Cytokine 118 (2019) 35–41

Contents lists available at ScienceDirect

Cytokine

journal homepage: www.elsevier.com/locate/cytokine

Review article

Methylation of RNA N^6-methyladenosine in modulation of cytokine responses and tumorigenesis

Guoqiang Chang[a], Jia-Shiun Leu[b], Li Ma[b], Keping Xie[a,c], Suyun Huang[b,c,*]

[a] Department of Gastroenterology, Hepatology & Nutrition, The University of Texas MD Anderson Cancer Center, Houston, TX 77030, United States
[b] Department of Neurosurgery, The University of Texas MD Anderson Cancer Center, Houston, TX 77030, United States
[c] Program in Cancer Biology, The University of Texas Graduate School of Biomedical Sciences at Houston, Houston, TX 77030, United States

ARTICLE INFO

Keywords:
m^6A modification
m^6A writers
m^6A erasers
m^6A readers
Tumorigenesis

ABSTRACT

Among myriads of distinct chemical modification in RNAs, the dynamic, reversible and fine-tuned methylation of N^6-methyladenosine (m^6A) is the most prevalent modification in eukaryotic mRNAs. This RNA mark is generated by proteins that act as m^6A writers and can be reversed by proteins that act as m^6A erasers. The RNA m^6A modification is also mediated by another group of proteins capable of recognizing m^6A that act as m^6A readers. The m^6A modification exerts direct control over the RNA metabolism including mRNA processing, mRNA exporting, translation initiation, mRNA stability and the biogenesis of long-non-coding RNA (LncRNA), thereby can influence various aspects of cell function. Evidently, m^6A is intimately associated with cancer development and progression such as self-renewal capacity of cancer stem cells, proliferation, apoptosis and therapeutic resistance, and immune response. In this review, we will discuss the regulation and function of m^6A, the various functions ascribed to these proteins and the emerging concepts that impact our knowledge of these proteins and their roles in the epitranscriptome. Conceivably, m^6A may play pivotal roles in cytokine and immune response and carcinogenesis.

1. Introduction

The central dogma of translating sequence information between biopolymers, including DNA, RNA, and proteins [1], has undergone a major change due to the discovery of reverse transcriptase [2,3]. The statement of the dogma is still subject to updating and revising. Epigenetic modifications occurring on DNA or histone proteins could modulate the gene expression that affects the cellular status in response to differentiation, development, and other stresses. In RNA, most of the modifications are found on non-coding RNA, including rRNA, tRNA, and snoRNA [4]. The N^6-methyladenosine (m^6A) is widely found in eukaryotic cells and some virus in 1970s [5,6]. At present, there are already more than 100 chemical modifications characterized in RNA. Although mRNA has a crucial role in the dogma, the discussion about the regulation of chemical modification on mRNA is only highlighted recently, due to the lack of suitable biotechnology for such analysis. Since 2010, the development of specific antibody targeting m^6A sites and the next generation sequencing (NGS) initiates the map of the distinct methylation sites in genomics [7,8]. The antibody can immunoprecipate the mRNA fragments with m^6A modification, then reverse transcribed into cDNA for high-throughput sequencing analyses. Those studies have found that the m^6A modification is present in over 25% human transcripts, most of which are located near stop codon and 3′-untranslated regions (3′-UTR) [7]. The 5′-UTR close to the start site also has the unique chemical modification in different levels (see Fig. 1).

Recent studies have shown that m^6A modification could regulate multiple RNA-related processes, such as RNA stability [9], cellular localization [10], and alternative splicing [11,12]. The RNA methylation on adenosine is usually present in consensus sequence G [G/A] m^6ACU [7]. The functions of m^6A RNA modification are regulated by methyltransferases (“writers”), demethylases (“erasers”), and m^6A binding proteins (“readers”). In mammalian cells, m^6-modification on adenosine is induced by METTL3/METTL14/WTAP protein complex. In 2011, He and colleagues have first discovered that fat mass and obesity-associated protein (FTO) could play as a demethylase to downregulate the methyl level of RNA [13]. Thereafter, they have found another demethylase protein, AlkB Homolog 5 (ALKBH5), which could distinctly

Abbreviations: m^6A, N^6-methyladenosine; FTO, fat mass and obesity-associated protein; ALKBH5, AlkB Homolog 5; Eif3, eukaryotic initiation factor 3; hnRNPs, heterogeneous nuclear ribonucleoprotein; IGF2, insulin-like growth factor 2; GSCs, glioma stem cells; WTAP, Wilms’ tumor 1-associating protein; METTL3, methyltransferase-Like Protein 3; METTL14, METTMethyltransferase like 14

* Corresponding author at: Department of Neurosurgery, The University of Texas MD Anderson Cancer Center, Houston, TX 77030, United States.
E-mail address: suyunhuang68@gmail.com (S. Huang).

https://doi.org/10.1016/j.cyto.2018.06.018
Received 20 March 2018; Received in revised form 25 May 2018; Accepted 12 June 2018
Available online 13 July 2018

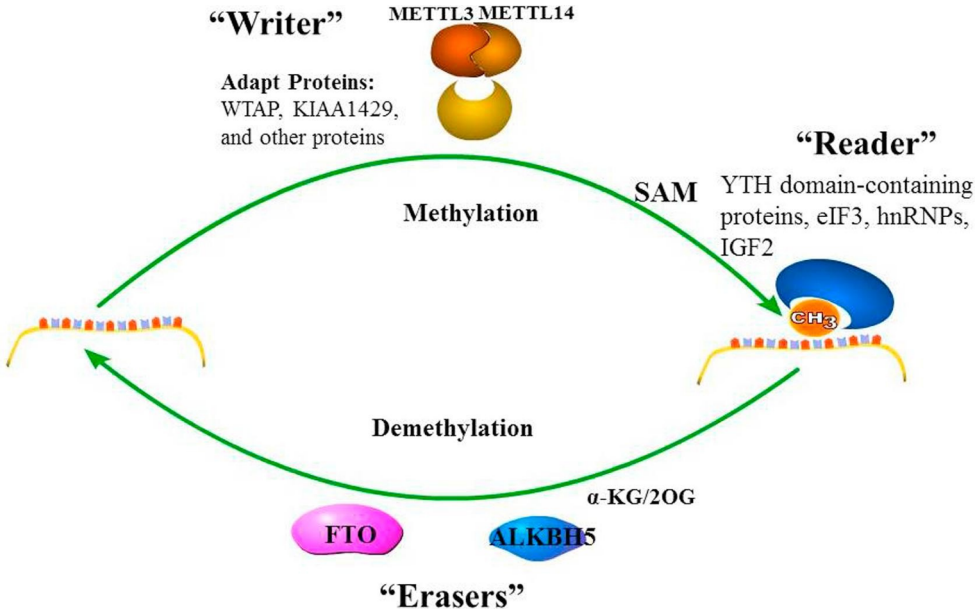

Fig. 1. m^{6}A modulation by m^{6}A writers, erasers and readers. m^{6}A modification is added by m^{6}A writers and can be reversed by m^{6}A erasers, and preferentially bound with m^{6}A readers. METTL14 shares an almost identical topological conformation with that of METTL3 and assists METTL13 for substrate recognition after forming a complex with METTL3. Adaptor proteins including WTAP, KIAA1429 and others pilot METTL3/METTL14 complex to specific mRNAs; Both FTO and ALKBH5 is well-conserved α-KG-dependent dioxygenases and demethylate the m^{6}A sites; YTH domain-containing proteins including YTHDF1-3 and YTHDC1-2, eIF3, hnRNPs and IGF2 recognize and bind to m6A sites.

reduce m^{6}A modification [14]. FTO is also regarded as one member of AlkB protein family and highly expressed in the central nervous system. The dysfunctions of FTO correlate with obesity and brain malformations, which implicate that m^{6}A modification may be involved in these diseases [15,16]. In virology, m^{6}A in virus RNA could also significantly mediate virus-host interactions [17–19]. In addition, scientists also found that m^{6}A modification is indispensable for stem cell to maintain their naïve and pluripotent status [20,21].

2. Linking m^{6}A modification to cytokine response and carcinogenesis

The dysregulation of the immune system is involved in nearly all known human disease including tumorigenesis, infection diseases, inflammation diseases, metabolism syndromes and autoimmune disease. Now, it is not clear due to very limited studies how m^{6}A-dependent functions for m^{6}A readers, writers and erasers regulate the immune system through affecting various cytokines output. The immune system includes both innate and adaptive immune responses. The innate immune responses are rapid and non-specific. However, the adaptive reaction requires antigen presentation, clone expansion and differentiation to perform antigen-specific reaction [22,23]. Indeed, upon antigen recognition, the immune cells release gigantic amount of cytokines within a little time, which is called as cytokine storm and is unable to be regulated from *de novo* gene transcription. As mentioned above, the rapid output of mRNA is an energy-cost-effective process in reaction to the microenvironment changes as compared with protein production. Given that m^{6}A plays an important function in mRNA splicing, translation and stability, m^{6}A may also play a considerable role in immune response, including cytokine response. Conversely, YTHDC2 can be regulated by tumor necrosis factor-α (TNF-α), and ALKBH5 has also been shown to be critical for the initiation of antiviral innate immunity by regulating interferon β (IFN-β) [24,25]. It is also shown that m^{6}A could stimulate type I primary dendritic cells and protect RNA from recognition by TLR3 and TLR7 for degradation [26,27]. Therefore, the roles of m^{6}A in immune response warrant more studies, particularly those using experimental immune disease models, such as genetically engineered mice with FTO, Alkbh5 or Mettl3 knockout.

Moreover, our group have recently found that ALKBH5 is highly expressed in glioma stem-like cells to maintain glioblastoma (GBM) tumorigenesis [28,29]. We suggest that m^{6}A could modify a group of mRNAs to timely regulate cellular processes in response to stress including hypoxia, tumor microenvironment, and stemness [30–32]. Because immune response is uniquely important to GBM, an aggressive and common brain tumor derived from neuroglial stem or progenitor cells, we expect that m^{6}A could at least in part impact glioma development and progression through regulating cytokine response. In this review, we will discuss m^{6}A methylation of mRNA and its potential influence on tumor progression.

3. m^{6}A readers

RNA modifications are reversible and dynamic, which can modulate the interaction between RNA and protein and mediate rapid responses to environmental changes. The major mechanisms of m^{6}A effects are by recruiting m^{6}A-binding proteins. The proteins that can recognize m^{6}A are those containing an YTH (YTH-B homology) domain, eukaryotic initiation factors 3 (eIF3) [33–35] and others. In contrast to eIF3, YTH proteins identify m^{6}A by a specific well-characterized YTH domain and have much higher binding affinity to methylated RNA sequence than unmethylated sequences.

3.1. YTH domain-containing proteins

The first YTH protein is discovered in a yeast two-hybrid screening and the recovered protein from that screening is designated as YT521-B [36,37]. Among YT521-B homologs, YTH domain is discovered as an approximately 140 amino acid domain that is highly conserved. YTH domain is predicated as an original RNA-binding domain based on the similarity with the RNA-binding RRM (RNA recognition motif) [38].

YTH domain-containing proteins can be divided into three categories: YTHDF (YTH domain-containing family protein) family, YTHDC1 (YTH domain-containing protein 1, also called DC1), and YTHDC2 (YTH domain-containing protein 2, also called DC2). DC1 and DC2 could not be combined into one family in that they are so different with each other based on amino acid sequence, size, and overall domain organization except the YTH domain. In contrast, the YTH family has three paralogs with high amino acid identity and entire length: YTHDF1, YTHDF2 and YTHDF3.

The first research to investigate the effects of YTHDF proteins is about the YTHDF2 [39], which is expressed abundantly in almost all cell types. The m^{6}A containing-mRNAs have reduced half-lives as compared with non-methylated mRNAs [20,40,41] and the half-lives of mRNA is increased in YTHDF2-knockout cells. This effect is presumed

to originate from the interaction between YTHDF2 and P-bodies [42]. YTHDF2 depletion decreases the viability of HeLa cells via MTase complex, which acts as anti-proliferative gene and is degradated by YTHDF2. YTHDF2 is required for maternal to zygote transition (MZT) in zebra fish embryos. Depletion of YTHDF2 in zebrafish embryos decreases the decay of m^{6}A-modified maternal mRNAs and impairs zygotic genome activation [43]. During stress conditions such as heat shock, YTHDF2 could be re-positioned into nucleus, protect the transcripts of m^{6}A-modified 5′UTR from demethylation by FTO and facilitate cap-independent translation initiation [44]. In hepatocellular carcinoma, YTHDF2 is degraded by the elevated tumor suppressor miR-145 and protects m^{6}A-containing RNA species [45].

In contrast to YTHDF2, YTHDF1 initially does not manifest considerable effects on mRNA stability based on mRNA half-life assays in YTHDF1-knockout cells. YTHDF1 interacts with some translation factors including eIF3, which could permit YTHDF1 to affect translation initiation directly [46]. However, the binding sites of YTHDF1 are mainly concentrated near stop codon and 3′-UTR. In contrast, the initiation factors are canonical enrolled to the 5′-UTR to promote the ribosome to the start codon. Question is how YTHDF1 could mediate translation by interacting with initiation factors. Therefore, the model of YTHDF1 function may represent a potentially novel style of translation initiation, in which the initiation factors are first recruited to stop codon-adjacent area. Recently, the three members (YTHDF1, YTHDF2 and YTHDF3) of YTHDF family proteins have essentially identical binding m^{6}A sites in mRNAs [47], which is consistent with the high identical sequence of YTH domain shared by these three proteins. All three YTHDF proteins could not only regulate mRNA initiation but also mediate mRNA degradation, which could further affect protein expression [19,48–50].

3.2. eIF3

There are many recent studies on the translation-promoting effects of m^{6}A through eukaryotic initiation factor 3 (eIF3), a component of 43S pre-initiation complex [51]. In those studies, template RNA are combined with purified translation initiation factors, to induce ribosome binding and formation of a translation pre-initiation complex at the start codon, which can be measured by a reverse transcription-based assay [52]. The primary proof that eIF3 is an m^{6}A reader emerged from the studies that cross-linking of eIF3 to the m^{6}A-containing RNA is significantly increased as compared with the non-methylated RNA and eIF3 preferentially bound to Gm^{6}AC nucleotides [53]. The eIF3-binding sites are mostly positioned to 5′-UTR of mRNAs and exhibit enhanced translation initiation in cells. These researches have revealed a novel function for m^{6}A in facilitating an unparalleled mode of translation initiation from canonical eIF4E-dependent translation initiation to eIF4E-independent translation initiation. Because stress and disease state could inhibit eIF4E activities, m^{6}A could be a potential mechanism to achieve a selective and disease-specific translation [54].

Remarkably, eIF3 is related to two manners of m^{6}A-induced translation. In one condition, m^{6}A directly binds and enlists eIF3 to the 5′-UTR; in another condition, m^{6}A residues close to the stop codon combine with YTHDF1 and then potentially deliver eIF3 to the 5′-UTR [46,55,56].

3.3. YTHDC1 and YTHDC2

Before YTHDC1 is recognized to interact with m^{6}A-containing mRNA, it is primarily defined as a nucleus-enriched splicing regulator. YTHDC1 mediates various styles of alternative splicing events in endogenous transcripts through over-expression assays [57], which regulation requires the YTH domain. When the YTH domain is defined as an m^{6}A-binding component, YTHDC1 is also investigated for its function in m^{6}A-mediated splicing [12,58–60]. The mRNA splicing process is impaired by YTHDC1 depletion, which could be rescued only by an YTH domain-containing YTHDC1 protein. Thus, YTHDC1 regulates, at least partly, splicing events mediated by m^{6}A. Until now, the functions of YTHDC2 remain poorly understood. Initially, YTHDC2 is characterized as a cellular factor which is required during the replication of HCV genome [61]. In recent studies including the iCLIP studies, YTHDC2 is found to increase the translation of HIF-1a mRNA through its helicase function [62]. However, it is not known whether these functions require the YTH domain or need m^{6}A interact with HIF-1a mRNA.

3.4. Other proteins

In addition to the YTH domain-containing proteins, some members of the nuclear proteins are recently defined as m^{6}A binding proteins, such as heterogeneous nuclear ribonucleoprotein (hnRNP) protein family and insulin-like growth factor 2 (IGF2) [63,64]. HnRNPs belongs to a cluster of proteins that pack pre-mRNAs into stable bundles and mediate mRNA processing and sorting, which also function as nuclear m^{6}A readers. For instance, hnRNPA2B1, a member of the nuclear hnRNP protein family, play an important role on processing a subset of modified primary miRNAs to precursor miRNAs in collaboration with microprocessor DGCR8 [65]. HnRNAPA2B1 could recruit DGCR8 protein complex to the step loop domain of m^{6}A methylated primary miRNAs and protect them from ribonuclease degradation. Even though, the RNA-binding sites in hnRNPA2B1 comprise m^{6}A consensus motif, it is still unknown whether hnRNPA2B1 bind m^{6}A directly or indirectly, so more structural and biochemistry data may be needed to validate the results [66]. IGF2BPs are comprised with two RNA recognition motif domains and four K homology (KH) domains, which are regarded as a new family of m^{6}A readers that protect m^{6}A-modified mRNAs from degradation. Huang *et al.* found IGF2BPs could directly bind to m^{6}A RNAs through KH domain in a new m^{6}A-dependent mode on top of the primary sequence. IGF2BPs inhibit degradation, increase the stability, and promote translation of m^{6}A-modified mRNAs, thereby globally affecting target gene expression [64].

4. m^{6}A writers, adenosine methyltransferases

A multi-factor methyltransferase complex is shown to regulate m^{6}A methylation, which contains two core components, METTLE3 and METTLE14, in addition to some accessory adapter proteins, including WTAP [67,68], REM15 [47,69,70], and KIAA1429 [71]. The METTL3/METTL14 complex preferentially binds the sequence motif G(G/A)ACU in RNA substrates and manifests low selectivity to the secondary structure of mRNA [72–74].

4.1. Mettl3

Early studies on m^{6}A methylation have revealed that a full-length METTL3 is required, and only the MTase domain of METTL14 is indispensable for the catalytic activity of the complex. METTL3 is one of members in a large family of putative SAM-dependent methyltransferases, which is highly conserved in mammals [75]. Genetic depletion of METTLE3 induces complete or near-complete loss of m^{6}A methylation [66,76,77]. Thus, METTL3 appears to play the core m^{6}A-catalytic role in poly-adenylated mRNA. The altered METTL3 expression level influences various aspects of cancer cell biology, including cell proliferation, viability, stem cell maintenance, transformation, translation and mRNA stability.

Most of m^{6}A sites are well conserved across normal and tumor tissues and even in response to various stimuli. And also some m^{6}A sites can be mediated by specific stimulus. When the m^{6}A sites are diversified, the splicing pattern of mRNA may be changed and affect gene expression. In human dental pulp cells, the global m^{6}A content and METTL3 expression can be up-regulated upon LPS treatment, while METTL3 inhibition reduces the accumulation of inflammatory

cytokines including IL-6 and IL-8 [78]. In HepG2 cells, the level of MDM4 is down-regulated by METTL3 silence, resulting in modulation of p53 signaling pathway and induction of apoptosis [8,79,80]. In lung cells, METTL3 as an oncogene increases the translation rate of some key proliferative modulator including EGFR, TAZ and DNMT3A [14,81]. METTL3 is indispensable for the cell survival after UV-induced DNA damage. Genetic deletion of METTL3 impairs the removal of cyclobutane pyrimidine dimers, affects timely translation re-initiation, induces cell death, and decreases colony-formation ability. The cell death of UV-treated U2OS osteosarcoma cells with METTL3 knockout is rescued by the increased expression of methylation catalytic METTL3 but not catalytic domain-truncating METTL3 [82]. DNA polymerase κ (Pol κ) is localized to the damage sites consistent with m^{6}A RNA. The over-expression of Pol κ overcomes the defect in the removal of cyclobutane pyrimidine dimers that is associated with METTL3 loss, suggesting that Pol κ may be a core effector of METTL3 in DNA damage repair [83]. The modification of m^{6}A could regulate the cell fate transition in stem cells, and METTL3 plays a considerable role in that process. In glioblastoma stem cells (GSC), the m^{6}A levels can be elevated by METTL3 as compared with those differentiated glioma cells. The SOX2 mRNA could be methylated in its 3′UTR by METTL3 in a HuR dependent manner, which results in its stabilization and promotes GSC stemness maintenance [84].

4.2. Mettl14

Although the methyltransferase domain of METTL14 share an almost identical topological conformation and approximately 22% sequence similarity with that of METTL3, some studies suggest that METTL14 is a pseudo-methyltransferase in the METTL3/METTL14 complex. First, from the crystal structure of the complex, the hydrophobic pocket inserted by the adenine moiety is in METTL3 [85]. Second, the EPPL motif in METTL14, which is a conserved sequence corresponding to the catalytic domain of METTL3, had no effects on ligand binding and enzyme activity from the mutagenesis studies [86,87]. However, METTL14 does have an important role in tumorigenesis. In uterine/endometrial cancers, a R298P mutation is detected in the RNA-binding domain of METTL14, resulting in decreased methyltransferase activity, which is partially due to inefficient RNA binding [88]. Moreover, METTL14 inhibition decreases 40% m^{6}A levels in transcripts. In HeLa cells, METTL14 manifests 56% common binding sites as compared with those of METTL3, which suggests that they may have separate effectors and mediate different aspects of mRNA function [67]. In hepatocellular carcinoma (HCC) tissue, the expression of m^{6}A is reduced as compared with adjacent non-tumor or normal hepatic tissue due to the significantly low METTL14 expression. The expression of METTL14 is negatively correlated with the survival rate of HCC patients [89]. Although METTL3 is the key catalytically active subunit, its capacity can be ignored without forming stoichiometric complex with METTL14. Thus, METTL14 not only plays a structural role to support METTL3′s catalytic activity, but also has other functions which need further investigation.

4.3. WTAP and other regulators

Wilms' tumor 1-associating protein (WTAP) is identified as the second major component of the human m^{6}A methylation complex, which is a binding partner of the methyltransferase and is substantial for RNA methylation. And the WTAP-METTL3 interaction is identified in various mammalian cells [90,91]. Thus, the major function of WTAP is to localize the METTL3/METLL14 complex to the nuclear speck [68]. WTAP depletion markedly induces malposition of the methylation complex from the speckles and decreases the global m^{6}A level in human cells, which indicates its importance in generating the specific landscape of mRNA methylation [71]. In zebrafish, genetic depletion of WTAP homolog results in cell death and impairs the tissue differentiation [68]. The FKBP12-interaction protein (FIP37), a homolog of WTAP, mediates shoot stem cell fate via m^{6}A modification [92]. However, WTAP has no effect on methylation enzyme activity *in vitro* and changing the RNA substrate of the METTL3/METTL14 complex. Furthermore, more and more studies suggest that additional effectors such as KIAA1429 participates in mRNA methylation and further research about the regulation of the m^{6}A writer complex will shed light on RNA epigenetics [93,94].

5. m^{6}A erasers, adenosine methyltransferases

A fundamental breakthrough in m^{6}A research is the identification of two dissimilar enzymes, which can demethylate m^{6}A: fat mass and obesity-associated protein (FTO) and AlkB homologue 5 (ALKBH5) [13,14]. FTO and ALKBH5 are the only two known m^{6}A demethylation enzymes, belonging to the AlkB family of Fe(II)/α-ketoglutarate(α-KG)-dependent dioxygenases [16,95–98].

5.1. Fto

FTO is the first enzyme linked to m^{6}A demethylation, which discovery further supports the concept that m^{6}A methylation is a reversible modification [99]. The crystal structure reveals that C-terminal domain of FTO contains a novel fold that is distinct from that of other proteins in AlkB family [100,101]. This C-terminal domain may engage in substrate selection through protein-protein or protein-RNA interaction [102]. Genetic depletion of FTO significantly increases m^{6}A peaks and dysregulation of FTO directly correlates with obesity, brain malformations, impaired proliferation and tumor development [31,103–105].

Increased levels of FTO in MLL-rearranged AML result in an accelerated removal of global m^{6}A modification, which leads to decreased expression of crucial differentiation markers such as Ankyrin repeat and SOCS box-containing 2 (ASB2) and retinoic acid receptor a (RARA). ASB2 and RARA are increased during normal hematopoiesis and are core effector in all-trans-retinoic acid (ATRA)-induced differentiation of leukemia cells. Thus, the decreased expressions of ASB2 and RARA induced by FTO increase leukemic oncogene-modulated cell transformation, leukemogenesis and decrease ATRA-induced cell differentiation. Knockdown of FTO results in retarded cell growth and increased apoptosis in MLL-rearranged AML [105]. Notably, metabolite D-2-hydroxyglutarate (D2-HG) is a competitive inhibitor of FTO, which aberrantly accumulates in isocitrate dehydrogenase 1 or 2 (IDH1/2)-mutant tumors. In vitro, FTO depletion elevates the m^{6}A level in HEK293T with IDH2 wild-type but not the mutated IDH2. Similar results are also obtained from AML cells that down-regulation of FTO only increases the m^{6}A level in IDH1/2-WT AMLs but not IDH1/2-mutant AMLs. Therefore, IDH1/2 mutation increases m^{6}A level by generating more D2-HG to competitively prohibit the functions of RNA demethylase FTO [106,107]. Furthermore, FTO may play an important role in the carcinogenesis of breast cancer, and there is a direct correlation between the development and aggressiveness of breast cancer and high expression of FTO, especially in HER2$^+$ breast cancer [108]. The expression of FTO is also elevated in human cervical squamous cell carcinoma (CSCC) tissues, which causes the chemo-radiotherapy resistance via decreasing the methylation of m^{6}A in β-catenin mRNA transcript and in turn increasing excision repair cross-complementation group 1 (ERCC1) activity [109].

5.2. Alkbh5

Initial investigation in a biochemical screening of demethylase enzymes indicates that ALKBH5 exhibits m^{6}A demethylase activity and directly removes the methyl group from m^{6}A-methylated adenosine instead of oxidative demethylation [14,104]. ALKBH5 localized in the nucleus and its demethylation capacity plays fundamental roles in

mRNA export as well as in the relation with nuclear speckle proteins and RNA metabolism [110]. The genetic silence of ALKBH5 increases the m^{6}A level of global mRNA as well as individual mRNA isolated from cells [14]. ALKBH5 could not only demethylate the m^{6}A in mRNA but also demethylate m^{6}A in ncRNA, which is enriched in nucleus, small nucleolar RNA (snRNA) and others [111]. Thus, ALBH5 could have various biological functions in an m^{6}A modification-dependent mode.

Glioblastoma is deadly primary brain tumor and the median survival time of glioblastoma patients is shorter than 15 months after diagnosis. Glioblastoma stem cells (GSCs) are the principal cause for the tumor recurrence and are resistant to therapeutics and promote tumor growth [112–114]. In our recent studies, we have found that the expression of ALKBH5 is elevated in GSCs, which predicts poor survival outcome as a negative prognostic factor for glioblastoma patients [29]. Genetic depletion of ALKBH5 could impair the self-renewal capacities of GSCs and inhibit the proliferation of GSCs and tumorigenesis, which could be rescued only by wild-type ALKBH5 not the catalytic inactive mutant. Moreover, we have found the fork head box M1 (FOXM1) is a direct substrate for ALKBH5-mediated GSC growth and ALKBH5 mainly affects FOXM1 expression through its demethylation activity. The binding between HUR and the pre-mRNA of FOXM1 is increased after ALKBH5 overexpression. Meanwhile, the stability of FOXM1 pre-mRNA is also enhanced due to the reduced m^{6}A level. The FOXM1-AS, a nuclear LncRNA, facilitates the interaction between ALKBH5 and FOXM1 nascent transcripts, resulting in FOXM1 pre-mRNA demethylation and stabilization. Silence of FOXM1-AS inhibits the GSCs growth like ALKBH5 deletion. The inhibition of GSCs tumor growth induced by FOXM1 overexpression could be rescued by FOM1-AS and ALKBH5 depletion, which further substantiate the critical role of FOXM1 in GSC tumorigenesis. FOXM1 belongs to the Forkhead box (Fox) transcription factor family and is ubiquitously expressed in embryonic tissues [115–117]. FoxM1 as a key cell-cycle effector is involved in the self-renewal and proliferation of stem cells. In our previous studies, we have found that FOXM1 is directly correlated with tumor grade in human glioma tissue and inversely correlated with patient survival outcome [118–120]. Furthermore, FOXM1 could mediate growth factor- and cytokine-induced STAT3 activation by enhancing β-catenin/TCF4 binding to the STAT3 gene promoter to maintain the stemness of GSCs, increase GSCs growth and decrease their chemo-sensitivity [121,122].

In addition, the Alkbh5 deficiency leads to aberrant spermatogenesis and apoptosis in testes of Alkbh5$^{-/-}$ mouse due to the increased m^{6}A [123]. In breast cancer cells, hypoxia, which is a critical feature of the tumor microenvironment, could induce m^{6}A demethylation by ALKBH5 and stabilization of NANOG mRNA, thereby promoting the breast cancer stem cell phenotype [32]. And ALKBH5 also plays an important role in innate immunity [25]. Upon viral infection, ALKBH5 could be recruited by DDX46 via DEAD domain to obliterate m^{6}A methylation of targeted transcripts. The m^{6}A modification inhibits the turnover of type I interferon and antiviral innate immune reaction. Those are closely related to cytokine responses.

6. Future perspective

The RNA methylation landscapes are regulated by the m^{6}A readers, writers and erasers in human cancer cells. The reversible RNA methylation, which influences transcription initiation, splicing, mRNA stability and translation, mediates the fundamental biological features of cancer cells. Although RNA modifications have been known for many decades, the epitranscriptomic modifications are recently emerging as a widely underestimated part of molecular regulation and we just start to understand the extent and complexity of both the modulation and significance of mRNA methylation. RNA modification represents a crucial interface of regulation of gene expression, which enables them to modulate gene expression beyond the regulation of mRNA abundance. Even we have made enormous progress in our understanding of the function and modulation of m^{6}A, much work lies ahead to obtain a comprehensive map of this mark and how it precisely regulates gene expression. Through consistent efforts to ameliorate m^{6}A detection methods, to discern additional readers, writers and erasers, and to discover potential functions for m^{6}A, we will undoubtedly enlarge our knowledge of biological features of m^{6}A and its contribution to human health and disease, including cytokine regulation and cancer development.

Declarations of interest

None.

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