complex, WTAP and RBM15, are both highly expressed in GC; WTAP expression is associated with worse outcomes, and RBM15 expression is associated with better survival [66,67]. By contrast, METTL14 is downregulated in GC cells and tissues [68]. This may be because METTL14 can suppress the progression of GC by deactivating the PI3K/AKT/mTOR pathway and epithelial-mesenchymal transition (EMT) through regulating m⁶A modification. Through gene set enrichment analysis and in vitro experiments, Zhang et al. [69] demonstrated that METTL14 could be a potential tumour suppressor by blocking Wnt and PI3K/AKT signalling. In addition, they demonstrated that reduced m⁶A (represented by METTL14 knockdown) promotes the proliferation and invasiveness of GC cells, which could be reversed by m⁶A elevation (represented by FTO knockdown). m⁶A regulators seem to directly

control the PI3K/AKT/mTOR signalling pathway, suggesting their potential as targets for antitumor therapy. The writer METTL5 was also proved to be significantly decreased in GC in a study of 168 GC patients [70]. As a tumour suppressor gene, high METTL5 protein expression is closely related to better prognosis ($P = 0.0051$ based on 168 GC samples, $P = 0.048$ based on TCGA, and $P = 0.00042$ based on the Gene Expression Omnibus [GEO]). However, the pathways regulated by METTL5 in GC remain to be elucidated [70].

Increased expression of m⁶A erasers, such as FTO, is associated with the tumorigenesis and progression of GC. Xu et al. [71] confirmed that FTO mRNA ($P < 0.001$) and protein levels (IHC: $P = 0.023$; WB: $P = 0.024$) were significantly elevated in GC tissues compared to corresponding adjacent non-tumour tissues. Moreover, high FTO expression was closely related to poor differentiation ($P < 0.001$), lymph node metastasis ($P = 0.029$), TNM stage ($P < 0.001$), and poor prognosis ($P < 0.001$). Li et al. [72] reported that high FTO ($P = 0.0017$) and ALKBH1 ($P = 0.0241$) levels were closely related to worse overall survival in GC patients; however, FTO and ALKBH1 were significantly downregulated at the protein level in GC tissues and closely correlated with clinicopathological features and prognosis of GC patients. The expression pattern of ALKBH5 in GC remains unclear. Shimura et al. [73] and Hu et al. [74] reported decreased ALKBH5 levels in GC samples, which were correlated with worse survival (both $P < 0.001$), but Jing et al. [75] and Zhang et al. [76] showed that GC patients with ALKBH5 overexpression had poor prognosis. Similar arguments exist regarding the expression of other m⁶A regulators. Therefore, multi-omics and multicenter studies are needed to further clarify the differential expression of m⁶A regulators in GC.

Readers are also dysregulated in GC, which leads to biological changes in an m⁶A-dependent manner. According to one analysis based on datasets from TCGA, the levels of *YTHDF1/2/3*, *YTHDC1*, *hmRNPC*, and *hmRNPA2B1* were significantly increased in GC [77]. GEO dataset analysis showed that *YTHDF1* was significantly upregulated in three of five GEO datasets; *hmRNPC*, *hmRNPA2B1*, *YTHDC1*, and *YTHDF3* were significantly upregulated in one of five GEO datasets, but *YTHDF2* was not significantly altered in any of the datasets examined [77]. IHC analysis verified that *hmRNPC* and *YTHDF1/2/3* were overexpressed at the protein level, whereas *hmRNPA2B1* and *YTHDC1* were not differentially expressed in GC. Moreover, there was no significant relationship between the expression of m⁶A readers and prognosis in GC [77]. Different readers have been reported to have different biological functions in GC, which makes it difficult to determine the clinical value of readers as biomarkers. The functions of GC-related m⁶A readers, as well as their target RNAs, are explained in detail in Chapter 3 and shown in Figure 1.

As mentioned above, the increase in total RNA m⁶A levels in GC is a complex result of differential expression of writers, readers, and erasers. It is not possible to explain the oncological mechanisms of m⁶A in GC by studying the abnormal expression of one or two m⁶A regulators because of the complexity and heterogeneity of GC. Various m⁶A writers, erasers, and

Table 1. Changes in total RNA m⁶A in GC.

Samples	Quantitative method	Change in m ⁶ A ^a	References
12 pairs of cancerous and adjacent tissues	colorimetry	elevated	Sun et al
5 pairs of cancerous and adjacent tissues	LC-MS/MS	elevated	Yue et al
17 pairs of cancerous and adjacent tissues	colorimetry	elevated	Liu et al
5 pairs of cancerous and adjacent tissues	colorimetry	elevated	Hu et al
40 pairs of cancerous and adjacent tissues	colorimetry	elevated	Zhou et al

^a Levels in GC tissues compared to those in adjacent tissues

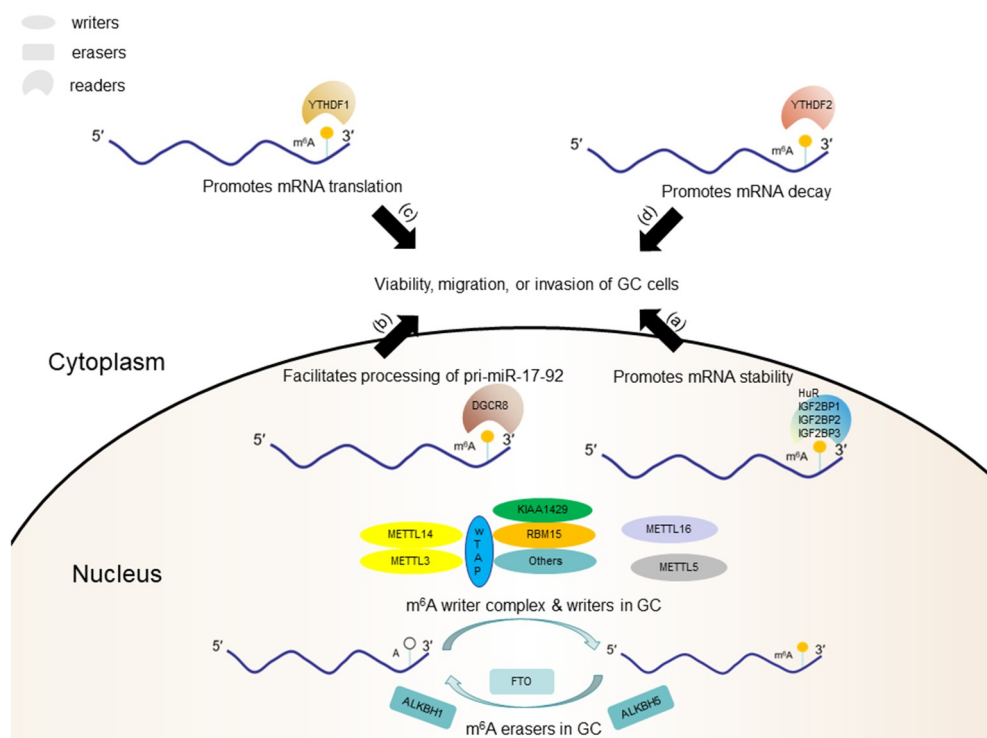


Figure 1. m⁶A regulators in GC cell

Different m⁶A regulators have been confirmed to get involved in the process and molecular functions of RNA m⁶A modification in GC. Writers (including METTL3, METTL14, WTAP, RBM15, et al) and erasers (including FTO, ALKBH1, ALKBH5) make RNA m⁶A modification a dynamic and reversible process in GC cells. And readers are responsible for different downstream effects of m⁶A in GC cells, such as (a) HuR, IGF2BP1, IGF2BP2 and IGF2BP3 in the nucleus facilitate the proliferation, metastasis, or EMT program of GC cells by promoting target mRNA stability; (b) DGCR8 facilitates processing of m⁶A modified pri-miR-17-92, which promotes the proliferation of GC cells; (c) The deficiency of YTHDF1, which regulates FZD7 and USP14 translation, inhibits the proliferation and metastasis of GC cells; (d) YTHDF2 in the cytoplasm enhances GC cell proliferation, migration and invasion by promoting PTEN mRNA decay.

readers are involved in the biological progression of GC and maintain a relationship with each other. In addition, although abnormal m⁶A levels in the transcriptome are known to be related to GC, more work is needed for m⁶A levels to be a viable biomarker because their increase and decrease are affected by many different factors, which makes them unreliable and inaccurate. To advance the study of m⁶A regulators in GC, we should pay attention to the specific downstream molecules regulated by abnormally expressed enzymes and their functions.

3. Biological functions of m⁶A RNA modifications in GC

3.1. Regulation of mRNA

The epigenetic modification of oncogenic and tumour suppressor genes is considered a novel mechanism related to the occurrence, development, and outcome of GC. The role of m⁶A modification in the regulation of gene expression depends on the mRNA, the locations of m⁶A modification, and the involved m⁶A regulators [78]. Myelocytomatosis viral oncogene homolog (MYC) is an oncogene associated with advanced tumour stage, diffuse type, deeper tumour extension, and lymph node metastasis in GC [79]. A recent study showed that the m⁶A eraser FTO could stabilize MYC mRNA through m⁶A demethylation in GC cells, which enhanced

their ability to proliferate, migrate, and invade [80]. Upstream of the FTO/m⁶A/MYC axis, another oncogene, histone deacetylase 3, promotes GC progression by degrading forkhead box transcription factor A2 (FOXA2), and FOXA2 directly mediates FTO transcriptional repression. In contrast, MYC methylation by METTL3 in the 3' UTR enhances MYC translation and stability, which promotes proliferation, migration, and invasion in GC cells [64]. Therefore, different m⁶A modification sites in MYC mRNA exhibit different functions [81]. Identifying precise m⁶A positions on a transcriptome-wide level is important for exploring the functions of different m⁶A sites on the same mRNA. Fortunately, novel detection techniques, such as miCLIP-seq and MAZTER-seq, can map m⁶A residues at single-nucleotide resolution [10].

Many other m⁶A-modified mRNAs participate in the biological processes of GC. The methylation of zinc finger MYM-type containing 1 by METTL3 enhances its stability, which facilitates GC EMT and metastasis [62]. Additionally, METTL3 methylates hepatoma-derived growth factor, which enhances its stability and recognition by IGF2BP3, which leads to tumour angiogenesis [65]. Conversely, METTL3 knockdown leads to increased basic leucine zipper ATF-like transcription factor 2 (BATF2) expression in GC cells, because m⁶A modification suppresses BATF2 translation [82]. High expression of YTHDF1 leads to an increase in the translation of frizzled 7 (FZD7), a key Wnt receptor, in an m⁶A-dependent manner [83]. As the expression of FZD7

increases, the Wnt/ β -catenin pathway becomes hyperactivated, which contributes to gastric carcinogenesis. PKMYT1, an oncogene that plays a role in the growth of multiple tumours, is a target of ALKBH5 in GC [74]. With the significant downregulation of ALKBH5 in GC, the m⁶A levels of *PKMYT1* are increased, increasing its recognition by IGF2BP3 and enhanced stability. This increased PKMYT1 promotes invasion and migration in GC. Furthermore, one study revealed a special function of mRNA m⁶A modification in GC, indicating that methylation of E2F transcription factor 3 (*E2F3*) at the 3' UTR mediates the inhibitory effect of miR-660 on *E2F3* [84]. This m⁶A modification is required for the interaction of miR-660 and *E2F3*. The mechanisms by which GC-related mRNAs are regulated by m⁶A modification are summarized in Table 2.

3.2. Regulation of ncRNA

High-throughput technology has verified that ncRNAs, such as lncRNAs, miRNAs, and circRNAs [85–87], are involved in tumorigenesis, progression, and metastasis in GC. Indeed, methylation of RNAs, including ncRNAs, has been recognized as a unique 'epitranscriptomic language' in oncology, although its mechanisms are not completely understood [88]. Recently, Hu et al. [89]

found that m⁶A could upregulate the lncRNA *LINC01320*, which promotes the proliferation, migration, and invasion of GC cells, implying that GC-related ncRNAs can be regulated by m⁶A modification. Pulldown assays and RIP experiments verified that *LINC01320* is methylated by METTL14. Zhang

et al. [76] determined that demethylation of the lncRNA *NEAT1* by ALKBH5 increases *NEAT1* levels and promotes GC invasion and metastasis. Sun et al. [97] showed that the methylation of *pri-miR-17-92* by METTL3 facilitates its processing into the miR-17-92 cluster in an m⁶A/DGCR8-dependent manner and that the miR-17-92 cluster inhibits PTEN/TMEM127 expression and activates the AKT/mTOR pathway. Moreover, they demonstrated that everolimus, an mTOR inhibitor, suppressed the viability of METTL3-high GC cells significantly better than that of METTL3-low GC cells ($P < 0.05$ on days 2 and 3), which was consistent with the results of an in vivo experiment, which measured tumour volume of mice carrying METTL3-high or control tumours ($P = 0.0465$). Therefore, everolimus has the potential to treat patients with m⁶A/METTL3-high GC. Zhang et al. [98] analysed differentially expressed circRNAs in poorly differentiated GC and found that most differentially expressed circRNAs had m⁶A modifications, which was consistent with the circRNA expression levels. Recently, *circORC5* was identified as a target of METTL14 in GC¹⁰². Low expression of METTL14 in GC reduced the m⁶A level of *circORC5*, which resulted in increased *circORC5* expression and poor survival of patients with GC. In addition, m⁶A in circRNAs mediates cap-independent translation, and proteins or peptides encoded by these circRNAs can play an important role in human cancers [99]. However, there has been little research on the mechanisms and functions of proteins translated from m⁶A-modified circRNAs in GC. All recent studies on m⁶A-modified ncRNAs in GC are summarized in Table 3.

ncRNAs can also interact with RNA-binding proteins, including m⁶A regulators, to regulate cancer cell phenotypes

Table 2. m⁶A-modified mRNAs in GC.

mRNA	m ⁶ A Regulator	Function of Regulators	Pathway	Role of target	Reference
<i>PKMYT1</i>	ALKBH5	Eraser	Demethylating m ⁶ A	Oncogene	[74]
	IGF2BP3	Reader	Promoting mRNA stability		
<i>IGF1R</i>	IGF2BP2	Reader	Promoting mRNA stability	Oncogene	[90]
<i>YAP1</i>	METTL3	Writer	Enhancing m ⁶ A which promotes mRNA translation	Oncogene	[91]
<i>USP14</i>	YTHDF1	Reader	Promoting mRNA translation	Oncogene	[92]
<i>Nanog</i>	ALKBH5	Eraser	Demethylating m ⁶ A which promotes mRNA decay	Oncogene	[104]
<i>SPHK2</i>	METTL3	Writer	Enhancing m ⁶ A Promoting mRNA translation	Oncogene	[93]
	YTHDF1	Reader			
<i>HK2</i>	WTAP	Writer	Enhancing m ⁶ A which promotes mRNA stability	Oncogene	[94]
<i>C-Jun</i>	KIAA1429	Writer	Enhancing m ⁶ A which promotes mRNA stability	Oncogene	[95]
<i>Cyclin D1</i>	METTL16	Writer	Enhancing m ⁶ A which promotes mRNA stability	Oncogene	[96]
<i>FZD7</i>	YTHDF1	Reader	Promoting mRNA translation	Oncogene	[83]
<i>MYC</i>	METTL3	Writer	Enhancing m ⁶ A which promotes mRNA translation	Oncogene	[64,80]
	FTO	Eraser	Demethylating m ⁶ A which promotes mRNA stability		
<i>ZMYM1</i>	METTL3	Writer	Enhancing m ⁶ A Promoting mRNA stability	Oncogene	[62]
	HuR	Reader			
<i>HDGF</i>	METTL3	Writer	Enhancing m ⁶ A Promoting mRNA stability	Oncogene	[65]
	IGF2BP3	Reader			
<i>ARHGAP5</i>	METTL3	Writer	Enhancing m ⁶ A	Oncogene	[102]
	HuR	Reader	Promoting mRNA stability		
<i>CDCP1</i>	METTL3	Writer	Enhancing m ⁶ A which promotes mRNA translation	Oncogene	[105]
<i>SEC62</i>	METTL3	Writer	Enhancing m ⁶ A Promoting mRNA stability	Oncogene	[106]
	IGF2BP1	Reader			
<i>E2F3</i>	MiR-660	miRNA	Silencing mRNA	Oncogene	[84]
<i>DDIT3</i>	FTO	Eraser	Attenuating m ⁶ A	Suppressor	[113]
<i>BATF2</i>	METTL3	Writer	Enhancing m ⁶ A which represses mRNA expression	Suppressor	[82]
<i>PTEN</i>	METTL3	Writer	Enhancing m ⁶ A Promoting mRNA decay	Suppressor	[103]
	YTHDF2	Reader			

Table 3. m⁶A-modified ncRNAs in GC.

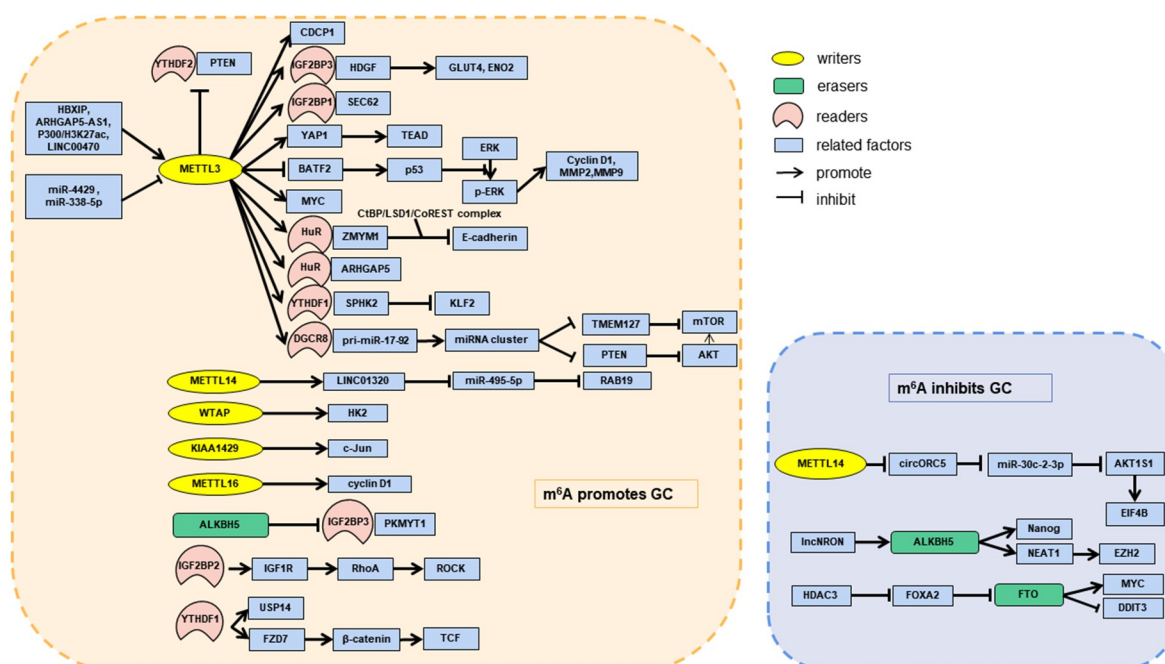
ncRNA		m ⁶ A Regulator	Function of Regulator	Pathway	Role of target	Reference
<i>LINC01320</i>	lncRNA	METTL14	Writer	Enhancing m ⁶ A which upregulates lncRNA	METTL14/m ⁶ A/LINC01320/miR-495-5p/RAB19	Oncogene [89]
<i>NEAT1</i>	lncRNA	ALKBH5	Eraser	Demethylating m ⁶ A	ALKBH5/m ⁶ A/lncRNA NEAT1/EZH2	Oncogene [93]
<i>pri-miR-17-92</i>	pri-miRNA	METTL3 DGCR8	Writer	Enhancing m ⁶ A	METTL3/m ⁶ A/pri-miR-17-92/DGCR8/PTEN or TMEM127/mTOR	Oncogene [97]
<i>circORC5</i>	circRNA	METTL14	Writer	Enhancing m ⁶ A which inhibits circRNA expression	METTL14/m ⁶ A/circORC5/miR-30c-2-3p/AKT1S1/EIF4B	Oncogene [100]

[101]. Zhu et al. [102] reported that the lncRNA *ARHGAP5-AS1* can recruit METTL3 to facilitate methylation of *ARHGAP5*, which stabilizes *ARHGAP5* in the cytoplasm. The upregulation of *ARHGAP5* promotes chemoresistance of GC cells, whereas its downregulation significantly reverses chemoresistance. Their study provided a potential strategy for addressing chemotherapy resistance in GC. The lncRNA *LINC00470*, which is correlated with distant metastasis, TNM stage, and poor prognosis in GC, promotes *PTEN* methylation via association with METTL3 [103]. Then, the m⁶A reader YTHDF2 is involved in the degradation of *PTEN*, and the half-life of *PTEN* is increased after YTHDF2 knockdown. The lncRNA *lncNRON* was recently suggested to exert oncogenic functions in GC by recruiting ALKBH5 to inhibit Nanog mRNA decay [104]. With regard to miRNAs, miR-338-5p can inhibit the methylation of *CDCP1* by suppressing METTL3, which is important because m⁶A modification stimulates *CDCP1* translation and accelerates the proliferation and invasion of GC cells [105]. Upstream of the miR-338-5p/METTL3/*CDCP1* axis, embryonic ectoderm development protein promotes GC progression by downregulating miR-338-5p through histone methylation. METTL3 is also suppressed by miR-4429, and reduced

METTL3 prevents GC progression by attenuating m⁶A-induced stabilization of *SEC62* [106]. These ncRNAs may serve as promising targets for molecular therapy of GC. Overall, interactions between RNAs (mRNAs and ncRNAs) and m⁶A regulators are indeed related to the biological behaviours of GC, which are potential targets for molecular diagnosis and treatment (Figure 2).

4. Application of RNA m⁶A modification in GC

Since increasing evidence demonstrates that RNA m⁶A modification is correlated with tumorigenesis and the progression of GC, the detection of dysregulated m⁶A levels may provide a promising method for diagnosing, predicting, and assessing GC. For example, Zhang et al. [107] constructed an m⁶A scoring system to quantify the m⁶A modification pattern in individual GC patients and characterize immune cell infiltration. The m⁶A scoring system was divided into three distinct patterns that were highly consistent with the three types of immune cell-infiltrating characteristics. In this system, GC patients with a low m⁶A score tend to have better survival and more effective immune infiltration than those with a high m⁶A score. Moreover, analysis of two cohorts receiving

**Figure 2.** Aberrant m⁶A promotes or inhibits GC by interacting with GC-related RNAs and regulating the corresponding pathways.

immunotherapy demonstrated that patients with a lower m⁶A score exhibited significantly more clinical benefits and better prognosis after treatment with anti-PD-1/L1 immunotherapy ($P = 0.002$ and 0.013 , respectively), implying the ability of m⁶A analysis to guide which patients should receive immunotherapy. Ge et al. [108] evaluated the dysregulation of m⁶A modification in the peripheral blood of GC patients and found that m⁶A levels were related to the progression and metastasis of GC. As a novel biomarker, m⁶A levels in peripheral blood RNA (AUC = 0.929) had a better ability to distinguish GC patients from healthy individuals than the conventional tumour markers CEA and CA19-9 (AUC = 0.694 and 0.603, respectively). In addition, the combination of m⁶A, CEA, and CA19-9 improved the AUC to 0.955 (95% CI, 0.91–0.98; $P < 0.0001$). Subsequently, a serum diagnostic signature based on m⁶A target miRNAs was developed for the mass detection of cancer [109]. The m⁶A-miRNAs signature showed a promising ability to distinguish GC patients from non-cancer controls and from mixed samples with other cancers (AUC = 0.989 and 0.791, respectively). When the signature was used to diagnose early GC, its AUC reached 0.989 (95% CI, 0.987–0.990), which was higher than those of CEA and CA19-9. This study provides a new direction for further investigation of the diagnostic value of m⁶A.

Aberrant expression or genetic variants of m⁶A regulators can also serve as valuable sources for screening and prognosis assessment. By analysing the expression profiles of m⁶A regulator genes in TCGA cohorts, Liu et al. [110] constructed an m⁶A-gene based diagnostic signature for GC. The signature showed high diagnostic accuracy (AUC = 0.986) in distinguishing between 375 tumour samples and 32 normal samples; however, there was no external validation cohort. Wang et al. [111] reported a significant increase in the m⁶A readers *IGF2BP1*, *IGF2BP2*, and *IGF2BP3* at the RNA level ($P < 0.05$) in GC tissues and identified the single nucleotide polymorphism rs9906944 in *IGF2BP1*, which is associated with GC risk ($P < 0.05$). Analysis of 2,900 GC cases and 3,536 controls indicated that the T allele of rs9906944 was significantly associated with decreased GC risk (odds ratio = 0.75, 95% CI 0.64–0.88, $P = 5.76 \times 10^{-4}$). Furthermore, elevated *IGF2BP1* levels are associated with worse overall survival in GC patients (HR = 1.49, 95% CI 1.16–1.91, $P = 1.50 \times 10^{-3}$). In addition, Li et al. [112] established a risk ratio model of potential m⁶A regulators through differential analysis based on TCGA datasets and least absolute shrinkage and selection operator Cox regression analysis. After adding another risk model, patients with GC were divided into high- and low-risk groups. The OS in the low-risk group was significantly higher than that in the high-risk group ($P < 0.05$), and the AUC of this risk model was 0.667. Another recent study demonstrated the presence of multiple differentially expressed m⁶A regulators in GC tissues by analysing data from UALCAN and Oncomine web [75]. High expression of FTO and YTHDC2 could be used as unfavourable prognostic markers in patients with GC. Interestingly, omeprazole, a proton pump inhibitor, enhances m⁶A levels by decreasing FTO and promotes chemosensitivity in GC cells [113]. Mechanistically, FTO inhibition increases DDIT3 expression and activates mTORC1, which promotes pro-survival autophagy [114]. Further studies on the dysregulation

of m⁶A regulators in GC may provide more promising biomarkers and treatment strategies.

5. Conclusions

With the recent development of epitranscriptomic research, the mechanism of RNA m⁶A modification, which is the most common post-transcriptional modification, has been largely clarified. Epitranscriptomic research has subsequently been extended to various types of malignant tumours, including GC. GC-related mRNAs and ncRNAs are regulated by m⁶A regulators, and these m⁶A-modified RNAs exert pro- and anti-tumorigenic downstream functions. Moreover, ncRNAs can affect the methylation of downstream RNAs by regulating the expression of m⁶A regulators. Clinically, abnormal changes in RNA m⁶A modification levels or m⁶A regulators have an important impact on tumorigenesis, development, metastasis, and prognosis in GC. By detecting and evaluating specific m⁶A-related molecules, clinicians can diagnose GC at an early stage, develop appropriate treatment strategies, and accurately predict individual prognoses. Future research should focus on a comprehensive understanding of the interaction of various m⁶A regulators, m⁶A-modified RNAs, and m⁶A-modified sites in the biological process of GC to identify better m⁶A markers with high sensitivity and specificity and develop novel molecular therapies targeting the pathways of m⁶A modification.

Abbreviations

(m ⁶ A)	N6-methyladenosine
(GC)	gastric cancer
(mRNA)	messenger RNA
(ncRNA)	non-coding RNA
(3' UTRs)	3' untranslated regions
(LC-MS/MS)	liquid chromatography-tandem mass spectrometry
(meRIP-seq)	methylated RNA immunoprecipitation sequencing
(LAIC-seq)	m ⁶ A-level and isoform-characterization sequencing
(miCLIP-seq)	m ⁶ A individual-nucleotide-resolution cross-linking and immunoprecipitation sequencing
(RIP)	RNA immunoprecipitation
(APA)	alternative polyadenylation
(m ⁶ A _m)	N6, 2'-O-dimethyladenosine
(lncRNAs)	long non-coding RNAs
(miRNAs)	microRNAs
(circRNAs)	circular RNAs
(rRNAs)	ribosomal RNAs
(MTase)	methyltransferase
(METTL3)	methyltransferase like 3
(SAM)	S-adenosyl methionine
(METTL14)	methyltransferase like 14
(H3K36me3)	histone H3 on Lys36
(WTAP)	Wilms Tumour 1-associated protein
(METTL16)	Methyltransferase like 16
(YTH)	YT521-B homology
(HNRNPs)	heterogeneous nuclear ribonucleoproteins
(IGF2BPs)	insulin-like growth factor 2 mRNA-binding proteins
(eIF3)	eukaryotic Initiation factor 3
(FMRP)	fragile X retardation protein
(FTO)	Fat mass and obesity-associated protein
(hm ⁶ A)	n6-hydroxymethyladenosine
(f ⁶ A)	N6-formyladenosine
(ALKBH5)	α-ketoglutarate-dependent dioxygenase homolog 5
(snRNA)	small nuclear RNAs

(SFPQ)	splicing factor, proline- and glutamine-rich
(TCGA)	The Cancer Genome Atlas
(EMT)	epithelial-mesenchymal transition
(GSEA)	gene set enrichment analysis
(IHC)	immunohistochemistry
(MYC)	myelocytomatosis viral oncogene homolog
(HDAC3)	histone deacetylase 3
(FOXA2)	forkhead box transcription factor A2
(ZMYM1)	zinc finger MYM-type containing 1
(HDGF)	hepatoma-derived growth factor
(BATF2)	basic leucine zipper ATF-like transcription factor 2
(FZD7)	frizzled 7
(E2F3)	E2F transcription factor 3
(EED)	embryonic ectoderm development protein
(TME)	tumour microenvironment
(SNP)	single-nucleotide polymorphism
(LASSO)	least absolute shrinkage and selection operator

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