Research progress concerning m^{6}A methylation and cancer (Review)

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Abstract. N6-methyladenosine (m^{6}A) methylation is a type of methylation modification on RNA molecules, which was first discovered in 1974, and has become a hot topic in life science in recent years. m^{6}A modification is an epigenetic regulation similar to DNA and histone modification and is dynamically reversible in mammalian cells. This chemical marker of RNA is produced by m^{6}A 'writers' (methylase) and can be degraded by m^{6}A 'erasers' (demethylase). Methylated reading protein is the 'reader', that can recognize the mRNA containing m^{6}A and regulate the expression of downstream genes accordingly. m^{6}A methylation is involved in all stages of the RNA life cycle, including RNA processing, nuclear export, translation and regulation of RNA degradation, indicating that m^{6}A plays a crucial role in RNA metabolism. Recent studies have shown that m^{6}A modification is a complicated regulatory network in different cell lines, tissues and spatio-temporal models, and m^{6}A methylation is associated with the occurrence and development of tumors. The present review describes the regulatory mechanism and physiological functions of m^{6}A methylation, and its research progress in several types of human tumor, to provide novel approaches for early diagnosis and targeted treatment of cancer.

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Abbreviations: m^{6}A, N6-methyladenosine; FTO, fat mass and obesity-associated protein; ALKBH5, AlkB homologous protein 5; YTHDF2, YTH N6-methyladenosine RNA binding protein 2; eIFs, eukaryotic initiation factors; MeRIP-Seq, methylated RNA immunoprecipitation sequencing; miCLIP-seq, m^{6}A individual nucleotide resolution cross-linking and immunoprecipitation; HCC, hepatocellular carcinoma; RCC, renal cell carcinoma; SAM, S-adenosylmethionine; PDAC, pancreatic ductal adenocarcinoma; CSCC, cervical squamous cell carcinoma; HIF, hypoxia-inducible factors; IGF2BP2, insulin like growth factor 2 mRNA binding protein 2; snRNA, small nuclear RNA; BCSCs, breast cancer stem cells; ZFP217; zinc-finger protein 217

Key words: RNA methylation, m^{6}A methylation, tumor

Contents

1. Introduction

Cancer is a gene-related disease with heredity and can be caused by various physical, chemical or biological factors (1). It is usually characterized by abnormal differentiation and proliferation of cells, which differ from normal cell proliferation and apoptosis (2). In addition, tumor cells have invasive and metastasis abilities, and promote angiogenesis. N6-methyladenosine (m^{6}A) methylation is an epigenetic modification of RNA, first discovered in the mRNA of eukaryotes in the 1970s (3). However, due to the limitation of technology, scientists hypothesized that the m^{6}A methylation site only existed in mRNA. In recent years, m^{6}A methylation has been found in different types of RNA, such as long non-coding RNA (lncRNA) (4), microRNA (5) and mRNA (6). With the rapid development of high-throughput sequencing technology, a variety of bioinformatics platforms, for predicting m^{6}A methylation sites, have been developed, which increased the investigation of m^{6}A methylation (7). In the process of carcinogenesis, m^{6}A methylation has been associated with the occurrence and development of cancer by regulating the expression level of oncogenes and cancer suppressor genes. For example, in leukemia, METTL-14 causes the occurrence and development of leukemia via m^{6}A RNA modification of *MYB*/*myc* (8). In liver cancer, METTL-3 promotes cancer progression through YTHDF2 dependent posttranscriptional silencing of SOCS2 (9). Similar to DNA methylation, m^{6}A methylation is regulated by methyltransferase and demethylase, which modulates post-transcriptional modifications without alternating the gene sequence (10). However, its regulatory mechanism is more complicated than DNA methylation. The present review explains the m^{6}A-related enzymes, research methods and research progress of m^{6}A methylation and cancer, and to describe the association between m^{6}A methylation and tumor occurrence and development. An improved

understanding of m^{6}A methylation could assist with identifying potential biomarkers and targets for molecular diagnosis and targeted therapy of cancer.

2. Composition and function of m^{6}A modified enzyme

m^{6}A is a dynamic and reversible modification process, which mainly involves three types of catalytic enzymes: Methyltransferase, demethylase and methylated reading protein (Table I). Methyltransferase, also known as mRNA 'writer', methylates adenosine in mRNA (11). *METTL-14* and *METTL-3* can form a methyltransferase complex by binding to the regulatory protein, WT1 associated protein (*WTAP*) and subsequently promote methylation. m^{6}A modified mRNA specifically binds to methylated reading protein, termed as 'reader', and results in various effects on gene expression (12). Demethylase (mRNA erasers) can remove the methyl group on adenosine bases for m^{6}A demethylation. The mRNA writers and erasers make m^{6}A modification a dynamic and reversible process. Previous studies hypothesized that m^{6}A modification could change the secondary structure of RNA, promote the combination of RNA binding protein with RNA segments, interfere with RNA modification and subsequently regulate gene expression (13,14). However, the underlying mechanism remains unclear.

mRNA writers. m^{6}A methylation is catalyzed by mRNA writers, including *METTL-3*, *METTL-14*, *WTAP*, *VIRMA* and *RBM15*. The core components of the m^{6}A methyltransferase complex (*METTL-3*, *METTL-14*, *WTAP* and *VIR*) are highly conserved in most eukaryotes (15). The study of the m^{6}A site on human small nuclear (sn) RNA U6 showed that human cells express at least one activated m^{6}A methyltransferase, apart from *METTL-14* and *METTL-3*. However, these enzymes have not been identified due to the limitation in technology.

METTL-3. *METTL-3*, also known as MT-A70, is the earliest reported m^{6}A methylase. Barbieri *et al* (16) reported that the upregulation of *METTL-3* expression significantly promoted the m^{6}A methylation of mRNA transcribed by the oncogene *SP1*, resulting in an increased expression of the *SP1* protein, which was associated with the differentiation of hematopoietic stem cells into acute myeloid leukemia (AML) cells. In addition, Vu *et al* (17). confirmed that downregulation of *METTL-3* gene expression increased the phosphorylation of *AKT* and promoted the differentiation of hematopoietic stem cells into AML cells. The two studies provide novel directions for the diagnosis and treatment of AML.

In addition, some studies have shown that under hypoxia, the transcription factor zinc-finger protein 217 (*ZNF217*) inhibited the m^{6}A modification of *KLF4* and *NANOG* by binding to *METTL-3*, leading to elevated expression of *KLF4* and *NANOG*, and the promotion of breast cancer (18). Cai *et al* (19) have found that high expression of *METTL-3* in breast cancer cells induced m^{6}A modification on *HBXIP* mRNA. *HBXIP* promoted the m^{6}A modification of *METTL-3* by reducing the expression level of tumor suppressor gene *LET-7G*, which forms a positive feedback pathway with *HBXIP*/*LET-7G*/*METTL-3*/*HBXIP* and promoted the malignant biological behaviors of breast cancer. These studies provide new approaches for the diagnosis and treatment of breast cancer.

Furthermore, Chen *et al* (9) found that overexpressed *METTL-3* in primary hepatocellular carcinoma (HCC) could change the m^{6}A modification of the tumor suppressor gene, *SOCS2*, leading to degradation of *SOCS2* mRNA and promotion of cancer cell proliferation and migration. This study showed that hypermethylation was associated with the progression of HCC. Taketo *et al* (20) found that, in pancreatic cancer cell lines with low expression of *METTL-3*, the cancer cells were more sensitive to gemcitabine and other anticancer drugs [Everolimus (21) or Elemene (22)] and external radiation. In addition, *METTL-3* was associated with cell cycle regulation, mitogen-activated protein kinase cascade and RNA splicing, suggesting that *METTL-3* may be one of the potential targets to improve the therapeutic efficacy in patients with pancreatic cancer.

However, in renal cell carcinoma (RCC), *METTL-3* exhibited tumor-suppressing activity (23). *In vivo* experiments confirmed that lower expression of *METTL-3* was significantly associated with tumor histological grade and tumor size. In addition, patients with RCC and overexpression of *METTL-3* had a higher overall survival rate and good prognosis. Downregulation of the *METTL-3* gene expression in a RCC cell line could promote cell epithelial-mesenchymal transition (EMT), proliferation, invasion and metastasis. It was suggested that *METTL-3* could be used as a new marker for the treatment of RCC, however, further studies are required to investigate the role of *METTL-3* and related factors in carcinogenesis to further understand the biological mechanism of the occurrence and development of RCC.

The aforementioned studies have investigated the different activities of *METTL-3* in various types of cancer, indicating that m^{6}A methyltransferase, *METTL-3* could be a potential target for developing novel therapeutic strategies, and investigating the mechanism of the occurrence and development of cancer.

METTL-14. *METTL-14* is a homologous heterodimer of *METTL-3* in the MT-A70 methyltransferase family (24). It has been reported that knockout of the *METTL-14* gene in HeLa cells led to a decrease in m^{6}A methylation level, suggesting that *METTL-14* was an important part of the m^{6}A methyltransferase complex (25). Since *METTL-3* is a subunit with catalytic activity, *METTL-14* is responsible for identifying substrates. The two proteins are combined to form a stable methyltransferase complex with a ratio of 1:1 *In vivo*, which allows the catalyzation of m^{6}A modification in target RNA (26). Weng *et al* (8) found that the knockout of *METTL-14* in AML cell lines could effectively inhibit the proliferation of the AML cells. *METTL-14* was negatively regulated by *SP1* at the protein level and induced cancer promotion by regulating target genes via m^{6}A modification. This study firstly revealed the role of the *SP1-METTL-14-MYB*/*MYC* signal axis in the progression, maintenance, and self-renewal of leukemia, providing new ideas for the diagnosis and treatment of AML. In addition, Ma *et al* (27) proved that the decrease in expression of *METTL-14* in HCC tissue was an independent factor in predicting cancer recurrence. The reduction of *METTL-14* led to a decreased level of m^{6}A methylation, which inhibited

Table I. m^{6}A modification-related enzymes and their biological functions in tumors.

Type	Gene	Function
Writer	METTL-3	Methyltransferase
	METTL-14	Enhancing radiotherapy and chemosensitivity
	METTL-16	Enhancing mRNA initiation translation
	WTAP	Plays a role in both transcriptional and post-transcriptional regulation of certain cellular genes
Eraser	WTAP	Demethylase, downregulating mRNA transcription levels. Promoting chemotherapy resistance
	ALKBH5	Mediating m^{6}A demethylation modification, maintaining tumorigenicity of tumor cells and pre-mRNA stability
Reader	YTHDF1/2	Recognition and binding of m^{6}A sites selectively and mediating mRNA degradation
	eIF3	Promoting translation independent of 5'-UTR under stress conditions

eIF3, eukaryotic initiation factors; UTR, untranslated region; YTHDF2, YTH N6-methyladenosine RNA binding protein 2; ALKBH5, AlkB homologous protein 5; WTAP, WT1 associated protein; m^{6}A, N6-methyladenosine.

cell proliferation and promoted apoptosis of HCC cells. In a HCC cell line, *METTL-14* mediated the decreased expression of miR-126, leading to the invasion and metastasis of HCC. Furthermore, Ma *et al* also found that the expression level of *METTL-14* and demethylase *WTAP* in HCC was decreased, indicating that m^{6}A modification has a complicated feedback regulation mechanism. Therefore, the investigation into the interaction between *METTL-14* and micro (mi) RNA could provide novel targets for the treatment of HCC.

METTL-16. *METTL-16* is a newly discovered m^{6}A methyltransferase (28). The downregulated expression of *METTL-16* led to a decrease in the level of m^{6}A methylation in cells. Warda *et al* (29) found that *METTL-16* could bind to snRNA U6, long non-coding (lnc) RNA and pre-mRNA via cDNA cross-linking analysis, which deepened the understanding of the interaction between m^{6}A and other RNA.

S-adenosylmethionine (SAM) is an important methyl donor of DNA methylation and acts as a key regulator controlling gene expression (30). It has previously been reported that SAM played an important role in RNA methylation (31). The study suggested that *METTL-16* maintained the stability of intracellular SAM by regulating the alternative splicing of MAT2A. The absence of SAM increased the residence time of *METTL-16* in the hairpin of *MAT2A 3'* untranslated region (UTR) and promoted the alternative splicing of *MAT2A*, subsequently regulating the homeostasis of intracellular SAM content (32). The association between RNA modification and alternative splicing was established by this mechanism. However, the association between *METTL-16* and the occurrence and development of cancer remains unclear. Therefore, further studies are required to investigate the role of *METTL-16* in cancer development.

WTAP. *WTAP* is an essential component in m^{6}A methylation modification. Ping *et al* (33) proved that *WTAP* assisted with the accurate location of the *METTL-3-METTL-14* heterodimer and promoted m^{6}A methylation. In addition, either knockdown or overexpression of *METTL-3* led to an elevation of *WTAP* expression, indicating that *METTL-3* plays an important role in the regulation of *WTAP* function (34). However, the upregulation of *WTAP* could not promote cancer cell proliferation in the absence of *METTL-3*. Therefore, the carcinogenic effect of *WTAP* is associated with the m^{6}A methyltransferase complex. The association between *WTAP* and the occurrence and development of cancer is unclear. Xi *et al* (35) found that *WTAP* was highly expressed in glioma tissue and was associated with pathological grade and poor postoperative survival rate. Li *et al* (36) confirmed that the expression level of *WTAP* was significantly increased in both the cytoplasm and nucleus of pancreatic ductal adenocarcinoma (PDAC), whereas the high expression level in the nucleus was significantly associated with sex and tumor stage and was considered to be an independent prognostic factor of PDAC. Tang *et al* (37) reported that the high expression level of *WTAP* in patients with RCC was associated with poor overall survival rate and prognosis. The study also found that *WTAP* may promote the proliferation of RCC cells by regulating the stability of *CDK2* mRNA, leading to the occurrence and development of cancer. Therefore, *WTAP* may become a new target for the diagnosis and treatment of RCC.

mRNA erasers. α-ketoglutarate-dependent dioxygenase FTO protein (*FTO*) encoded by the obesity gene, FTO was the first demethylase found in mammals, which proved that m^{6}A modification was dynamically reversible (38). Similarly, AlkB homologous protein 5 (*ALKBH5*) in mammals could also catalyze the restoration of m^{6}A methylation (39). Currently, it is not clear whether demethylases exist in lower-grade eukaryotes. Some studies have found that when the first nucleotide adjacent to the cap in the nucleotide sequence is adenosine,

FTO cannot induce demethylation, but the specific mechanism is unclear.

FTO. The *FTO* gene is located on chromosome 16 (16q12.2) and is widely expressed in all stages of human growth (40). Its main functions are to regulate the rate of fat consumption, promote the overall metabolic rate, and ensure the energy balance of the body (41). Jia *et al* (42) first confirmed that the FTO protein was a crucial demethylase in both DNA and RNA modification, especially in m^{6}A demethylation. This report ushered in the era of m^{6}A research. Selberg *et al* (26) confirmed that the level of m^{6}A in mRNA was increased in *FTO* knockout leukemia cells or gastric cancer cells and vice versa. However, the expression of m^{6}A methylase *METTL-3* was not affected. Based on these results, researchers preliminarily proved that the methylation process of m^{6}A was reversibly and dynamically regulated. Previous studies have shown that the expression of the *FTO* gene was associated with breast cancer (41), thyroid cancer (43), endometrial cancer (44), gastric cancer (45), and other types of cancer (46,47). Li *et al* (48) found that *FTO* increased leukemia oncogene-mediated cell transformation and leukemogenesis by reducing the m^{6}A modification of ASB2 and RARA genes, which led to the inhibition of AML cell differentiation induced by all-trans retinoic acid. In another study, Zhou *et al* (49) found that the expression of the *FTO* gene was significantly increased in patients with cervical squamous cell carcinoma (CSCC), and the increased expression of *FTO* and β-catenin indicated a poor prognosis. Therefore, the expression level of *FTO* and β-catenin could predict the prognosis of CSCC. In short, few studies have focused on the mechanism of *FTO*-induced m^{6}A modification in the carcinogenesis and development of cancer. More studies are required to clarify the relevant molecular biological mechanisms involved in FTO-induced m^{6}A modification. However, it remains controversial whether the activity of methylase and demethylase is limited to catalyzing m^{6}A modification on RNA.

ALKBH5. *ALKBH5* belongs to the AIkB family, but unlike other family members, *ALKBH5* only has demethylation ability on single-stranded RNA/DNA (50). With the participation of hypoxia-inducible factors (HIF), *ALKBH5* can induce the transformation of breast cancer cells into tumor stem cells by reducing the m^{6}A methylation of NANONG, which improves the stability of *NANONG* mRNA and elevates its expression (51). Similarly, Zhang *et al* (52) found that *ALKBH5* was significantly overexpressed in glial stem cell-like cells (GSCs) and the interference of *ALKBH5* could inhibit the proliferation of GSCs. In addition, the study also found that lncRNA *FOXM1*-AS promoted the interaction between *ALKBH5* and *FOXM1*, indicating that m^{6}A demethylase *ALKBH5* acted as an oncogene in glioma. Recently, low expression of *ALKBH5* in pancreatic cancer cell lines was found to promote the m^{6}A demethylation of lncRNA *KCNK15*-AS1, resulting in a decreased ability of cancer invasion and metastasis (53). This provided a new direction for the diagnosis and treatment of pancreatic cancer.

In summary, further studies are required to investigate whether *ALKBH5* is associated with the occurrence and development of other types of cancer and whether these key demethylation modifications are associated with the stability, translation, and alternative splicing of mRNA.

mRNA readers. The term, mRNA readers, refers to proteins that can specifically bind to mRNA with m^{6}A methylation. The YTH domain is the marker of m^{6}A binding protein on mRNA. Their affinity with m^{6}A methylated mRNA is higher than that of unmethylated mRNA (54). The carboxyl terminal domain of YTH N6-methyladenosine RNA binding protein 2 (*YTHDF2*) selectively binds to the m^{6}A modified mRNA, which assists the *YTHDF2*-mRNA complex to move to the RNA decay site of the cell, thus inducing the degradation of mRNA. The degradation of mRNA plays an important role in stem cell differentiation by regulating key pluripotent factors (55). In different situations, the YTH protein can interact with different subsets of the m^{6}A locus and induce different effects on gene expression. Insulin like growth factor 2 mRNA binding protein 2 (*IGF2BP2*) is another m^{6}A reader using the Khomlog (KH) domain to selectively bind m^{6}A modified RNA and promote mRNA translation, which is different from proteins with the YTH domain (56). This discovery increased the understanding of the mechanism and function of the m^{6}A binding protein. In addition, m^{6}A modification could destroy the complementary pairing of nucleotides, improve the accessibility of single-stranded RNA motifs, and promote the recognition of m^{6}A binding proteins heterogeneous nuclear ribonucleoprotein C and G.

YT521-B homology. The YTH domain recognizes m^{6}A methylation in a methylation-dependent manner (57). A total of five proteins in the human body contain YTH domains. *YTHDC1* can regulate the expression of mRNA in the nucleus by affecting the alternative splicing of mRNA precursors (58). Zhao *et al* (35) found that the expression of *YTHDF1* was significantly increased in patients with advanced HCC. In addition, potential target genes regulated by the *YTHDF1* protein may be associated with the cell cycle of the tumor, degradation of different amino acids and metabolism of various lipids. Li *et al* (59) reported that overexpression of miR-493-3p in *YTHDF2* knockdown prostate cancer cell lines promoted m^{6}A modification and thereby inhibited the proliferation and migration of the cancer cells. These findings lay a foundation for further investigation of the biological function of m^{6}A and RNA epigenetics and provide a new direction for investigating the underlying mechanism of cancer development. Currently, the role of YTH family members in m^{6}A methylation has become a hot topic, which provides novel approaches for investigating cancer-related mechanisms.

Eukaryotic initiation factors (*eIF3*). There are numerous and complex *eIFs*. Up to now, a total of 13 *eIFs* have been identified (60). *eIF3* is the most complex factor in *eIF* translation and plays an important role in the initiation of protein translation. Li *et al* (61) first found that *eIF3e* was an independent prognostic factor for overall survival and disease-free survival time in patients with colon cancer. Downregulation of *eIF3e* expression could inhibit proliferation and promote apoptosis of colon cancer cells. Furthermore, the interaction between *METTL-3* and *eIF3h* could increase mRNA translation and form dense polyribosomes, which was necessary for

Table II. Methods to detect and predict m^{6}A methylation.

Method	Application	Characteristic
m^{6}A-Seq	The region of m^{6}A hypermethylation was identified, but the resolution of single base could not be achieved	High-throughput
MeRIP-Seq	The RNA fragments containing m^{6}A were enriched with m^{6}A antibody, and then the enriched fragments were sequenced	High-throughput
miCLIP-Seq	M^{6}A antibody was used to enrich m^{6}A modification, combined with UV crosslinking technology to identify m^{6}A modification at single base level in the whole genome	High-throughput unit point
m^{6}A-CLIP	M^{6}A antibody was used to enrich m^{6}A modification, combined with UV crosslinking technology to identify m^{6}A modification at single base level in the whole genome	High-throughput unit point
PA-m^{6}A-Seq	M^{6}A antibody was used to enrich m^{6}A modification, combined with UV crosslinking technology to identify m^{6}A modification at single base level in the whole genome	High-throughput unit point

m^{6}A, N6-methyladenosine; seq, sequencing; MeRIP, methylated RNA immunoprecipitation sequencing; miCLIP, m^{6}A individual-nucleotide-resolution cross-linking and immunoprecipitation; PA, photo-cross-linking.

carcinogenic transformation (62). The study by Chao *et al* (39) revealed the regulatory mechanism of protein translation based on the mRNA cycle and indicated that *METTL-3-eIF3h* could be a potential therapeutic target for patients with lung cancer.

3. Detection methods of m^{6}A methylation

In the early days, due to the limitation of technology, researchers could not detect m^{6}A methylation sites. As m^{6}A methylation of RNA does not affect its reverse transcription and it cannot be specifically cleaved, like M7G methylation, it is very difficult to identify the m^{6}A site in the initial study (63). However, with the emergence of second-generation sequencing (seq), two techniques, screening m^{6}A methylation site-m^{6}A-seq (64) and methylated RNA immunoprecipitation sequencing (MeRIP-seq) have been developed (65). These methods were designed to capture RNA fragments with m^{6}A methylation using co-immunoprecipitation then identify the sequences by second-generation sequencing. Subsequently, a multitude of m^{6}A methylation sites were found, and researchers found up to 12,000 m^{6}A signal peaks in >7,000 genes in humans and mice, all of which were enriched near the stop codon at the 3' end (66). These sites were highly conserved in both humans and mice. This finding provided strong evidence for the post-transcriptional regulation of m^{6}A methylation for gene expression and the modification may be associated with various genetic diseases (67,68). A limitation of this technique is that the RNA fragments captured are limited to 100-200 nucleotides and the technique cannot identify two very-close m^{6}A sites, so this method cannot accurately identify the m^{6}A methylation sites in the full transcriptome (69). In addition, a novel m^{6}A modification was found at the 5' end of mRNA (70). Asm^{6}A modification has the same methyl site with m^{6}A modification, both m^{6}A-seq and MeRIP-seq may misinterpret this modification as m^{6}A modification.

Based on the aforementioned limitations, the detection technology was improved by researchers. In 2015, three laboratories reported that the application of purple diplomatic co-immunoprecipitation could accurately capture m^{6}A methylation sites on a single base of RNA, which is the core technology of m6A individual-nucleotide-resolution cross-linking and immunoprecipitation (miCLIP) (69), photo-cross-linking-m^{6}A-seq (71), and m^{6}A-CLIP (or UV-CLIP) (72). Another technique for detecting m^{6}A methylation site is m^{6}A-LAIC-seq, which introduces spike-in-RNAs as an internal reference on the basis of m^{6}A-seq to calculate the m^{6}A methylation level of each gene in the full transcriptome (73). The disadvantage of this method is that a single m^{6}A methylation site cannot be detected. In addition to Qualcomm sequencing, the methods detecting the m^{6}A methylation site of a single gene are also important. The most famous one is site-specific cleavage and radioactive-labeling followed by ligation-assisted extraction and thin-layer chromatography (SCARLET) test (74), which can accurately detect a single m^{6}A methylation site in mRNA and lncRNA, and calculate the m^{6}A methylation level of the whole RNA (73). SCARLET is a low-throughput test with high expenses; however, its high accuracy makes it a common method for testing the accuracy of high-throughput detection of m^{6}A methylation sites. In addition, SCARLET can be used to detect other types of epigenetic modifications of RNA, such as M5C modification and ψ modification (75). Fluorescence quantitative PCR can also be used to detect the level of m^{6}A methylation. Golovina *et al* (76) found that different m^{6}A methylation levels in the same RNA will produce different melting curves under fluorescence quantitative PCR detection, which is due to the different melting temperatures of the RNA-DNA complex with different m^{6}A methylation levels. Therefore, Golovina *et al* proposed a high-resolution melting detection method, which could detect the alternation of the known m^{6}A methylation level in RNA. However, the experiments were only performed with ribosomal RNA, total RNA and snRNA,

so whether this technology can be extended to other types of RNA remains to be verified.

With the progress of high-throughput sequencing and antibody-specific enrichment technology, a new detection method, methylated RNA immunoprecipitation sequencing (MeRIP-seq), was developed with the advantage of identifying almost all m^{6}A modifications in different types of RNA, such as mRNA (77), lncRNA (78) and circular RNA (79). In MeRIP-seq, specific antibody of m^{6}A is used to extract co-immunoprecipitated RNA fragments, which are further identified using high-throughput sequencing (80). Then, the m^{6}A modification can be systematically investigated in combination with bioinformatics analysis. However, the main limitation of MeRIP-seq is that MeRIP-seq can only identify hypermethylated regions of the RNA, but cannot locate a certain m^{6}A site (81).

Subsequently, a novel sequencing method, miCLIP-seq, was developed to identify the specific site of m^{6}A modification. Apart from the application of m^{6}A antibodies to identify and enrich m^{6}A modification, miCLIP-seq also uses UV cross-linking method to identify m^{6}A modification at the single-base level in the whole genome. Therefore, miCLIP-seq can efficiently detect the m^{6}A residue with high resolution and perform m^{6}A cluster analysis on the whole RNA, which provides a novel technique to investigate the unique epigenetic trait of RNA. Furthermore, miCLIP can also detect the m^{6}A modification in a class of small non-coding RNA, such as small nucleolus RNA, which cannot be obtained using previous techniques. High-throughput sequencing technology has improved and is effective, but fluorescence quantitative detection is still one of the most economical and convenient molecular detection methods. There is clear importance to develop fluorescence quantitative PCR for m^{6}A detection. With the gradual improvement of the detection methods of m^{6}A methylation sites, a deeper understanding of m^{6}A methylation will develop, which lays a solid foundation for the study into the association between m^{6}A methylation and various diseases, particularly cancer. Table II lists the characteristics of five detection methods.

4. Prediction methods of m^{6}A methylation sites

As the detection of the m^{6}A methylation site is expensive and time consuming, bioinformatics prediction has been used to improve the research efficiency with high cost-effectiveness. In recent years, bioinformatics has developed rapidly and been widely used in molecular biology research. The following methods can assist with the prediction of the methylation sites of m^{6}A more effectively. Yu-Chen *et al* (82) first proposed the use of the Hidden Markov Model (HMM) to predict the residual sites around known sites. Li *et al* (83) developed the pRNAm-PC method to predict loci faster and was more stable. In addition, Chen *et al* (84) developed the iRNA-Methyl method. Based on these, Jia *et al* (85) developed the RNA-methylPred method, which is more stable and efficient than the former. After that, Li *et al* (83) proposed an improved Target m^{6}A method, but this method could only predict the methylation site of m^{6}A in the primary RNA sequence. On the other hand, Zhou *et al* (86) synthesized several mathematical models and proposed the sequence-based RNA adenosine methylation site predictor (SRAMP) method, which could effectively predict m^{6}A methylation sites in mammalian RNA. Recently, an online database called, RMBase-V2.0, has been established (http://rna.sysu.edu.cn/rmbase/), which contains a number of RNA epigenetic modification sequence data of 13 species, including a high amount of data on m^{6}A methylation sites.

5. Association between m^{6}A modification and malignancies

m^{6}A modification and breast cancer. Breast cancer stem cells (BCSCs) can proliferate indefinitely via self-renewal and forming recurrent or metastatic tumors (87). In the hypoxic tumor microenvironment, *ALKBH5* could reduce m^{6}A methylation in *NANOG* mRNA, increase the expression of *NANOG* mRNA and mediate the enrichment of BCSCs in a HIF-dependent manner (51). Zinc-finger protein 217 (*ZFP217*) and *ALKBH5* play complementary roles in regulating m^{6}A methylation in RNA (18). Hypoxia-induced *ZNF217* inhibited m^{6}A methylation of *NANOG* mRNA, whereas *ALKBH5* induced m^{6}A demethylation. Taken together, they can increase the expression of *NANOG* mRNA and protein and enrich BCSCs. In addition, *ZFP217* and *ALKBH5* were associated with a more malignant phenotype of breast cancer by inhibiting m^{6}A methyltransferase-related modifiers or inducing HIF-dependent hypoxia (88). A recent study reported that m^{6}A modification regulated the expression of early polyadenylation (premature polyadenylation; PPA), which blocked the expression of tumor suppressor genes and lead to carcinogenesis (89). In breast cancer cells, premature polyadenylation causes oncogenic truncations of the tumor suppressor genes *MAGI3* (90), *LATS1* (91) and *BRCA1* (92). The activation and truncation of PPA in tumor suppressor genes was regulated by m^{6}A modification. Compared with that in normal breast cells, m^{6}A methylation, activated by PPA significantly, was decreased in tumor suppressor gene-related exons. However, there are no conclusions on how breast cancer cells regulate the level of m^{6}A in exons to trigger PPA.

m^{6}A modification and colon cancer. As an ATP-dependent RNA helicase and a member of the YTH family, *YTHDF2* promoted initial translation by unlocking the 5'-UTR of mRNA, and the transcription and translation of metastasis-related factors by inducing m^{6}A methylation, thereby enhancing the metastasis of cancer cells (93). In colon cancer, *YTHDF2* promoted metastasis by promoting the translation of HIF-1α. Knockdown of the *YTHDF2* gene could reduce the expression level of metastasis-related genes, such as HIF-1α and inhibit the metastasis of colon cancer cells *in vitro* and *In vivo* (94). In addition, the expression level of *YTHDF2* was positively associated with the stage of colon cancer. At present, few studies have identified the function and target of *YTHDF2* in the progression and metastasis of colon cancer. However, these findings will provide new insights into the role of RNA demethylase in tumorigenesis.

m^{6}A modification and liver cancer. Hou *et al* (95) have revealed that *YTHDF*2 was positively associated with the malignant grade of HCC. miR-145 could increase the level of m^{6}A methylation by targeting the 3'-UTR of *YTHDF2* mRNA in HCC cells, leading to the malignant progression of HCC.

In addition, *YTHDF1* was highly expressed in human HCC tissues and associated with the regulation of the cell cycle and metabolism of HCC cells. Furthermore, the deletion of m^{6}A methylation was associated with the metastasis of HCC with the downregulation of *METTL-14*. In HCC, *METTL-3* mediated the methylation of m^{6}A in the mRNA of the chromosome (or critical) region in DiGeorge syndrome (96). *METTL-14* could significantly upregulate the level of miR-126 modified by m^{6}A methylation, thus promoting the maturation of miR-126 and inhibiting the metastasis of HCC cells (97). At present, the mechanism of how *METTL-14* has a low expression in liver cancer cells remains unclear. More in-depth investigation is required to clarify the structure and biological function of *METTL-14* in cancer to determine whether *METTL-14* can be used as a therapeutic target for the treatment of liver cancer. However, the interaction between *METTL-14* and *YTHDF1*/2 with miRNA still provides clues for identifing new approaches to treat liver cancer.

m^{6}A modification and pancreatic cancer. The role of *METTL-14* in pancreatic cancer has also been confirmed. The methylase, *METTL-1*4 was highly expressed in pancreatic cancer tissues. *METTL-14* could promote the proliferation, invasion, and metastasis of pancreatic cancer cells by increasing the level of m^{6}A methylation, inhibiting the expression of miR-1-3p, and activating the mitogen-activated protein kinase (MAPK) pathway (98). In addition, a new mechanism of lncRNA with m^{6}A methylation was found. *ALKBH5* inhibited the progression of pancreatic cancer by promoting m^{6}A demethylation of lncRNA potassium two-pore domain channel subfamily K member 15 (*KCNK15*) and WNT1-induced signal pathway protein 2 (*WISP2*) antisense lncCNK15-AS1 (53). This finding reveals a new area for investigating the role of lncRNA methylation in cancer development.

m^{6}A modification and hematopoietic tumor. *WTAP*, as an m^{6}A demethylase, plays a carcinogenic role in AML. Both *In vivo* and *in vitro* research has proved that *WTAP* was associated with cell transformation and all-trans retinoic acid (ATRA)-mediated leukemia cell differentiation (48). In addition, *METTL-3* inhibited the differentiation of hematopoietic stem/progenitor cell in patients with AML by inducing m^{6}A methylation, which maintained the undifferentiated phenotype of the leukemia cells and promoted the occurrence of AML. On the other hand, the knockdown or deletion of *METTL-3* could activate a translation process to promote cell differentiation and apoptosis, leading to the suppression of leukemia cells without affecting normal hematopoietic cells (17). Similarly, *METTL-14* plays a key role in both normal myelopoiesis and pathogenesis of AML (8). *METTL-14* could block normal myeloid differentiation and promote malignant bone marrow formation by mediating m^{6}A methylation. These studies provide new insights into the molecular mechanism of hematological tumorigenesis, suggesting that inhibition of *METTL-3*/14 may be used as a strategy for the treatment of malignant myeloid tumors.

m^{6}A modification and endometrial carcinoma. In 2018, a study found that m^{6}A methylation in mRNA played a crucial role in endometrial carcinogenesis with the activation of protein kinase B (PKB) signal (99). m^{6}A methylation reduced the expression of PKB negative regulator PH domain and leucine-rich repetitive protein phosphatase 2, whereas the expression of the positive PKB regulator mammalian target of rapamycin c2 was elevated, which promoted the proliferation and invasion endometrial cancer cells (49).

m^{6}A modification and cervical cancer. Previous studies have found that a low level of m^{6}A was associated with the occurrence of cervical cancer. In addition, the decrease in m6A level was positively associated with The International Federation of Gynecology and Obstetrics stage, tumor size, degree of differentiation, lymph node invasion and tumor recurrence (100). The results suggested that m^{6}A methylation site in mRNA may serve as a potential therapeutic target for cervical cancer and could be used as an independent prognostic factor for predicting disease-free survival and overall survival times in patients with cervical cancer (101,102).

m^{6}A modification and gastric cancer. In gastric cancer, the expression level of *ALKBH5*, a m^{6}A 'eraser', was significantly decreased in highly invasive diffuse gastric adenocarcinoma compared with that in adjacent tissues. The knockdown of *ALKBH5* could decrease the mRNA and protein expression levels of E-cadherin and increase the expression level of interstitial markers, such as snail (103) and N-cadherin (104). Further investigation showed that the downregulation of *ALKBH5* could decrease the ability of mRNA demethylation and promote the methylation level, which reduced the stability of E-cadherin mRNA and promoted the invasion of tumor cells. Furthermore, *ALKBH5*, as a tumor suppressor gene in gastric cancer (105), could suppress EMT, migration, and invasion of gastric cancer cells by inhibiting the mRNA and protein expression levels of *MMP-2* and *MMP-9*. In addition, *WTAP* was found to play an important role in the progression and metastasis of gastric cancer, and was associated with poor differentiation, lymph node metastasis, high TNM stages and poor prognosis (106). M^{6}A may also be an important molecular marker for monitoring gastric cancer. Furthermore, the expression level of *METTL-3* was positively associated with the prognosis, tumor grade and tumor stage in patients with gastric cancer (107). In addition, *METTL-3* was associated with the mRNA and protein expression levels of a-smooth muscle actin to regulate the proliferation and migration of gastric cancer cells, which could be a potential target for the treatment of gastric cancer in the future (108,109).

m^{6}A modification and other types of cancer. They regulate the level of m^{6}A through direct or indirect modification and participate in tumor progression. *WTAP* enhanced the expression of marrow zinc finger 1 (MZF1) by reducing the level of m^{6}A and destabilizing MZF1 mRNA in bone, thus promoting the progression of lung squamous cell carcinoma. *YTHDF2* and miR-495 inhibited the progression of prostate cancer by indirectly downregulating the level of m^{6}A methylation (110). *ALKBH5* maintained the expression level of fork box protein M1 mRNA by promoting m^{6}A demethylation, which retained the tumorigenicity of glioblastoma stem cells (52). The aforementioned studies revealed the importance of m^{6}A

modification in different types of cancer. The dynamic change of m^{6}A methylation has various regulatory effects on cancer cells (111). By revealing the previously unidentified regulation mechanism in tumors, further studies will provide bases for exploring the pathogenesis of tumors and developing new potential targets for cancer treatment (112,113).

6. Conclusions

Since m^{6}A methylation plays an important role in numerous types of malignant tumor, m^{6}A modification could be used as a diagnostic/prognostic target. Due to the effect of various related factors, the results of from several researchers are sometimes contradictory. This requires more multicenter and large-scale research for further investigation, thus laying the foundation for accurate treatment of human tumors.

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Author's contributions

YZ conceived and designed this study. The literature search was carried out by JY. ZT and JZ were involved in drafting the manuscript or revising it critically for important intellectual content, in addition they resolved any disagreements. The manuscript was drafted by YZ and WS. Manuscript revisions and modifications were carried out by YZ. Final changes were made by JY and WS. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

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Not applicable.

Competing interests

The authors declare that they have no competing interests.

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