Am J Cancer Res 2019;9(4):752-764
www.ajcr.us /ISSN:2156-6976/ajcr0092945

Original Article

Epitranscriptomic m6A modification in the stem cell field and its effects on cell death and survival

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Received February 23, 2019; Accepted March 14, 2019; Epub April 1, 2019; Published April 15, 2019

Abstract: The reversible N6-methyl-adenosine (m6A) modification of messenger RNAs (mRNAs) has generated much interest in the field of stem cell modulation in recent years. Meanwhile, mounting evidence has shown that many physiopathological processes concerning cell death and survival harbor this chemical mark. Our review provides an overview of the m6A epitranscriptomic field and the updated mechanisms of m6A decoration in stem cell regulation. Furthermore, we focus on the role of m6A in DNA damage and the immune response, cell apoptosis, autophagy, and senescence, followed by recent advancements in m6A-induced viral replication. The function of abundant RNA-binding proteins (RBPs) identified in m6A regulatory systems will also be discussed in this review, highlighting their far-reaching implications in cellular m6A machinery and disease treatment.

Keywords: m6A, stem cell, DNA damage response, immune response, viral replication, apoptosis and autophagy, RNA-binding proteins

Introduction

Regulatory RNA modifications mainly consist of methylation, pseudouridylation (ψ), A-to-I editing, and nicotinamide-adenine dinucleotide (NAD+) capping. Five types of RNA methylation have been identified involving N1-methyladenosine (m1A), m6A, 5-methylcytosine (m5C), N7-methylguanine (m7G), and 2-O-methylation (2'OME) [1]. Among them, m6A is the most pervasive and highly conserved RNA modification in eukaryotic cells, and affects almost all aspects of mRNA metabolism including stability, alternative splicing, nuclear export, and translational efficiency [2]. Generally, m6A sites are predominantly enriched within long internal exons, near stop codons, and 3'-untranslated regions (3'UTRs) [2, 3].

Increasing evidence suggests that m6A participates more deeply in diverse cellular processes than previously expected [4]. On one hand, m6A decoration acts as a fate determiner in stem cell regulation [5]. Stem cells are a small subpopulation within diverse cell types, which have the dual properties of self-renewal and the ability to differentiate into most cell types. These features make them valuable tools for clinical therapies [6, 7]. However, cancer stem cells (CSCs) have an extraordinary capacity to promote tumor proliferation and invasion. There is a high correlation with cancer recurrence, probably due to their resistance to most radio- and chemotherapies. Therefore, novel treatments that target CSCs are urgently needed [8]. On the other hand, various processes related to cell death and survival contain m6A modification, such as apoptosis, autophagy, and the immune response system. The dynamic interplay among "writers", "erasers", and "readers" determines the effects of m6A on both sides [3].

RNA m6A is created by methyltransferases (writers) consisting of methyltransferase-like 3 (Mettl3), Mettl14, and Wilms Tumor 1-Assoc-iated Protein (WTAP), which constitute the core complex, and other proteins such as Virilizer (Kiaa1429) and RNA-binding motif protein 15 (RBM15) [3, 9]. Recently, a zinc-finger protein Zc3h13 (zinc-finger CCCH domain-containing protein 13) was newly identified as a nuclear m6A writer in mouse embryonic stem cell (mESC) regulation. Zc3h13 is indispensable for

nuclear localization of the m6A writer complex and may contribute to mESC self-renewal by mediating target mRNA stability [9, 10]. In addition, natural dioxygenases, including fat mass and obesity-associated gene (FTO) and α-ketoglutarate-dependent dioxygenase alkB homolog 5 (ALKBH5) demethylate m6A in transcripts, thus acting as erasers [11]. Recently, Flavin mononucleotide (FMN) was identified as a novel demethylase of m6A residues [12]. Besides direct chemical modifications by writers and erasers, m6A regulates RNA processing by recruiting several reader proteins. A group of YT521-B homology (YTH) domain-containing proteins that recognize m6A-marked transcripts through the conserved YTH domain have been well documented [13]. In the nucleus, Ythdc1 selectively binds precursor RNAs containing m6A to regulate splicing while in the cytoplasm, other members such as Ythdf1-3 and Ythdc2 act as readers of the mature mRNA m6A signal [14]. Among them, Ythdf2 can accelerate the decay of m6A-tagged transcripts at the post-transcriptional level and is recognized as a vital regulator in the development of different types of stem cells such as hematopoietic stem/progenitor cells (HSPCs) and neural stem/progenitor cells (NSPCs) (**Figure 1A**) [15-17].

In this review, we provide an updated summary of RNA m6A modulation, focusing on the regulatory role of m6A RNA methylation in stem cell fate and cellular processes affecting death and survival.

Stem cell regulation

m6A modification in ESCs

Embryonic stem cells (ESCs) that originate from the inner cell mass of the blastocyst are considered the gold standard of pluripotent stem cells (PSCs) [18]. Studies have proved that m6A likely influences embryonic development. Mettl3-deficient ESCs fail to exit pluripotency despite differentiation cues, likely because m6A reduces the stability of vital pluripotency-promoting transcripts such as Nanog, sex determining region Y-box 2 (SOX2), and insulin-like growth factor-binding proteins (IGFBPs) [19-21]. One mechanism was uncovered by Wang et al. in Mettl3/Mettl14-knockdown (KD) mouse ESCs (mESCs). Loss of m6A on the mRNAs of some developmental regulators enhanced the interaction with the well-established RNA stabilizer, human antigen R (HuR). HuR-binding promotes mRNA stability, likely by inhibiting microRNA (miRNA) targeting, as is exemplified by IGFBP3 which is a direct target of miRNAs (**Figure 1B**) [20]. In addition, Bertero et al. showed that the transforming growth factor-β (TGF-β) pathway is responsible for neuroectoderm differentiation in an m6A-dependent manner. Activin and Nodal phosphorylate the intracellular effector SMAD2/3 (drosophila mothers against decapentaplegic protein 2/3), which then interacts with the Mettl3-Mettl14-WTAP (M-M-W) complex and promotes m6A deposition on mRNAs, including Nanog. The subsequent decay of these transcripts in human PSCs results in rapid exit from pluripotency (**Figure 1C**) [22].

A renewed interest in m6A-modified long noncoding RNAs (lncRNAs) revealed its role in ESC stemness and differentiation. For example, researchers demonstrated that some ESC-specific lincRNAs are m6A modified and are also key players in the mESC pluripotency regulatory circuit. Yang et al. revealed that the m6A, which is dependent on Mettl3, is highly enriched within linc1281 and crucial for mature let-7 miRNA binding. Upregulation of linc1281 with m6A enrichment results in let-7 miRNA sponging and releases the expression of protein lin28, which is crucial for the maintenance of pluripotency and is a differentiation-inhibiting factor in mESCs (**Figure 1D**) [23]. In addition, Zhou et al. have identified a novel, cell-specific methylation pattern in circular RNAs in human ESCs (hESCs) (**Figure 1E**). Although they share readers and writers, m6A circular RNAs (circRNAs) are always generated from long single exons that do not contain m6A peaks in mRNAs. Interestingly, a novel connection between m6A circRNAs and mRNA half-life, which is regulated by YTHDF2, was uncovered. Results showed that mRNAs encoded by genes that simultaneously encoded m6A circRNAs from the same or different exons had shorter half-lives than those encoded by genes not encoding any circRNAs [24]. However, whether circRNA m6A affects the development of hESCs warrants further investigation.

m6A modifications in NSCs

In the development of the nervous system, embryonic neural stem cells (NSCs), also known as radial glial cells (RGCs), are multipotent stem

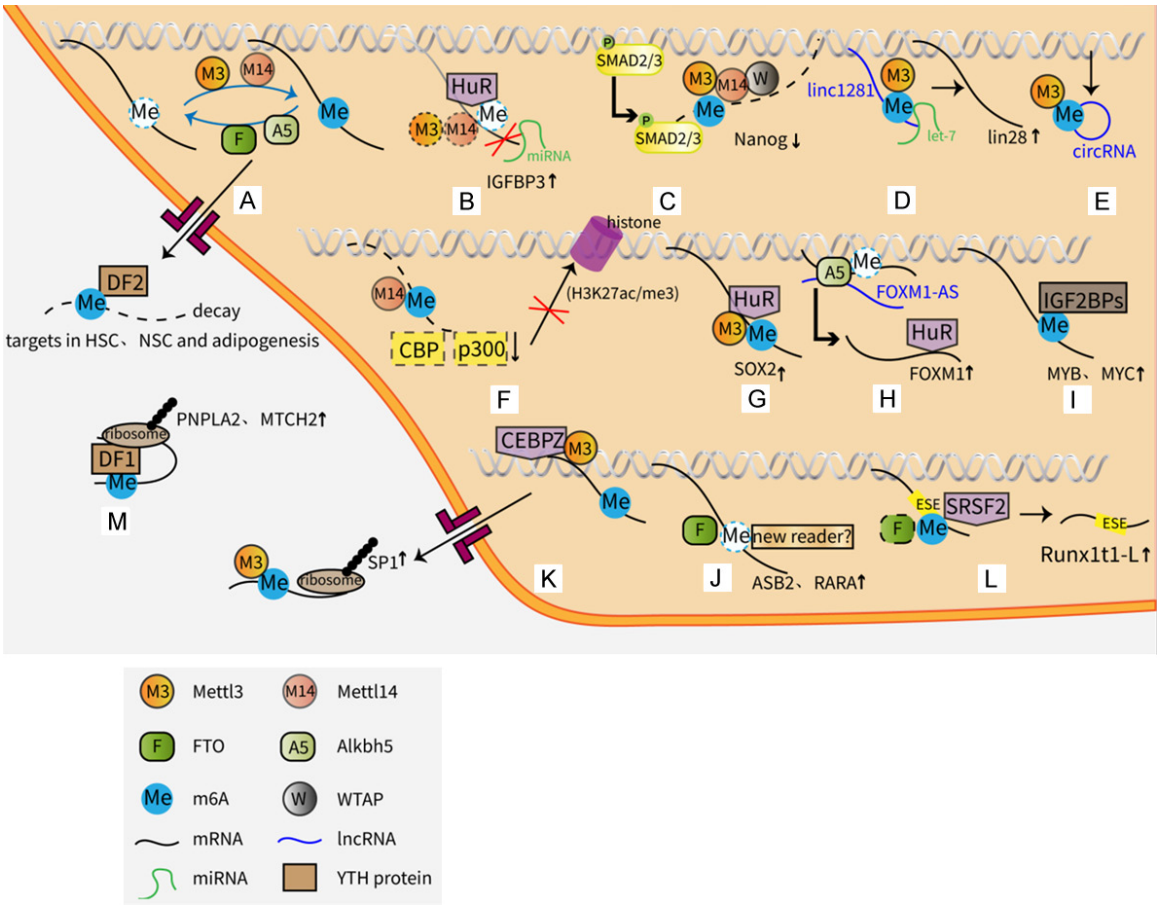

Figure 1. Effects of m6A on stem cell regulation. A. Key transcripts in ESC, NSC, GSC, and HSC development and adipogenesis are destabilized by "writers" (represented by Mettl3/Mettl14) or deficient "erasers" (represented by FTO/Alkbh5) and/or degraded by the "writer" protein Ythdf2. B. Mettl3/Mettl14-induced loss of m6A recruits HuR to repress miRNA targeting and protect transcripts. C. m6A destabilizes mRNAs via the M-M-W complex, activated by the TGFβ-SMAD2/3 pathway. D. Mettl3-induced m6A on linc1281 sponges let-7 to promote lin28 expression. E. m6A-circRNAs in ESCs with unknown functions. F. Mettl14-induced m6A destabilizes the acetyltransferases CBP and p300 and inhibits H3K27ac and H3K27me3. G. Mettl3 increases m6A and recruits HuR to stabilize SOX2 mRNA. H. Alkbh5 decreases m6A and recruits HuR and FOXM1-AS to stabilize FOXM1 mRNA. I. IGF2BPs act as m6A readers and promote translation in normal and malignant hematopoiesis. J. FTO demethylates and promotes the stability of ASB2 and RARA by an unidentified reader. K. The trans-acting factor CEBPZ recruits Mettl3 to promote mRNA translation. L. FTO depletion regulates m6A and promotes exon inclusion of RUNX1T1. M. Ythdf1-mediated translation promotion in adipogenesis.

cells and give rise to the entire brain [25]. Neurons designated for different cortical layers during early postnatal stages are derived from RGCs. Notably, various studies have produced conflicting results regarding the role of m6A in both driving differentiation and maintaining pluripotency [26].

On the one hand, m6A decoration promotes the proliferation and differentiation of NSCs simultaneously. Novel cross-talk between m6A-regulated RNA and histone modification has been validated to correlate with NSCs. Histone modification involves acetylation, which stimulates gene expression and methylation, which has dual functions [27]. One study showed that m6A deficiency achieved by Mettl14-knockout (KO) regulated the acetylation and methylation of histone 3 at lysine 27 in NSCs (H3K27ac and H3K27me3). Acetylation and methylation are associated with gene activation and repression, respectively [25, 27]. Additionally, an increase in transcript levels of acetyltransferases CBP and p300 was observed in Mettl14-KO mouse NSCs, suggesting that m6A can destabilize mRNAs that encode histone modifiers (**Figure 1F**). The altered histone modifications repressed proliferation-related genes and

activated differentiation-related genes, resulting in the decreased self-renewal and premature differentiation of NSCs [25]. In addition to indirect epigenetic modulation, m6A can induce similar results directly. Recent reports revealed that m6A depletion derived from Mettl14 or Mettl3-KO inhibits the decay of mRNAs related to transcription factors (TFs), the cell cycle, neurogenesis, and differentiation, leading to a protracted cell-cycle in RGCs and delayed cortical differentiation into postnatal stages. Consequently, the Mettl3-Mettl14 m6A writer complex mark affects the timing of cerebral cortex development in humans and mice [26, 28].

On the other hand, m6A modification inhibits both the proliferation and differentiation of NSCs. Evidence showed that in adult NSCs (aNSCs), loss of FTO enhanced m6A methylation of mRNAs from the brain derived neurotrophic factor (BDNF) pathway, such as PI3K, Akt, and mammalian target of rapamycin (mTOR), leading to degradation of these transcripts and decreased gene expression. This impairment of the BDNF/Akt signaling pathway resulted in neurogenic deficits and impaired learning and memory [29]. Furthermore, Ythdf2 is responsible for the normal proliferation and differentiation of neural stem/progenitor cells (NSPCs) during mouse embryonic neural development, since Ythdf2 deficiency delays the degradation of several mRNAs. Their upregulation inhibits the JAK/STAT (Janus kinase/signal transducer and activator of transcription) cascade and stimulates neuroprotection and neurite outgrowth [16].

m6A modifications inGSCs

Glioblastoma (GBM) is the most common malignancy of the human brain, and its high mortality rate may result from treatment resistance in glioblastoma stem cells (GSCs) [30]. Earlier experiments from Cui et al. recognized the crucial role of m6A in GSC regeneration and tumorigenesis. Knockdown of Mettl3 or Mettl14 promoted GSC self-renewal by altering mRNA expression of functional genes in GBM such as ADAM19, where inhibiting FTO or Alkbh5 generated opposite result [31]. Interestingly, recent reports indicated that the recruitment of specific partner RBPs that are either amiable to or repelled by m6A sites might be essential for mRNA stabilization and target translation. As was previously reported in mESCs, HuR is one of the m6A-mediated nuclear RBPs that affects RNA stability and translation. Results from another study then concluded that Mettl3 maintains the properties of glioma stem-like cells (GSCs) by methylating transcript of SOX2, a crucial transcription factor in the reprogramming of differentiated glioma cells to induced GSCs. Intriguingly, recruitment of HuR to m6A-modified sites is vital for SOX2 mRNA stabilization (**Figure 1G**) [32]. Nevertheless, HuR was also shown to disfavor binding with the m6A-enriched sites on RNAs. Zhang et al. demonstrated that elevated Alkbh5 induced GSC self-renewal by demethylating nascent transcript of Forkhead box protein M1 (FOXM1), an important transcription factor similar to SOX2. The interaction of Alkbh5 to FOXM1 is dependent on FOXM1-AS, a lncRNA antisense to FOXM1. Furthermore, HuR facilitates the maintenance of FOXM1 expression by binding to the unmethylated sites (**Figure 1H**) [33].

m6A modifications in HSCs and leukemia

Hematopoietic stem cells (HSCs) are essential for the maintenance of hematopoietic function in the human body under both homeostatic conditions and hematological stress, and there is emerging evidence concerning m6A involvement in the development of both hematopoietic and leukemic stem cells [34, 35]. As a key modulator of endothelial-to-hematopoietic transition (EHT), Notch 1a mRNA is methylated by endothelial-specific Mettl3, and the subsequent Ythdf2-driven mRNA decay inhibits the Notch signaling pathway and facilitates EHT and HSPC specification during embryogenesis [15, 36]. Furthermore, the enrichment of Yhtdf2 was shown to be critical for the regenerative capacity of HSCs. By conditional knockout (cKO) of Ythdf2, m6A-modified transcripts encoding transcription factors significant for stem cell self-renewal and mRNAs of Wnt target genes were rescued from degradation, leading to HSC expansion without skewing lineage differentiation (**Figure 1A**) [34, 37]. Moreover, alternative m6A reading processes may exist aside from the traditional YTH readers. Vu et al. identified that Mettl3 aids HSPCs and acute myeloid leukemia (AML) cells in sustaining pluripotency by a novel mechanism apart from modulating mRNA stability. Its upregulation in AML cells induced m6A of mRNAs necessary for regulating apoptosis and differentiation involving MYC, Bcl-2, and PTEN, leading to their

efficient translation and improvement of stem cell growth [38]. Similarly, overexpression of Mettl14 in normal HSPCs and various AMLs is responsible for a blockade of myeloid differentiation and acceleration of AML proliferation and survival. Mechanistically, Mettl14 is likely to regulate mRNA stability and translation of the essential targets MYB and MYC without the involvement of Ythdf proteins [35]. Results from Huang et al. suggested that insulin-like growth factor 2 binding proteins (IGF2BPs) are likely to be the potential m6A readers in the above studies, promoting stability and translation of target transcripts at the post-transcriptional level (**Figure 1I**) [39]. Consistent with the effects of Mettl3 and Mettl14, FTO may also play an oncogenic role in AML. The negative effect of FTO on two crucial tumor suppressors, ASB2 and RARA, occurs in an m6A-dependent manner and is likely to be mediated by an unidentified reader that can promote mRNA stability (**Figure 1J**) [40].

Studies proved that Mettl3 promotes mRNA translation through recruiting m6A readers such as YTH proteins or interacting with the translation initiation machinery, independent of its catalytic activity [41]. Recently, Barbieri et al. unveiled a novel mechanism through which Mettl3 regulates gene expression in the maintenance of a leukemic state. Recruited by the transcriptional activator CAATT-box binding protein (CEBPZ), Mettl3 localizes at promoter regions of specific genes like SP1, which activates the oncogene c-Myc, methylating mRNAs and promoting their translation by relieving ribosome stalling at specific codons (**Figure 1K**). This translation control role of chromatin-binding by Mettl3 facilitated AML cell growth [42].

m6A modifications in adipogenesis

To date, most of the research has focused on preadipocytes in adipose tissue, a progenitor cell isolated from the stromal vascular fraction (SVF) that is capable of proliferating constantly and differentiating into mature adipocytes. The mechanism that leads adipose-derived stem cells (ADSC) into preadipocytes is not clearly defined [43]. Multiple lines of evidence have identified the regulatory role of m6A on preadipocyte development and adipogenesis.

Various studies have observed inhibition of adipogenesis in FTO-deficient preadipocytes via different mechanisms. A study by Zhao et al. reported that deposition of m6A resulting from FTO deletion in alternatively spliced exons elevated the RNA-binding capacity of serine/arginine-rich splicing factor 2 (SRSF2), which recognizes mRNA exonic splicing enhancers (ESEs). Thus, FTO controls the inclusion of target exons in adipogenic regulatory factor termed Runt-related TF 1 (RUNX1T1), leading to decreased expression of a shorter protein type (RUNX1T1-S) and suppressed differentiation of preadipocytes (**Figure 1L**) [44, 45]. Another study indicated that the effect of FTO on adipogenesis was mediated by modulating cell-cycle progression. The expression of two key proteins in the mitotic clonal expansion (MCE), termed cyclin A2 (CCNA2) and cyclin dependent kinase 2 (CDK2), were reduced in an m6A-Ythdf2-dependent manner. In addition, exposing preadipocytes to Epigallocatechin gallate (EGCG) decreased the expression of FTO (**Figure 1A**) [46, 47]. Furthermore, Zhang et al. showed that the well-known adipogenic differentiating factor, peroxisome proliferator-activated receptor gamma (PPARγ), is likely to be a downstream target of FTO during adipogenesis [48]. Intriguingly, a novel study validated that PPARα gene regulation by RNA m6A impacts the cross-talk between circadian networks and lipid metabolism [49].

In addition, Wang et al. identified two unique genes containing m6A peaks named uncoupling protein-2 (UCP2) and patatin-like phospholipase domain containing 2 (PNPLA2) in pig models. m6A depletion inhibited adipogenesis in UCP2 while promoting adipogenesis in PNPLA2. Mechanistically, m6A negatively modulated UCP2 protein expression through neither Ythdf1 nor Ythdf2, while positively modulating PNPLA2 protein through Ythdf1 (**Figure 1M**) [50]. Further study from Jiang et al. found that the overexpression of mitochondrial carrier homology 2 (MTCH2) was activated by the m6A-Ythdf1 axis in intramuscular preadipocytes to promote adipogenesis [51]. Moreover, a recent study indicated that family with sequence similarity 134, member B (FAM134B) plays an essential role in porcine lipid homeostasis. Deletion of m6A facilitated the expression of FAM134B in a Ythdf2-dependent manner, resulting in porcine preadi-

pocyte differentiation and fat deposition, partly by increasing the expression of PPARγ and CEBPα [52].

m6A modifications in myogenesis

A recent study showed that FTO contributed to myogenic differentiation by affecting mitochondrial biogenesis. Dependent on its demethylase activity, FTO activates the expression of mTOR, mitochondrial DNA (mtDNA) encoding proteins, and peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1α) without altering mRNA stability. The involvement of the mTOR-PGC-1α axis facilitates mitochondrial biogenesis and energy production [53]. Furthermore, in myogenic stem/progenitor cells (MSPCs), Mettl3 induced m6A methylation within the 5'UTR of myogenic TF MyoDmRNA. The deletion of Mettl3 downregulated mature mRNA levels of MyoD by regulating RNA splicing, resulting in the absence of skeletal muscle differentiation [54].

Cell death and survival

An increasing number of studies have suggested that RNA methylation is interrelated with the regulation of cell death and survival [4]. Here, we sum up novel insights into the involvement of m6A in distinct biological processes ranging from DNA damage and immune responses to cell apoptosis, autophagy, and senescence. These processes maintain cellular homeostasis and provide therapeutic targets for various diseases such as cancer.

DNA damage response

Ubiquitous sources of DNA damage such as extracellular ionizing radiation, ultraviolet (UV) radiation, or intracellular reactive oxygen species threaten cell growth and survival. Therefore, a repair network termed the DNA damage response (DDR) is of great necessity to ensure genome integrity [4]. As early as 2012, Li et al. implied that Alkb may not only remove alkyl groups from nucleic acids such as DNA- and RNA-repair enzymes, but also participates in control of the cell replication cycle by demethylating DNA m6A [55]. According to conclusions from Xiang et al., Mettl3, together with Mettl14 and FTO, rapidly methylate a number of transcripts localized at DNA damage sites following UV irradiation. Moreover, an essential modulator termed poly ADP-ribose polymerase (PARP) is recruited upstream of DNA damage sites by Mettl3. Accumulated m6A recruits the repair and trans-lesion synthesis DNA polymerase (Pol κ) to damage sites, leading to promotion of DDR and cell survival. Interestingly, a new m6A reader may interact with Pol κ, stimulating its recruitment (**Figure 2A**) [56]. Within the field of cancer research, one study showed that Mettl3 promoted radioresistance in GSCs through increasing DNA repair. Increased SOX2 mediated by Mettl3 and HuR recruitment may activate transcription of DNA repair genes, resulting in improvement of the DDR [32]. Moreover, Zhou et al. revealed that FTO enhanced the chemoradiotherapy resistance of cervical squamous cell carcinoma (CSCC) through demethylating β-catenin mRNA and promoting its expression. This in turn increased the activity of excision repair cross-complementation group 1 (ERCC1), a significant regulator of the nucleotide excision repair (NER) pathway [57].

Immune response

The innate immune system provides the first response toward viral infections by distinguishing pathogen nucleic acids from the host. The role of RNA m6A in the innate immune response remains contradictory. On one hand, Zheng et al. unveiled that DDX46, a member of the DEAD-box RNA helicase family, interacts with Alkbh5 to demethylate m6A-marked antiviral transcripts including MAVS, TRAF3, and TRAF6, leading to their retention in the nucleus and subsequent inhibition of translation. Therefore, DDX46 suppresses the type I interferon (IFN-I) signaling cascade after viral infection, suggesting a positive role for m6A in the innate antiviral response (**Figure 2B**) [58]. On the other hand, Winkler et al. recognized m6A modulation as a negative regulator in the IFN-I-induced innate immune response. During viral infection, overexpression of Mettl3 and Ythdf2 elevated the m6A tag on transcripts of IFNB (encoding IFN-β), destabilizing it to inhibit IFN-β expression and the immune response [59]. Aside from host transcripts, viral RNAs containing nucleotide modifications such as m6A interrupt cytosolic retinoic acid-inducible gene I (RIG-I)-like innate immune signaling [60].

In addition, m6A is useful in adaptive immunity. Recently, Han et al. revealed participation of the m6A reader protein Ythdf1 in the process

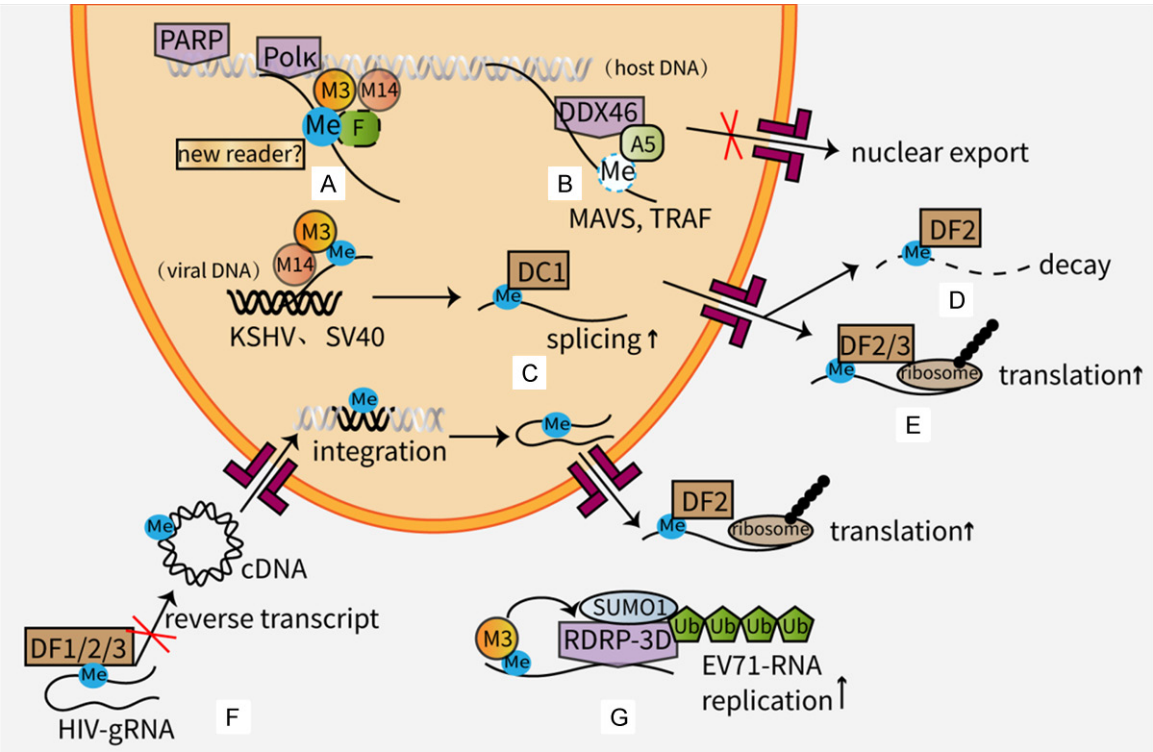

Figure 2. Effects of m6A on DNA damage and the immune response. (A) The polymerase PARP recruits the m6A "writer" complex and may activate Pol κ by a new "reader", facilitating UV-induced DDR. (B) DDX46 recruits Alkbh5 in the nucleus to inhibit nuclear export of mRNAs crucial to the innate immune response. In the viral genome system, viruses hijack host proteins in the m6A modification system to regulate viral replication and infection by (C) Ythdc1-induced RNA splicing, (D) Ythdf2-induced decay, (E) Ythdf2/3-induced translation promotion. (F) Ythdf1/2/3 inhibit HIV-1 infection by impairing both early and late reverse transcription. (G) Mettl3 promotes EV71 replication by interacting with RdRp-3D and facilitating its SUMOylation and ubiquitination.

of antigen presentation from dendritic cells (DCs) to CD8+ T-cells. Mechanistically, Ythdf1 promotes the translation of lysosomal cathepsins, impairing the cross-presentation of tumor neoantigens and the cross-priming of CD8+ T-cells, leading to tumor immune escape. Loss of Ythdf1 represses tumor development and enhances the therapeutic efficacy of checkpoint inhibitors [61]. Additionally, Li et al. unraveled that deletion of Mettl3 in T-cells attenuates decay of mRNAs of suppressor of cytokine signaling (SOCS) family genes, the essential inhibitors of Janus kinase (JAK) and signal transducer and activator of transcription (STAT) proteins. Consequently, m6A regulates the homeostatic differentiation ability of T-cells in the adaptive immune response by targeting the Interleukin 7 (IL-7)-induced JAK/STAT5 signaling pathway [62].

Rapid replication of viral mRNAs is essential for their spread before the host immune response is activated, enabling viruses to hijack host proteins to circumvent the immune system [63]. The m6A deposition in the genomes of RNA viruses and transcripts of DNA viruses, accompanied by m6A modulators, plays either a restricting or promoting role in the lifecycle of select viruses [64]. Chen et al. presented evidence that Ythdc1 improves splicing of chief replication transcription activator (RTA) of Kaposi's sarcoma-associated herpesvirus (KSHV) to regulate KSHV latency and induce cellular transformation and tumorigenesis (**Figure 2C**) [64]. In the cytoplasm, Ythdf2 from host cells suppresses viral lytic replication by facilitating the decay of KSHV RNAs (**Figure 2D**) [65]. In addition, Ythdf2 and Ythdf3 promote translation of m6A-marked RNAs and viral particle assembly, as is exemplified by human immunodeficiency virus type-1 (HIV-1) and prototypic polyomavirus simian virus 40 (SV40) (**Figure 2E**) [63, 66-68]. Hesser et al. determined that the m6A-Ythdf2 axis has cell type-specific pro- or antiviral impacts on the expression of ORF50, the chief replication trans-acti-

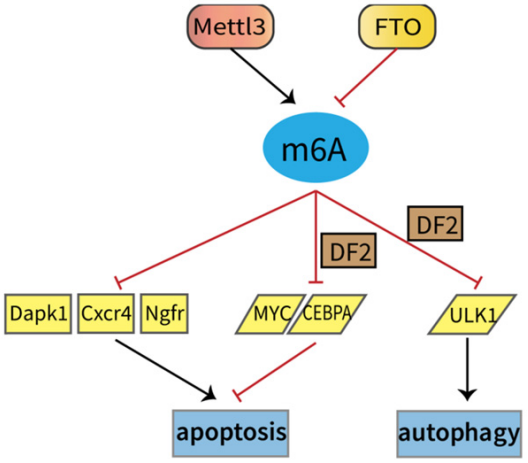

Figure 3. Effects of m6A on apoptosis and autophagy. Depletion of either methylating or demethylating enzymes can induce apoptosis, some of which act through Ythdf2-induced transcript decay; FTO deletion promotes ULK1 degradation by Ythdf2 to inhibit autophagy initiation.

vator of KSHV [69]. The reason for this conflicting role of Ythdf2 is still unknown. Furthermore, Ythdf1/2/3 are reported to inhibit HIV-1 infection by impairing both early and late reverse transcription (RT) of its viral genomic RNA (gRNA) (**Figure 2F**) [70]. A recent study generated novel insights into the viral-m6A field. The results showed that host Mettl3 interacts in the cytoplasm with a vital replication modulator of enterovirus 71 (EV71), termed RNA-dependent RNA polymerase 3D (RdRp-3D), leading to SUMOylation and ubiquitination of RdRp-3D to enhance its stability and promote EV71 replication (**Figure 2G**) [71, 72].

Apoptosis and autophagy

m6A modulation participates in diverse pathophysiological processes by controlling apoptosis-related genes and depletion of methylating or demethylating enzymes, both of which result in apoptosis (**Figure 3**) [73]. Research results identified that genes enriched in a Mettl3-knockdown model belong to the p53 signaling cascade and are implicated in apoptosis [20]. Taketo et al. also found that Mettl3 depletion accelerates apoptosis in pancreatic cancer (PC) cells [74]. Furthermore, studies showed the role of m6A in the development of not only the cerebral cortex but the cerebellum as well. Recent evidence by Wang et al. showed that a deficiency of Mettl3 induced abnormal postmitotic apoptosis of newborn cerebellar granule cells (CGCs) by extending the half-lives of apoptosis-promoting genes such as Dakp1, Cxcr4 and Ngfr, causing cerebellar hypoplasia [75]. Furthermore, loss of FTO dramatically increased the apoptosis rate of human ovarian granulosa cells (hGCs), impairing ovarian function and causing premature ovarian insufficiency (POI) [76]. Additionally, R-2-hydroxyglutarate (R-2HG), known as an oncometabolite, facilitated cell-cycle arrest and apoptosis in leukemia cells by inhibiting FTO-induced demethylation of target transcripts such as the oncogene MYC and the transcription factor CEBPA. This decreased mRNA stability and increased Ythdf2-mediated protein decay [77]. Similar results in lung squamous cell carcinoma (LSCC) revealed that silencing of FTO promoted apoptosis, while preventing growth and invasion via regulating myeloid zinc finger protein 1 (MZF1) mRNA stability [78].

Because autophagy is a crucial cellular process governing cell fate, the correlation between m6A and autophagy is worth investigating. Lately, research has identified an oncogenic role of FTO via autophagy (**Figure 3**). Elevated m6A modification in the mRNA 3'UTR of Unc-51-like kinase 1 (ULKI), a protein kinase essential to autophagy initiation, is catalyzed by FTO deletion. Subsequently, the m6A-marked transcript is targeted for degradation by Ythdf2. In addition, FTO positively regulates autophagy by enhancing LC3B accumulation and p62 consumption [79]. Other m6A-targeted autophagic genes warrant further investigation.

Senescence

A study compared m6A profiles in human peripheral blood mononuclear cells (PBMCs) and diploid fibroblasts (HDFs) from young and old cohorts and found a global decrease of m6A methylated mRNAs in the old cohorts. Among them, suppression of m6A destabilized AGO2 mRNA leading to under-expression of its protein, cell senescence, and decreased miRNA abundance during the aging process [1]. Furthermore, Li et al. indicated a new synergistic effect of RNA 5-methylcytosine (m5C) and m6A in senescence regulation. Nsun2 is a primary RNA methyltransferase catalyzing m5C in both coding and non-coding RNAs. Nsun2-mediated m5C and Mettl3/Mettl14-mediated m6A mutually promoted and collectively facilitated oxidative stress-induced cellular senes-

cence by overexpressing p21, a vital inhibitor of cyclin-dependent kinase (CDKN1A), at the translational level [80].

Conclusions and perspective

m6A modulation acts as a double-edged sword in regulating stem cell fate and variant cellular processes relative to cell death and survival. This discrepancy is likely due to interactions with abundant RBPs, involving m6A reader proteins and non-reader proteins.

Besides the well-established YTH domain proteins, IGF2BPs newly identified as m6A readers are likely to play critical roles in HSCs and leukemia development by promoting mRNA target stability and protein translation [35, 38, 40, 42]. Moreover, the m6A-mediated interaction between linc1281 and miRNA in mESC behaviors may require specific RBPs bound to linc1281 [23]. Novel reader proteins in many biological processes such as adipogenesis and DDR also require further investigation [50, 56]. Many studies have confirmed the existence of various other readers such as Leucine-rich pentatricopeptide repeat-containing protein (LRPPRC) [81], fragile X-related proteins (FMR1) [82, 83], eukaryotic initiation factor 3 (eIF3) [84], heterogeneous nuclear ribonucleoproteins (hnRNPs) [85], and proline-rich coiled-coil 2A (Prrc2a) [86]. Whether these proteins are involved in the stem cell field or cellular processes concerning cell death and survival remains unclear.

In addition, many non-reader RBPs are noteworthy. Using HuR, target transcripts marked by m6A are stabilized in ESCs and GSCs [20, 32, 33]. Splicing factors such as SRSF2 and trans-acting factor CEBPZ are also recognized as functional factors in the stem cell field, according to this review [42, 44]. Furthermore, enzymes such as the polymerases (PARP, Pol κ and RdRp-3D) and helicases (DDX3 and DDX46) can interact with variant RNA m6A modulators during various biological processes [56, 58, 87]. Identifying crosstalk between m6A modulators and non-reader RBPs, or protein recruitment directly to RNAs is challenging since interactions can be of low affinity and protein complexes make it difficult to distinguish direct from indirect binding. Accordingly, more studies are necessary to expand the m6A-related RBP family.

This review summarizes the current knowledge of the abundant functions of RNA m6A in determining stem cell fate and regulating diverse cellular processes involved in cell death and survival. More research is needed to identify novel m6A-associated RBPs and relevant signaling pathways.

Acknowledgements

This work was supported by grants from National Natural Science Foundation of China (grant No. 81672362).

Disclosure of conflict of interest

None.

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