Molecular Therapy
Nucleic Acids
Review

Epigenetic Regulation of m^{6}A Modifications in Human Cancer

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N6-methyladenosine (m^{6}A) is the most prevalent internal RNA modification, especially within eukaryotic messenger RNAs (mRNAs). m^{6}A modifications of RNA regulate splicing, translocation, stability, and translation into proteins. m^{6}A modifications are catalyzed by RNA methyltransferases, such as METTL3, METTL14, and WTAP (writers); the modifications are removed by the demethylases fat mass and obesity-associated protein (FTO) and ALKBH5 (ALKB homolog 5) (erasers); and the modifications are recognized by m^{6}A-binding proteins, such as YTHDF domain-containing proteins and IGF2BPs (readers). Abnormal changes in the m^{6}A levels of these genes are closely related to tumor occurrence and development. In this paper, we review the role of m^{6}A in human cancer and summarize its prospective applications in cancer.

N6-methyladenosine (m^{6}A) is an epigenetic modification of RNA molecules that was discovered in the early 1970s in messenger RNAs (mRNAs) from eukaryotes.[1] Due to the limitations of detection technology, scientists initially thought that m^{6}A modification sites existed only in mRNA, but recent studies have found that m^{6}A modifications exist in various types of RNA,[2] such as rRNAs, small nucleolar RNAs (snRNAs), and microRNAs. With the rapid development of high-throughput sequencing technology and the gradual progress of epigenetic research, the functions of m^{6}A modification in different biological processes have attracted people's attention again. At present, there are mature single-site or even high-throughput single-site detection methods that can accurately and effectively detect m^{6}A sites.

DNA carries the genetic information that is transcribed to generate mRNAs, which are then translated to generate functional proteins. In the process of cancer transformation, m^{6}A modification can influence the development of tumors by regulating the mRNA expression level of oncogenes and suppressor genes. Similar to DNA methylation, m^{6}A modification is regulated by methyltransferase and demethylase, and regulates the posttranscriptional expression level of genes without changing the base sequence, but the regulatory mechanism of m^{6}A is complex. Emerging evidence suggests that m^{6}A is involved in various aspects of RNA metabolism, including pre-mRNA splicing, 3′ end processing, nuclear export, translation regulation, mRNA decay, and noncoding RNA (ncRNA) processing.[3] Furthermore, we summarize the association between m^{6}A and human cancer occurrence and development to discover new markers and potential targets for molecular pathological diagnosis and molecular targeted therapy. The present review introduces m^{6}A and describes the detection methods of m^{6}A modification and its relationship with human cancer.

m^{6}A Modification of RNA

Molecular Mechanism of m^{6}A Modification

m^{6}A modification mainly occurs at the consensus motif of RRm^{6}ACH ([A/G/U][A/G]m^{6}AC[A/C/U]), and the modification is enriched in 3′ untranslated regions (3′ UTRs), near stop codons and within internal long exons.[4]

The first type of protein involved in m^{6}A regulation is the m^{6}A methyltransferase, which possesses the ability to modify RNA with m^{6}A modification; coding genes for these enzymes are called writing genes (writers). m^{6}A modification is catalyzed by a multicomponent methyltransferase complex.[5] METTL3, METTL14, and WTAP were the earliest writers discovered, and the complexes they form jointly promote the incorporation of the m^{6}A methylated group into RNA.[6–8] A newly discovered methyltransferase, METTL16, the paralog of METTL3, controls cellular SAM levels and methylates U6 snRNA.[9] Subsequently, more new proteins, such as KIAA1429, RBM15[9–12] (and its paralog RBM15B[12]), and ZC3H13,[13,14] have been identified as components of the methyltransferase complex and as essential for m^{6}A modification.

The second type of protein is the m^{6}A demethylase, which can remove m^{6}A methylated groups from RNA; its coding genes are known eraser genes (erasers), indicating that m^{6}A modification is actually a dynamic reversible process Figure 1. The common erasers include FTO[15] and ALKBH5 (ALKB homolog 5).[16] In addition, another

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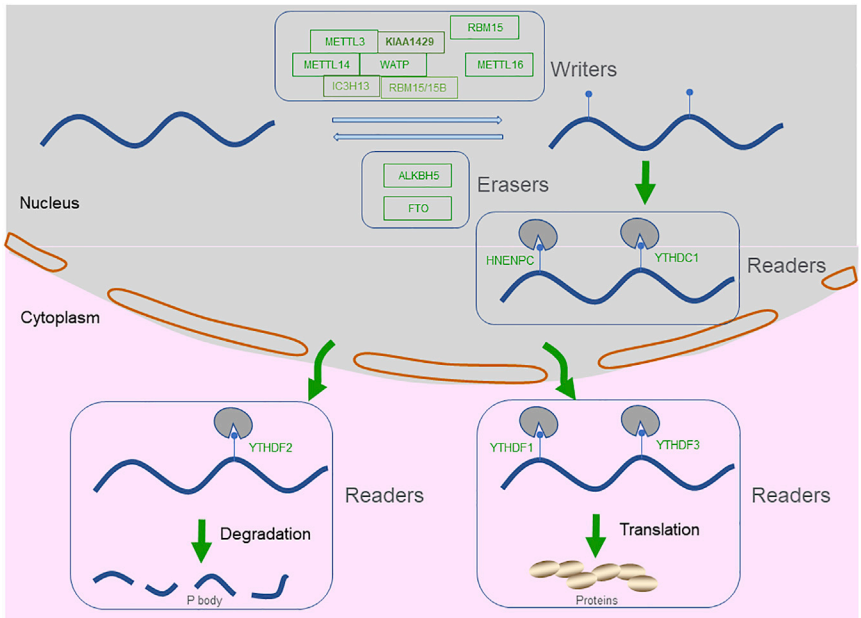

Figure 1. A Schematic Diagram Illustrates the Molecular Mechanisms and Functions of m^{6}A Methylation

Complexes formed by METTL3, METTL14, WTAP, RBM15, and METTL16 are named writers. ALKBH5 and FTO are common erasers. YTHDC1, YTHDF1, and YTHDF3 are common readers. Different readers binding to m^{6}A sites can produce different biological effects.

m^{6}A demethylase, ALKBH3 (ALKB homolog 3), has been found in recent studies.[17] Interestingly, ALKBH3 preferentially acts on m^{6}A in tRNA rather than in mRNA or rRNA. Because there are other members of the ALKB subfamily with unknown functions,[18] the discovery of more m^{6}A demethylases is unsurprising.

The last type of m^{6}A regulatory protein can bind to the m^{6}A modification site in RNA and play a specific role. The coding genes for these proteins are called readers. For the m^{6}A group to have a biological function, it needs to be identified by variable "readers," which initiate different downstream effects.[19] The earliest readers were coding genes in the YTH domain family proteins, including YTHDF and YTHDC subtypes, such as YTHDC1, YTHDC2,[12,20] YTHDF1, YTHDF2, and YTHDF3.[21] Later, several readers were found: eIF3, HNRNP C, and HNRNP A2/B1.[22–24] The reading protein binds to the m^{6}A site on RNA to perform various biological functions. At present, the biological functions of m^{6}A modification are not fully understood. However, it has been found that the proteins of the YTHDF subtype are mainly located in the cytoplasm, and the YTHDF1 and YTHDF3 proteins can improve the efficiency of mRNA translation. The binding of YTHDF2 protein to the m^{6}A site is associated with shortening the half-life of mRNA.[25,26] YTHDF2 acts as a multivalent scaffold, and when m^{6}A binds to a YTHDF protein, the protein juxtaposes its low-complexity domain, resulting in phase separation for the bound RNA.[27] However, it has also been found that the YTHDF2 protein can prevent m^{6}A modification in the 5′ UTR of the FTO protein by binding to the m^{6}A site in the nucleus, thereby promoting the cap-independent translation of RNA.[28,29] The YTHDC subtype of protein mainly functions in the nucleus. YTHDC1 regulates transcription of both coding and noncoding genes, and it regulates mRNA expression levels by affecting alternative splicing of mRNA precursors.[20,30] Little is known about YTHDC2 and m^{6}A modification. It has been reported that the YTHDC2 protein can exist both inside and outside the nucleus, and it can selectively bind to the m^{6}A site of ncRNAs,[12] but the biological functional result of binding remains a mystery. In the HNRNP family, it was found that the HNRNP A2/B1 protein may be involved in the regulation of the transcription of precursor miRNAs,[22] whereas the HNRNP C protein may affect the local secondary structure of mRNAs and long ncRNAs (lncRNAs).[23] Recently, another study verified that mRNA binding proteins of insulin-like growth factor 2 (IGF2BPs, including IGF2BP1, IGF2BP2, and IGF2BP3), which serve as a distinct family of m^{6}A readers, could also recognize m^{6}A modifications.[31] IGF2BPs promote the stability and storage of their target mRNAs in a m^{6}A-dependent fashion, thus affecting gene regulation and cancer biology. At present, new writing and reading proteins related to m^{6}A modification are still being identified, suggesting a broad area of research left to explore regarding the dynamic regulation of m^{6}A modification levels of RNA and the potential regulation of biological functions. Recently, Zhou et al.[32] revealed an integrated mechanism of cotranscriptional m^{6}A-mediated splicing regulation: a m^{6}A reader protein uses RGG motifs to cotranscriptionally interact with both RNAPII and a m^{6}A-modified nascent pre-mRNA to modulate RNAPII occupancy and alternative splicing. In addition, a finding reveals that multivalent m^{6}A-containing RNAs can promote the phase separation potential of YTHDF proteins *in vitro* and in cells. Multivalent m^{6}A-driven phase separation of YTHDFs is likely important for many functions of m^{6}A.[27,33]

It is expected that in the future, there will be a more comprehensive understanding of the biological function of m^{6}A modification so that epigenetics can generate more knowledge regarding the regulation of gene expression at the posttranscriptional level.

Methods of m^{6}A Modification Detection

The identification of m^{6}A sites was initially very difficult, because m^{6}A modification of RNA does not affect its reverse transcription. However, with the emergence of second-generation sequencing, two similar m^{6}A modification site detection technologies were created: m^{6}A sequencing (m^{6}A-seq)[34] and combine m^{6}A-specific methylated RNA immunoprecipitation with next-generation sequencing (MeRIP-seq).[35] In the two detection methods, m^{6}A methylated RNA fragments are captured by immunoprecipitation

technology and then identified by second-generation sequencing. These two methods revealed m^{6}A to be a pervasive and dynamically reversible modification, particularly enriched in 3′ UTRs and near mRNA stop codons. The shortcoming of this method is that the RNA fragments captured by this technique are limited to approximately 100–200 nt, and the technique cannot identify two m^{6}A sites that are very close to each other; therefore, this method cannot accurately identify m^{6}A modification sites in the whole transcriptome. Another technique for detecting m^{6}A modification level is m^{6}A-level and isoform-characterization sequencing (m^{6}A-LAIC-seq),[36] which is similar to m^{6}A-seq but introduces spiked-in RNAs as an internal reference; the spiked-in RNAs are used to calculate the m^{6}A modification level of each gene in the whole transcriptional group, but single m^{6}A modification sites cannot be detected.

Other methods have been developed to improve resolution, such as PA-m^{6}A-Seq (photo-crosslinking-assisted m^{6}A sequencing),[37] m^{6}A individual-nucleotide-resolution cross-linking and immunoprecipitation (miCLIP),[38] and m^{6}A-CLIP.[39] In these methods, UV strengthens the crosslink between m^{6}A-containing RNA fragments and m^{6}A antibodies, and then antibody-RNA complexes are obtained through affinity purification. m^{6}A modification sites can be more accurately detected at a single-base resolution of RNA using site-specific cleavage and radioactive-labeling followed by ligation-assisted extraction and thin-layer chromatography (SCARLET) immunoprecipitation techniques.

In 2013, SCARLET[40] was developed. This method can accurately detect a single m^{6}A modification site in mRNAs and lncRNAs, and can calculate the m^{6}A modification level of the whole RNA. Although SCARLET is incapable of high-throughput screening and is time-consuming, it is relatively expensive, and its high accuracy makes it a common method used to check the accuracy of high-throughput detection of m^{6}A methylated sites.

Recently, Garcia-Campos et al.[41] developed MAZTER-seq, which allows systematic m^{6}A quantitation by digesting RNA via antibody-independent methods; the results revealed that antibody-based methods are of limited sensitivity. This method can quantitatively track m^{6}A in diverse biological settings, but it also has limitations. Only when a subset of RNA sites occurs at ACA sites and is within suitable distances of adjacent ACA sites can m^{6}A be quantified.

Furthermore, Liu et al.[42] reported RNAmod, which provides intuitive interfaces to show outputs, including the distribution of RNA modifications, modification coverage for different gene features, functional annotation of modified mRNAs, and comparisons between different groups or specific gene sets.

However, our current knowledge about dynamic changes in m^{6}A levels and how the change in m^{6}A levels for a specific gene can play a role in certain biological processes is largely elusive. To address this lack of knowledge, Zhang et al.[43] proposed FunDMDeep-m^{6}A, which is a novel pipeline for identifying context-specific (e.g., disease versus normal, differentiated cells versus stem cells, or gene knockdown cells versus wild-type cells) m^{6}A-modified functional genes.

Liu et al.[44] showed that, using direct RNA sequencing, m^{6}A RNA modifications could be detected with high accuracy in the form of systematic errors and decreased base-calling qualities. Specifically, they found that an algorithm, trained with m^{6}A-modified and unmodified synthetic sequences, could predict m^{6}A RNA modifications with ~90% accuracy.

Zhang et al.[45] developed a precise and high-throughput antibody-independent m^{6}A identification method based on an m^{6}A-sensitive RNA endoribonuclease that recognizes ACA motifs (m^{6}A-sensitive RNA-endoribonuclease-facilitated sequencing [m^{6}A-REF-seq]). Whole-transcriptomic, single-base m^{6}A maps generated by m^{6}A-REF-seq quantitatively displayed an explicit distribution pattern with enrichment near stop codons. They used independent methods to validate the methylation status and abundance of individual m^{6}A sites, confirming the high reliability and accuracy of the m^{6}A-REF-seq data.

Although multiple methods Table 1 have been developed to detect m^{6}A modification, many difficulties and challenges remain at both single-base and quantitative sequencing levels, and more methods are desperately needed.

Prediction of m^{6}A Modification Sites

Bioinformatics can greatly improve research efficiency by effectively predicting m^{6}A modification sites (Table 1). Zhang et al.[46] first proposed HMM (hidden Markov model) to predict residual sites around known sites. Subsequently, Liu et al.[47] developed a faster and more stable method, pRNAm-PC, to predict sites. Moreover, Chen et al.[48] developed the iRNA-Methyl method. The common feature of pRNAm-PC and iRNA-Methyl is the application of an SVM (support vector machine) model. On this basis, Jia et al.[49] developed a RNA-methylPred method, which is more stable and efficient than the previous two. Subsequently, Li et al.[50] proposed an improved TargetM^{6}A method, but this method could predict only the methylation site of m^{6}A in primary RNA sequences. Zhou et al.[51] proposed the SRAMP method, which could more effectively predict m^{6}A modification sites in mammalian RNA by integrating various mathematical models. Recently, a database website named rmbase-v2.0 (rmbase-v2.0: http://rna.sysu.edu.cn/rmbase/)[52] was established, and it contains the sequencing data of multiple kinds of RNA epigenetic modifications in 13 species. This database contains a substantial amount of data on m^{6}A modification sites, which can predict m^{6}A modification information of RNAs to be studied.

Song et al.[53] proposed a computational approach for the prediction of the target sites of m^{6}A enzymes. Their method achieved reasonable performance on both writer target prediction (as high as 0.918) and eraser target prediction (as high as 0.888) in a 5-fold cross-validation analysis, and the results of the gene ontology analysis of their

Table 1. Methods for Detection and Prediction of m^{6}A Methylation

Methods	Features
Detection	
m^{6}A-Seq[34]	high throughput
MeRIP-seq[35]	high throughput
m^{6}A-LAIC-seq[36]	high throughput, precise
PA-m^{6}A-Seq[37]	high throughput, single site
miCLIP[38]	high throughput, single site
m^{6}A-CLIP[39]	high throughput, single site
SCARLET[40]	high throughput, single site
MAZTER-seq[41]	high throughput, single site
RNAmod[42]	high throughput, single site
FunDMDeep-m^{6}A[43]	high throughput, single site
DART-seq	high throughput, single site
RNA sequencing[44]	high throughput, single site
m^{6}A-REF-seq[45]	high throughput, single site
Prediction	
HMM[46]	database
pRNAm-PC[47]	database
iRNA-Methyl[48]	database
RNA-methylPred[49]	database
TargetM^{6}A[50]	database
SRAMP[51]	database
RMbase-v2.0[52]	database
DART-seq[53]	database

preferential targets further revealed the functional relevance of different RNA methylation writers and erasers.

Meyer[54] presented DART-seq (deamination adjacent to RNA modification targets), which is an antibody-free method for detecting m^{6}A sites. In DART-seq, the cytidine deaminase APOBEC1 is fused to the m^{6}A-binding YTH domain. APOBEC1-YTH expression in cells induces C-to-U deamination at sites adjacent to m^{6}A residues, which are detected using standard RNA-seq.

m^{6}A Modification and Tumors

m^{6}A Hypermethylation and Tumors

m^{6}A modification is involved in the differentiation process of hematopoietic stem cells in vertebrates, suggesting that m^{6}A modification may be closely related to the occurrence and development of hematopoietic tumors in the blood system. The METTL3 gene has different effects on the proliferation and growth of mouse and human acute myelocytic leukemia cell lines; among them, Acute myeloid leukemia (AML) cell lines expressing the KMT2A-MLLT3 fusion gene and the Flt3 gene were most significantly affected.[55] The downregulation of METTL3 gene expression promotes the differentiation of AML cells. Due to the regulation of METTL3 expression by the transcription factor CEBPZ, the m^{6}A modification levels of mRNA transcribed by the oncogene SP1 increase significantly when METTL3 is downregulated, eventually leading to an increase of SP1 protein expression. SP1 protein can continuously affect the differentiation of hematopoietic stem cells into AML cells, suggesting that METTL3 may be a potential therapeutic target for KMT2A-MLLT3 and mutated Flt3 in AML. Vu et al.[56] also reported that METTL3 was associated with the occurrence and development of AML. This study found that the pAKT gene expression in CD34$^+$ hematopoietic stem cells increased after the METTL3 gene was downregulated, thus promoting the differentiation of normal hematopoietic stem cells into AML cells and vice versa. It was also found that m^{6}A modification promoted the transcription of c-MYC, BCL2, and PTEN in CD34$^+$ hematopoietic stem cells.[57] Recently, Han et al.[58] found that METTL3 may positively regulate the pri-miR221/222 process in a m^{6}A-dependent manner by interacting with the microprocessor protein DGCR8, thereby exerting a carcinogenic effect in bladder cancer. In addition, Jin et al.[59] demonstrated an oncogenic role of m6A-modified ITGA6, showing its regulatory mechanisms in bladder cancer development and progression, thus identifying a potential therapeutic target for bladder cancer. Yang et al.[60] confirmed the dynamic modification of m^{6}A in chemically induced malignant transformation and provided insight into the key role of the METTL3-m^{6}A-CDCP1 axis in chemical carcinogenesis. In addition, Cheng M et al.[61] recently found that the AFF4/NF-KB/MYC signaling network is modified by METTL3-mediated m^{6}A and provided insight into the mechanism of bladder cancer progression. Recently, Li et al.[62] found that m^{6}A modulates nonsense-mediated mRNA decay in human glioblastoma, revealing a novel function for m^{6}A in modulating NMD and uncovering the mechanism by which METTL3 promotes glioblastoma multiforme (GBM) tumor growth and progression. Li et al.[63] revealed that METTL3, acting as an oncogene, maintained SOX2 expression through an m^{6}A-IGF2BP2-dependent mechanism in CRC (colorectal carcinoma) cells, and they suggested a panel of potential biomarkers for prognostic prediction of CRC.

Weng et al.[64] found that the METTL14 gene in normal hematopoietic stem cells and mixed surface AML cells accompanied by t(11q23) t(15);17) or t (8;21) was highly expressed in normal differentiated bone marrow cells but poorly expressed in normal differentiated bone marrow cells. The METTL14 gene encodes a protein that increases m^{6}A modification levels in some transcriptomes. In this study, it was found that knocking down the expression of METTL14 in AML cell lines reduced the stability of mRNA transcribed by MYB and MYC, and accelerated the degradation of the RNA. This indicates that MYB and MYC are regulated by METTL14. METTL14 plays a role in the differentiation of hematopoietic stem cells into AML cells and plays a role in the development and maintenance of leukemia and self-renewal. Silent expression of METTL14 can effectively inhibit the proliferation of AML cell lines. The effect of the SPY1-METTL14-MYB/MYC pathway on AML was revealed, providing a new direction for the diagnosis and treatment of AML. Chen et al.[65] found that METTL3 can change the m^{6}A modification level of the tumor suppressor gene SOCS2. In addition, METTL3 overexpression can promote the proliferation and migration of hepatocellular carcinoma

Table 2. m6A Methylation-Related Tumor

Related Proteins that Encode Genes	Trend	Cancer	Regulatory Gene
Writers			
METTL3	↑	leukemia	*SP1*, *c-MYC*, *BCL2*, *PTEN*
	↑	hepatoma	*SOCS2*
	↑	bladder cancer	*DGCR8*, *CDCP1*, *AFF4/NF-kB/MYC*
	↓	glioblastoma	*ADAM19*, *EPHA3*, *KLF4*, *CDKN2A*, *BRCA2*, *TP53*
	↑	colorectal carcinoma	*IGF2BP2*
METTL14	↑	leukemia	*MYB*, *MYC*
	↓	glioblastoma	*ADAM19*, *EPHA3*, *KLF4*, *CDKN2A*, *BRCA2*, *TP53*
WTAP	↑	hepatoma	*ETS proto-oncogene 1*
Erasers			
FTO	↑	leukemia	*ASB2*, *RARA*, *TP53*
	↑	cervical carcinoma	β-*catenin* pathway
	↑	melanoma	autophagy and NF-κB pathway *BNIP3*
ALKBH5	↑	breast carcinoma	*KLF4*, *NANOG*
Readers			
YTHDF1	↑	colorectal cancer	
	↑	hepatoma	
YTHDF2	↑	hepatoma	*SOCS2*
	↑	AML	*LSC*

(HCC), whereas decreased METTL3 may inhibit the growth and metastasis of cells. SOCS2 acts as a cancer suppressor gene in HCC, whereas METTL3 accelerates the degradation of SOCS2 mRNA by regulating the m^{6}A-YTHDF2 pathway. This study suggests that m^{6}A hypermethylation is also correlated with the development of HCC. Chen et al.[66] first reported that WTAP-mediated m^{6}A modification has a crucial role in HCC oncogenesis, and they identified WTAP as a potential therapeutic target for HCC treatment.

m^{6}A Hypomethylation and Tumors

The concentrated expression of FTO at the mixed lineage leukemia (MLL) gene site in reprogrammed AML cells could reduce the methylation level of m^{6}A in the mRNA of ASB2 and RARA genes, thus leading to the occurrence and development of AML. Recently, Paris et al.[67] demonstrated that YTHDF2 is also a key factor for cancer stem cells in AML. They found that inhibition of YTHDF2 specifically compromises leukemic stem cell (LSC) development and propagation. At the same time, they found that the high expression of FTO could inhibit the differentiation of AML cells into normal blood cells following treatment with all-*trans*-retinoic acid.[68] This makes FTO, which is a demethylase, an oncogene related to MLL in AML. In cervical squamous cell carcinoma (CSCC), FTO expression was significantly increased, and it was found that these patients developed tolerance to radiotherapy and chemotherapy, which may be because of FTO reducing the m^{6}A modification level of some genes, thereby activating the β-catenin pathway and affecting the expression of ERCC1.[69] Increased expression of both FTO and β-catenin in patients with CSCC presented a worse prognosis than for patients with increased expression of both FTO and β-catenin alone. Therefore, the expression of FTO and β-catenin has a certain prognostic value for evaluating the clinical progression of CSCC. METTL3 and METTL14 were significantly reduced in CSCC, whereas FTO and ALKBH5 were significantly increased. It is speculated that m^{6}A hypomethylation may play an important role in the occurrence and development of cervical cancer. Disease-free survival (DFS) and overall survival (OS) of m^{6}A hypomethylated patients were significantly reduced, and hypomethylated patients had a higher recurrence rate ($p < 0.01$). Hypermethylation of m^{6}A in cervical cancer cells can significantly inhibit proliferation. Yang et al.[70] demonstrated a crucial role of FTO as an m^{6}A demethylase in promoting melanoma tumorigenesis and anti-PD-1 (PDCD-1) resistance, and the authors suggested that the combination of FTO inhibition with anti-PD-1 treatment may reduce the resistance to immunotherapy in melanoma. Niu et al.[71] showed that FTO, a key m^{6}A demethylase, was upregulated in human breast cancer. A high level of FTO was significantly associated with lower survival rates in patients with breast cancer. Because the methylation level of m^{6}A in tumors often depends on the levels of transmethylase and demethylase, the study of the gene expression levels of these two enzymes in tumors adds to the in-depth understanding of the correlation between the occurrence and development of tumors and m^{6}A modification, which could help establish a new method for early diagnosis and prognostic analysis of tumors Table 2.

m^{6}A Modification Readers and Tumors

Reading proteins can specifically bind to m^{6}A sites in RNA, and different reading proteins will produce different biological functions after binding to m^{6}A sites. For example, the YTHDF2 protein binds to the m^{6}A site in the mRNA to accelerate the degradation of mRNA. When the m^{6}A modification level of mRNA for the tumor suppressor gene SOCS2 in HCCs is upregulated, the YTHDF2 protein has more m^{6}A binding sites and ultimately promotes mRNA degradation.[65] The YTHDF1 gene is highly expressed in patients with colorectal cancer.[72] Stratification of clinicopathological data showed that the expression of the YTHDF1 gene was correlated with tumor diameter, lymph node metastasis, distant metastasis, and clinical stage, but the specific regulatory mechanisms were not studied. Yang et al.[73] collected samples from 31 HCC patients with stage III TNM and found that the increased expression rate of YTHDF2 was as high as 83.9% (26/31), suggesting that HCC patients at an advanced stage were most likely to have YTHDF2 protein binding to the m^{6}A site of some genes. Subsequently, the author overexpressed miR-145 in HepG2 cell lines to reduce the expression of YTHDF2, and found that the proliferation of HepG2 cells was significantly inhibited, suggesting that miR-145 may be used as a potential therapy for HCC.[74] Zhao et al.[75] studied the liver cancer data in The Cancer Genome Atlas (TCGA) and found that the expression of YTHDF1 was significantly upregulated in patients with stage III and IV TNM compared with patients with stage II HCC, and the

patients with high levels of YTHDF1 had a worse prognosis. It was found that the potential target genes regulated by YTHDF1 protein may be related to the degradation of various amino acids and lipid metabolism of tumor cell cycles, and the abnormal physiological functions of these cells may be related to the occurrence and development of HCC. Finally, the author verified that c-Myc is the upstream gene regulating YTHDF1 through analysis of the TCGA and chromatin immunoprecipitation (ChIP)-atlas database prediction data and experiments.

Conclusions

m^6A modification is one of many types of RNA epigenetic modification. Based on the current understanding of its association with tumors, m^6A modification is not good or bad in itself, and it mainly promotes or inhibits the production of tumor cells by regulating the mRNA levels of related oncogenes or suppressor genes. Li et al.[76] analyzed the foundational landscape of molecular alterations and clinically relevant m^6A regulators; they also provided insights into the development of related therapeutic targets. With increasing research on the m^6A modification mechanism, it was found that m^6A modification enables more complex and diverse regulation of related RNA levels. Interestingly, simply the binding of a protein to the m^6A modification site may not only promote the expression of related mRNAs but also reduce the stability of related mRNAs and accelerate degradation. In addition, ncRNAs can regulate the expression level of target genes, and when m^6A modification occurs in ncRNA, the expression level of the ncRNA itself is regulated. The two-sided regulation of m^6A modification, especially the regulation of ncRNA, might provide a new perspective to clarify some problems that could not be originally explained.[29] The m^6A modification level in RNA is closely related to the expression level of writing and erasing genes in cells, whereas the proteins that are readers bind to the m^6A modification site to initiate a series of biological functions. Therefore, in tumors, changes in the expression level of both m^6A-related genes and proteins are likely to be potential markers for tumor molecular diagnosis and may provide new targets for the research and development of clinical molecular targeted therapies.

AUTHOR CONTRIBUTIONS

W.Z., X.Q., and L.L. wrote and drafted the manuscript and figures. J.W., J.L., and Z.L. revised the manuscript. All authors read and approved the final manuscript.

CONFLICTS OF INTEREST

The authors declare no competing interests.

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REFERENCES

1. Dubin, D.T., and Taylor, R.H. (1975). The methylation state of poly A-containing messenger RNA from cultured hamster cells. Nucleic Acids Res. *2*, 1653–1668.
2. Lee, M., Kim, B., and Kim, V.N. (2014). Emerging roles of RNA modification: m(6)A and U-tail. Cell *158*, 980–987.
3. Shi, H., Wang, X., Lu, Z., Zhao, B.S., Ma, H., Hsu, P.J., Liu, C., and He, C. (2017). YTHDF3 facilitates translation and decay of N^6-methyladenosine-modified RNA. Cell Res. *27*, 315–328.
4. Kane, S.E., and Beemon, K. (1985). Precise localization of m6A in Rous sarcoma virus RNA reveals clustering of methylation sites: implications for RNA processing. Mol. Cell. Biol. *5*, 2298–2306.
5. Bokar, J.A., Rath-Shambaugh, M.E., Ludwiczak, R., Narayan, P., and Rottman, F. (1994). Characterization and partial purification of mRNA N6-adenosine methyltransferase from HeLa cell nuclei. Internal mRNA methylation requires a multisubunit complex. J. Biol. Chem. *269*, 17697–17704.
6. Bokar, J.A., Shambaugh, M.E., Polayes, D., Matera, A.G., and Rottman, F.M. (1997). Purification and cDNA cloning of the AdoMet-binding subunit of the human mRNA (N6-adenosine)-methyltransferase. RNA *3*, 1233–1247.
7. Wang, Y., Li, Y., Toth, J.I., Petroski, M.D., Zhang, Z., and Zhao, J.C. (2014). N6-methyladenosine modification destabilizes developmental regulators in embryonic stem cells. Nat. Cell Biol. *16*, 191–198.
8. Ping, X.L., Sun, B.F., Wang, L., Xiao, W., Yang, X., Wang, W.J., Adhikari, S., Shi, Y., Lv, Y., Chen, Y.S., et al. (2014). Mammalian WTAP is a regulatory subunit of the RNA N6-methyladenosine methyltransferase. Cell Res. *24*, 177–189.
9. Pendleton, K.E., Chen, B., Liu, K., Hunter, O.V., Xie, Y., Tu, B.P., and Conrad, N.K. (2017). The U6 snRNA m^6A Methyltransferase METTL16 Regulates SAM Synthetase Intron Retention. Cell *169*, 824–835.e14.
10. Horiuchi, K., Kawamura, T., Iwanari, H., Ohashi, R., Naito, M., Kodama, T., and Hamakubo, T. (2013). Identification of Wilms' tumor 1-associating protein complex and its role in alternative splicing and the cell cycle. J. Biol. Chem. *288*, 33292–33302.
11. Schwartz, S., Mumbach, M.R., Jovanovic, M., Wang, T., Maciag, K., Bushkin, G.G., Mertins, P., Ter-Ovanesyan, D., Habib, N., Cacchiarelli, D., et al. (2014). Perturbation of m6A writers reveals two distinct classes of mRNA methylation at internal and 5′ sites. Cell Rep. *8*, 284–296.
12. Patil, D.P., Chen, C.K., Pickering, B.F., Chow, A., Jackson, C., Guttman, M., and Jaffrey, S.R. (2016). m(6)A RNA methylation promotes XIST-mediated transcriptional repression. Nature *537*, 369–373.
13. Wen, J., Lv, R., Ma, H., Shen, H., He, C., Wang, J., Jiao, F., Liu, H., Yang, P., Tan, L., et al. (2018). Zc3h13 Regulates Nuclear RNA m^6A Methylation and Mouse Embryonic Stem Cell Self-Renewal. Mol. Cell *69*, 1028–1038.e6.
14. Knuckles, P., Lence, T., Haussmann, I.U., Jacob, D., Kreim, N., Carl, S.H., Masiello, I., Hares, T., Villaseñor, R., Hess, D., et al. (2018). Zc3h13/Flacc is required for adenosine methylation by bridging the mRNA-binding factor Rbm15/Spenito to the m^6A machinery component Wtap/Fl(2)d. Genes Dev. *32*, 415–429.
15. Jia, G., Fu, Y., Zhao, X., Dai, Q., Zheng, G., Yang, Y., Yi, C., Lindahl, T., Pan, T., Yang, Y.G., and He, C. (2011). N6-methyladenosine in nuclear RNA is a major substrate of the obesity-associated FTO. Nat. Chem. Biol. *7*, 885–887.
16. Huang, Y., Yan, J., Li, Q., Li, J., Gong, S., Zhou, H., Gan, J., Jiang, H., Jia, G.F., Luo, C., and Yang, C.G. (2015). Meclofenamic acid selectively inhibits FTO demethylation of m6A over ALKBH5. Nucleic Acids Res. *43*, 373–384.
17. Ueda, Y., Ooshio, I., Fusamae, Y., Kitae, K., Kawaguchi, M., Jingushi, K., Hase, H., Harada, K., Hirata, K., and Tsujikawa, K. (2017). AlkB homolog 3-mediated tRNA demethylation promotes protein synthesis in cancer cells. Sci. Rep. *7*, 42271.
18. Iyer, L.M., Tahiliani, M., Rao, A., and Aravind, L. (2009). Prediction of novel families of enzymes involved in oxidative and other complex modifications of bases in nucleic acids. Cell Cycle *8*, 1698–1710.
19. Allis, C.D., and Jenuwein, T. (2016). The molecular hallmarks of epigenetic control. Nat. Rev. Genet. *17*, 487–500.
20. Meyer, K.D., and Jaffrey, S.R. (2017). Rethinking m^6A Readers, Writers, and Erasers. Annu. Rev. Cell Dev. Biol. *33*, 319–342.

21. Wang, X., Lu, Z., Gomez, A., Hon, G.C., Yue, Y., Han, D., Fu, Y., Parisien, M., Dai, Q., Jia, G., et al. (2014). N6-methyladenosine-dependent regulation of messenger RNA stability. Nature *505*, 117–120.

22. Alarcón, C.R., Goodarzi, H., Lee, H., Liu, X., Tavazoie, S., and Tavazoie, S.F. (2015). HNRNPA2B1 Is a Mediator of m(6)A-Dependent Nuclear RNA Processing Events. Cell *162*, 1299–1308.

23. Liu, N., Dai, Q., Zheng, G., He, C., Parisien, M., and Pan, T. (2015). N(6)-methyladenosine-dependent RNA structural switches regulate RNA-protein interactions. Nature *518*, 560–564.

24. Meyer, K.D., Patil, D.P., Zhou, J., Zinoviev, A., Skabkin, M.A., Elemento, O., Pestova, T.V., Qian, S.B., and Jaffrey, S.R. (2015). 5′ UTR m(6)A Promotes Cap-Independent Translation. Cell *163*, 999–1010.

25. Li, Z., Huang, C., Bao, C., Chen, L., Lin, M., Wang, X., Zhong, G., Yu, B., Hu, W., Dai, L., et al. (2017). Corrigendum: Exon-intron circular RNAs regulate transcription in the nucleus. Nat. Struct. Mol. Biol. *24*, 194.

26. Wang, X., Zhao, B.S., Roundtree, I.A., Lu, Z., Han, D., Ma, H., Weng, X., Chen, K., Shi, H., and He, C. (2015). N(6)-methyladenosine Modulates Messenger RNA Translation Efficiency. Cell *161*, 1388–1399.

27. Ries, R.J., Zaccara, S., Klein, P., Olarerin-George, A., Namkoong, S., Pickering, B.F., Patil, D.P., Kwak, H., Lee, J.H., and Jaffrey, S.R. (2019). m^6A enhances the phase separation potential of mRNA. Nature *571*, 424–428.

28. Zhou, J., Wan, J., Gao, X., Zhang, X., Jaffrey, S.R., and Qian, S.B. (2015). Dynamic m(6)A mRNA methylation directs translational control of heat shock response. Nature *526*, 591–594.

29. Wu, J., Qi, X., Liu, L., Hu, X., Liu, J., Yang, J., Yang, J., Lu, L., Zhang, Z., Ma, S., et al. (2019). Emerging Epigenetic Regulation of Circular RNAs in Human Cancer. Mol. Ther. Nucleic Acids *16*, 589–596.

30. Xiao, W., Adhikari, S., Dahal, U., Chen, Y.S., Hao, Y.J., Sun, B.F., Sun, H.Y., Li, A., Ping, X.L., Lai, W.Y., et al. (2016). Nuclear m(6)A Reader YTHDC1 Regulates mRNA Splicing. Mol. Cell *61*, 507–519.

31. Huang, H., Weng, H., Sun, W., Qin, X., Shi, H., Wu, H., Zhao, B.S., Mesquita, A., Liu, C., Yuan, C.L., et al. (2018). Recognition of RNA N^6-methyladenosine by IGF2BP proteins enhances mRNA stability and translation. Nat. Cell Biol. *20*, 285–295.

32. Zhou, K.I., Shi, H., Lyu, R., Wylder, A.C., Matuszek, Ż., Pan, J.N., He, C., Parisien, M., and Pan, T. (2019). Regulation of Co-transcriptional Pre-mRNA Splicing by m^6A through the Low-Complexity Protein hnRNPG. Mol. Cell *76*, 70–81.e9.

33. Gao, Y., Pei, G., Li, D., Li, R., Shao, Y., Zhang, Q.C., and Li, P. (2019). Multivalent m^6A motifs promote phase separation of YTHDF proteins. Cell Res. *29*, 767–769.

34. Dominissini, D., Moshitch-Moshkovitz, S., Schwartz, S., Salmon-Divon, M., Ungar, L., Osenberg, S., Cesarkas, K., Jacob-Hirsch, J., Amariglio, N., Kupiec, M., et al. (2012). Topology of the human and mouse m6A RNA methylomes revealed by m6A-seq. Nature *485*, 201–206.

35. Meyer, K.D., Saletore, Y., Zumbo, P., Elemento, O., Mason, C.E., and Jaffrey, S.R. (2012). Comprehensive analysis of mRNA methylation reveals enrichment in 3′ UTRs and near stop codons. Cell *149*, 1635–1646.

36. Molinie, B., Wang, J., Lim, K.S., Hillebrand, R., Lu, Z.X., Van Wittenberghe, N., Howard, B.D., Daneshvar, K., Mullen, A.C., Dedon, P., et al. (2016). m(6)A-LAIC-seq reveals the census and complexity of the m(6)A epitranscriptome. Nat. Methods *13*, 692–698.

37. You, X., Vlatkovic, I., Babic, A., Will, T., Epstein, I., Tushev, G., Akbalik, G., Wang, M., Glock, C., Quedenau, C., et al. (2015). Neural circular RNAs are derived from synaptic genes and regulated by development and plasticity. Nat. Neurosci. *18*, 603–610.

38. Linder, B., Grozhik, A.V., Olarerin-George, A.O., Meydan, C., Mason, C.E., and Jaffrey, S.R. (2015). Single-nucleotide-resolution mapping of m6A and m6Am throughout the transcriptome. Nat. Methods *12*, 767–772.

39. Ke, S., Alemu, E.A., Mertens, C., Gantman, E.C., Fak, J.J., Mele, A., Haripal, B., Zucker-Scharff, I., Moore, M.J., Park, C.Y., et al. (2015). A majority of m6A residues are in the last exons, allowing the potential for 3′ UTR regulation. Genes Dev. *29*, 2037–2053.

40. Liu, N., Parisien, M., Dai, Q., Zheng, G., He, C., and Pan, T. (2013). Probing N6-methyladenosine RNA modification status at single nucleotide resolution in mRNA and long noncoding RNA. RNA *19*, 1848–1856.

41. Garcia-Campos, M.A., Edelheit, S., Toth, U., Safra, M., Shachar, R., Viukov, S., Winkler, R., Nir, R., Lasman, L., Brandis, A., et al. (2019). Deciphering the "m^6A Code" via Antibody-Independent Quantitative Profiling. Cell *178*, 731–747.e16.

42. Liu, Q., and Gregory, R.I. (2019). RNAmod: an integrated system for the annotation of mRNA modifications. Nucleic Acids Res. *47* (W1), W548–W555.

43. Zhang, S.Y., Zhang, S.W., Fan, X.N., Zhang, T., Meng, J., and Huang, Y. (2019). FunDMDeep-m6A: identification and prioritization of functional differential m6A methylation genes. Bioinformatics *35*, i90–i98.

44. Liu, H., Begik, O., Lucas, M.C., Ramirez, J.M., Mason, C.E., Wiener, D., Schwartz, S., Mattick, J.S., Smith, M.A., and Novoa, E.M. (2019). Accurate detection of m^6A RNA modifications in native RNA sequences. Nat. Commun. *10*, 4079.

45. Zhang, Z., Chen, L.Q., Zhao, Y.L., Yang, C.G., Roundtree, I.A., Zhang, Z., Ren, J., Xie, W., He, C., and Luo, G.Z. (2019). Single-base mapping of m6A by an antibody-independent method. Sci. Adv *5*, eaax0250.

46. Zhang, Y.C., Zhang, S.W., Liu, L., Liu, H., Zhang, L., Cui, X., Huang, Y., and Meng, J. (2015). Spatially Enhanced Differential RNA Methylation Analysis from Affinity-Based Sequencing Data with Hidden Markov Model. BioMed Res. Int. *2015*, 852070.

47. Liu, Z., Xiao, X., Yu, D.J., Jia, J., Qiu, W.R., and Chou, K.C. (2016). pRNAm-PC: Predicting N(6)-methyladenosine sites in RNA sequences via physical-chemical properties. Anal. Biochem. *497*, 60–67.

48. Chen, W., Feng, P., Ding, H., Lin, H., and Chou, K.C. (2015). iRNA-Methyl: Identifying N(6)-methyladenosine sites using pseudo nucleotide composition. Anal. Biochem. *490*, 26–33.

49. Jia, C.Z., Zhang, J.J., and Gu, W.Z. (2016). RNA-MethylPred: A high-accuracy predictor to identify N6-methyladenosine in RNA. Anal. Biochem. *510*, 72–75.

50. Li, G.Q., Liu, Z., Shen, H.B., and Yu, D.J. (2016). TargetM6A: Identifying N^6-Methyladenosine Sites From RNA Sequences via Position-Specific Nucleotide Propensities and a Support Vector Machine. IEEE Trans. Nanobioscience *15*, 674–682.

51. Zhou, Y., Zeng, P., Li, Y.H., Zhang, Z., and Cui, Q. (2016). SRAMP: prediction of mammalian N6-methyladenosine (m6A) sites based on sequence-derived features. Nucleic Acids Res. *44*, e91.

52. Xuan, J.J., Sun, W.J., Lin, P.H., Zhou, K.R., Liu, S., Zheng, L.L., Qu, L.H., and Yang, J.H. (2018). RMBase v2.0: deciphering the map of RNA modifications from epitranscriptome sequencing data. Nucleic Acids Res. *46* (D1), D327–D334.

53. Song, Y., Xu, Q., Wei, Z., Zhen, D., Su, J., Chen, K., and Meng, J. (2019). Predict Epitranscriptome Targets and Regulatory Functions of N^6-Methyladenosine (m^6A) Writers and Erasers. Evol. Bioinform. Online *15*, 1176934319871290.

54. Meyer, K.D. (2019). DART-seq: an antibody-free method for global m6A detection. Nat. Methods *16*, 1275–1280.

55. Barbieri, I., Tzelepis, K., Pandolfini, L., Shi, J., Millán-Zambrano, G., Robson, S.C., Aspris, D., Migliori, V., Bannister, A.J., Han, N., et al. (2017). Promoter-bound METTL3 maintains myeloid leukaemia by m^6A-dependent translation control. Nature *552*, 126–131.

56. Vu, L.P., Pickering, B.F., Cheng, Y., Zaccara, S., Nguyen, D., Minuesa, G., Chou, T., Chow, A., Saletore, Y., MacKay, M., et al. (2017). The N^6-methyladenosine (m^6A)-forming enzyme METTL3 controls myeloid differentiation of normal hematopoietic and leukemia cells. Nat. Med. *23*, 1369–1376.

57. Zhao, W., Cui, Y., Liu, L., Qi, X., Liu, J., Ma, S., Hu, X., Zhang, Z., Wang, Y., Li, H., et al. (2019). Splicing factor derived circular RNA circUHRF1 accelerates oral squamous cell carcinoma tumorigenesis via feedback loop. Cell Death Differ. , Published online September 30, 2019. https://doi.org/10.1038/s41418-019-0423-5.

58. Han, J., Wang, J.Z., Yang, X., Yu, H., Zhou, R., Lu, H.C., Yuan, W.B., Lu, J.C., Zhou, Z.J., Lu, Q., et al. (2019). METTL3 promote tumor proliferation of bladder cancer by accelerating pri-miR221/222 maturation in m6A-dependent manner. Mol. Cancer *18*, 110.

59. Jin, H., Ying, X., Que, B., Wang, X., Chao, Y., Zhang, H., Yuan, Z., Qi, D., Lin, S., Min, W., et al. (2019). N^6-methyladenosine modification of ITGA6 mRNA promotes the development and progression of bladder cancer. EBioMedicine *47*, 195–207.

60. Yang, F., Jin, H., Que, B., Chao, Y., Zhang, H., Ying, X., Zhou, Z., Yuan, Z., Su, J., Wu, B., et al. (2019). Dynamic m^6A mRNA methylation reveals the role of METTL3-m^6A-CDCP1 signaling axis in chemical carcinogenesis. Oncogene *38*, 4755–4772.

61. Cheng, M., Sheng, L., Gao, Q., Xiong, Q., Zhang, H., Wu, M., Liang, Y., Zhu, F., Zhang, Y., Zhang, X., et al. (2019). The m^{6}A methyltransferase METTL3 promotes bladder cancer progression via AFF4/NF-κB/MYC signaling network. Oncogene *38*, 3667–3680.

62. Li, F., Yi, Y., Miao, Y., Long, W., Long, T., Chen, S., Cheng, W., Zou, C., Zheng, Y., Wu, X., et al. (2019). N6-methyladenosine modulates nonsense-mediated mRNA decay in human glioblastoma. Cancer Res *79*, 5785–5798.

63. Li, T., Hu, P.S., Zuo, Z., Lin, J.F., Li, X., Wu, Q.N., Chen, Z.H., Zeng, Z.L., Wang, F., Zheng, J., et al. (2019). METTL3 facilitates tumor progression via an m^{6}A-IGF2BP2-dependent mechanism in colorectal carcinoma. Mol. Cancer *18*, 112.

64. Hengyou Weng, et al. (2018). METTL14 Inhibits Hematopoietic Stem/Progenitor Differentiation and Promotes Leukemogenesis via mRNA M 6 A Modification. Cell Stem, Cell *22*, 192–205.

65. Chen, M., Wei, L., Law, C.T., Tsang, F.H., Shen, J., Cheng, C.L., Tsang, L.H., Ho, D.W., Chiu, D.K., Lee, J.M., et al. (2018). RNA N6-methyladenosine methyltransferase-like 3 promotes liver cancer progression through YTHDF2-dependent posttranscriptional silencing of SOCS2. Hepatology *67*, 2254–2270.

66. Chen, Y., Peng, C., Chen, J., Chen, D., Yang, B., He, B., Hu, W., Zhang, Y., Liu, H., Dai, L., et al. (2019). WTAP facilitates progression of hepatocellular carcinoma via m6A-HuR-dependent epigenetic silencing of ETS1. Mol. Cancer *18*, 127.

67. Paris, J., Morgan, M., Campos, J., Spencer, G.J., Shmakova, A., Ivanova, I., Mapperley, C., Lawson, H., Wotherspoon, D.A., Sepulveda, C., et al. (2019). Targeting the RNA m^{6}A Reader YTHDF2 Selectively Compromises Cancer Stem Cells in Acute Myeloid Leukemia. Cell Stem Cell *25*, 137–148.e6.

68. Zhang, J., Liu, H., Hou, L., Wang, G., Zhang, R., Huang, Y., Chen, X., and Zhu, J. (2017). Circular RNA_LARP4 inhibits cell proliferation and invasion of gastric cancer by sponging miR-424-5p and regulating LATS1 expression. Mol. Cancer *16*, 151.

69. Zhou, S., Bai, Z.L., Xia, D., Zhao, Z.J., Zhao, R., Wang, Y.Y., and Zhe, H. (2018). FTO regulates the chemo-radiotherapy resistance of cervical squamous cell carcinoma (CSCC) by targeting β-catenin through mRNA demethylation. Mol. Carcinog. *57*, 590–597.

70. Yang, S., Wei, J., Cui, Y.H., Park, G., Shah, P., Deng, Y., Aplin, A.E., Lu, Z., Hwang, S., He, C., and He, Y.Y. (2019). m^{6}A mRNA demethylase FTO regulates melanoma tumorigenicity and response to anti-PD-1 blockade. Nat. Commun. *10*, 2782.

71. Niu, Y., Lin, Z., Wan, A., Chen, H., Liang, H., Sun, L., Wang, Y., Li, X., Xiong, X.F., Wei, B., et al. (2019). RNA N6-methyladenosine demethylase FTO promotes breast tumor progression through inhibiting BNIP3. Mol. Cancer *18*, 46.

72. Nishizawa, Y., Konno, M., Asai, A., Koseki, J., Kawamoto, K., Miyoshi, N., Takahashi, H., Nishida, N., Haraguchi, N., Sakai, D., et al. (2017). Oncogene c-Myc promotes epitranscriptome m^{6}A reader YTHDF1 expression in colorectal cancer. Oncotarget *9*, 7476–7486.

73. Yang, Z., Li, J., Feng, G., Gao, S., Wang, Y., Zhang, S., Liu, Y., Ye, L., Li, Y., and Zhang, X. (2017). MicroRNA-145 Modulates N^6-Methyladenosine Levels by Targeting the 3′-Untranslated mRNA Region of the N^6-Methyladenosine Binding YTH Domain Family 2 Protein. J. Biol. Chem. *292*, 3614–3623.

74. Wu, J., Zhao, W., Wang, Z., Xiang, X., Zhang, S., and Liu, L. (2019). Long non-coding RNA SNHG20 promotes the tumorigenesis of oral squamous cell carcinoma via targeting miR-197/LIN28 axis. J. Cell. Mol. Med. *23*, 680–688.

75. Zhao, X., Chen, Y., Mao, Q., Jiang, X., Jiang, W., Chen, J., Xu, W., Zhong, L., and Sun, X. (2018). Overexpression of YTHDF1 is associated with poor prognosis in patients with hepatocellular carcinoma. Cancer Biomark. *21*, 859–868.

76. Li, Y., Xiao, J., Bai, J., Tian, Y., Qu, Y., Chen, X., Wang, Q., Li, X., Zhang, Y., and Xu, J. (2019). Molecular characterization and clinical relevance of m^{6}A regulators across 33 cancer types. Mol. Cancer *18*, 137.