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REVIEW

Regulation of Virus Replication and T Cell Homeostasis by N^6-Methyladenosine

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Abstract

RNA modifications are abundant in eukaryotes, bacteria, and archaea. N^6-methyladenosine (m^6A), a type of RNA modification mainly found in messenger RNA (mRNA), has significant effects on the metabolism and function of mRNAs. This modification is governed by three types of proteins, namely methyltransferases as "writers", demethylases as "erasers", and specific m^6A-binding proteins (YTHDF1-3) as "readers". Further, it is important for the regulation of cell fate and has a critical function in many biological processes including virus replication, stem cell differentiation, and cancer development, and exerts its effect by controlling gene expression. Herein, we summarize recent advances in research on m^6A in virus replication and T cell regulation, which is a rapidly emerging field that will facilitate the development of antiviral therapies and the study of innate immunity.

Keywords RNA modification · N^6-methyladenosine (m^6A) · Virus replication · T cell homeostasis

Introduction

RNA modifications, which are abundant in cells, are regarded as tags for RNAs that are essential for gene expression (Engel and Chen 2018; Sanchez-Vasquez *et al.* 2018). N^6-methyladenosine (m^6A) is one of the most abundant modifications of RNAs, and especially messenger RNAs (mRNAs) (Gonzales-van Horn and Sarnow 2017; Ivanova *et al.* 2017; Shi *et al.* 2017). This modification expedites turnover of the transcript (Zhao *et al.* 2017). It can also regulate RNA fate including their transcription, translation (Wang *et al.* 2015), decay (Cao *et al.* 2016; Huang and Yin 2018), and involvement in biological processes (Shi *et al.* 2017) such as stem cell differentiation, embryonic development (Batista *et al.* 2014), and stress responses (Wu *et al.* 2017; Zhou *et al.* 2015).

In the cell, m^6A methylation is affected by three proteins; it is installed by methyltransferases, which function as "writers", reversed by demethylases termed "erasers", and recognized by specific m^6A-binding proteins that act as "readers" (Fig. 1) (Yue *et al.* 2015). The methyltransferase "writers'' are methyltransferase-like protein 3 (METTL3), METTL14, the associated protein Wilms' tumor 1-associated protein (WTAP), and METTL16, recently identified by Warda *et al.* (Huang *et al.* 2018; Liu *et al.* 2014; Scholler *et al.* 2018; Warda *et al.* 2017). For many years, METTL3 was only regarded as an AdoMet-binding subunit that participates in mRNA modifications (Bokar *et al.* 1997), until METTL14 was identified. In mammalian cells, METTL3 and METTL14 form a heterodimer complex to regulate the m^6A methylation of mRNA (Huang *et al.* 2018; Liu *et al.* 2014; Scholler *et al.* 2018); they add a methyl to the amidogen of adenosine, which occurs mainly in the 5′ untranslated region (UTR) and 3′ UTR near the stop codon, but can also be present in long exons (Gonzales-van Horn and Sarnow 2017). Interestingly, an mRNA with sites for m^6A methylation in the 5′UTR can be translated in a cap-independent manner, directly binding eukaryotic initiation factor 3 (eIF3) without cap-binding factor eIF4E, a protein normally associated with translation initiation (Meyer *et al.* 2015). Further, the methylation of METTL3 and METTL14 can be reversed by demethylases such as fat mass and obesity-associated protein (FTO) and

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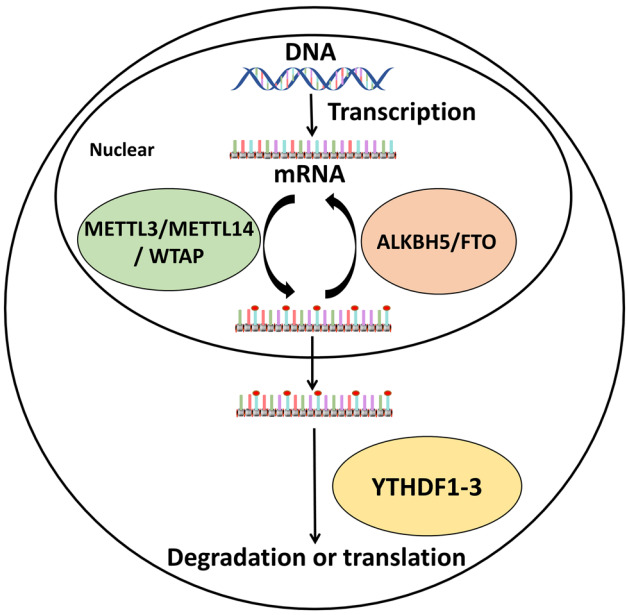

Fig. 1 The machinery of m^{6}A methylation. RNAs, especially mRNA, are methylated by the methyltransferases METTL3, METTL14 and WTAP, which is reversed by the demethylases FTO and ALKBH5. In addition, m^{6}A-modified mRNAs can be recognized by YTHDF1-3, leading to degradation or translation.

AlkB homolog 5 (ALKBH5), which function as "erasers" and belong to the ALKB family (Aik *et al.* 2014; Zhu and Yi 2014). FTO prefers to bind intronic regions in the pre-mRNA, and in particular sites near alternatively spliced exons. The m^{6}A then forms two intermediates, namely N^6-hydroxymethyladenosine (hm^{6}A) and N^6-formyladenosine (fm^{6}A) (Fu *et al.* 2013), and the absence of FTO leads to substantial changes and exon skipping in the pre-mRNA (Bartosovic *et al.* 2017). ALKBH5 directly binds adenosines of m^{6}A-modified mRNAs without any intermediate (Wu *et al.* 2017; Zheng *et al.* 2013). In addition, m^{6}A modification is regulated by "readers" that contain the YTH domain, specifically the proteins YTHDF1-3, which all specifically bind m^{6}A-modified mRNAs (Wu *et al.* 2017). YTHDF1 interacts with translation initiation factors to facilitate the formation of mRNA (Wu *et al.* 2017). YTHDF2 is also essential for the post-transcriptional regulation of mRNA (Ivanova *et al.* 2017) by directly recruiting the CCR4-NOT deadenylase complex to destabilize m^{6}A-RNA, which leads to mRNA degradation (Du *et al.* 2016). YTHDF3 is also a "reader", but its function is not yet clear. Some studies indicate that YTHDF3 accelerates mRNA translation (Li *et al.* 2017a) together with YTHDF1 and facilitates the decay of m^{6}A RNA (Shi *et al.* 2017). However, some studies show that YTHDF2 might play dual roles for RNAs. For example, YTHDF2 was found to enhance HIV-1 protein and RNA expression by promoting mRNA translation in HIV-producing-CD4$^+$ cells (Kennedy *et al.* 2017; Lu *et al.* 2018), but it also suppresses HIV infection by degrading gRNA in HIV-1 target cells (Lu *et al.* 2018; Tirumuru *et al.* 2016). Furthermore, there are other "readers", termed YTHDC proteins, with a YTH domain, including YTHDC1-2, which can also recognize m^{6}A sites through the preferential recognition of GG(m(6)A)C sequences in the nucleus (Xu *et al.* 2014).

Many scientists have focused their studies on the roles of N^6-methyladenosine in vital processes including T cell functions, virus activities, and cancers, with the goal of devising new therapies. We summarize the recent achievements in this field that systematically elaborate upon the roles of N^6-methyladenosine in such process.

Regulation of m^{6}A in Viruses

In the 20th century, it was found that m^{6}A modifications are widespread in viruses including influenza (Krug *et al.* 1976), vaccinia virus (Wei and Moss 1975), Rous sarcoma virus (Kane and Beemon 1985) and simian virus 40 (SV40) (Canaani *et al.* 1979). Recent studies indicate that m^{6}A is also present in other viruses such as human immunodeficiency virus (HIV) (Kennedy *et al.* 2017; Lichinchi *et al.* 2016a; Lu *et al.* 2018; Tirumuru *et al.* 2016), herpesvirus (Hesser *et al.* 2018; Ye 2017; Ye *et al.* 2017), and *Flaviviridae* (Gokhale *et al.* 2016; Lichinchi *et al.* 2016b); there has also been much progress in studies on polyomavirus (Finkel and Groner 1983; Tsai *et al.* 2018) and influenza A virus (IAV) (Courtney *et al.* 2017).

m^{6}A Modification in Human Immunodeficiency Virus

Recently, three groups revealed the vital roles of m^{6}A methylation in the HIV life cycle (Kennedy *et al.* 2017; Lichinchi *et al.* 2016a; Tirumuru *et al.* 2016). They confirmed that m^{6}A methylation is beneficial for virus replication and viral gene expression; specifically, this was increased when they suppressed the demethylases FTO and ALKBH5 and was decreased when they inhibited the methyltransferases METTL3 and METTL14 (Kennedy *et al.* 2017; Lichinchi *et al.* 2016a; Tirumuru *et al.* 2016). Furthermore, the three teams identified the locations of m^{6}A sites. Although they all mapped the sites to 5′UTRs, env/rev, and 3′UTRs, some differences were also noted among these publications. Kennedy *et al.* divided these sites into four m^{6}A clusters containing two or three potential m^{6}A methylation sites, including an env/rev cluster, a U3/NF-κB cluster, a transactivation response (TAR) cluster, and a Nef cluster. When using YTHDF proteins to bind m^{6}A sites, there was another edited site in

the Nef cluster in primary HIV-1 isolates BaL and JR-CSF (Kennedy *et al.* 2017). In contrast, Lichinichi *et al.* identified 14 distinct m^6A methylation peaks located in splicing junctions, coding regions, and noncoding regions, including two in the Rev-responsive element (RRE) bound by the viral Rev protein in a structural region (Lichinichi *et al.* 2016a). Tirumuru *et al.* identified multiple CLIP peaks of m^6A sites that bind YTHDF1-3, covering TAR in the 5′UTR leader sequence, env/rev, and the 3′UTR (Tirumuru *et al.* 2016). Moreover, two of these publications showed that the abundance of m^6A motifs changed after HIV-1 infection. Lichinichi *et al.* demonstrated that the frequency of the MGACK (A/C-GAC-G/U) motif was increased in infected T cells, and that the UGAC motif and MGACK motif were then preferred in HIV-1 RNA (Lichinichi *et al.* 2016a). Tirumuru *et al.* reported a slight increase in RRACH motif enrichment and a decrease in the GGACU motif in infected primary $CD4^+$ T cells. In two of these studies, the roles of YTHDF1-3 proteins were found to be very different. Kennedy *et al.* showed that overexpression of YTHDF proteins in $CD4^+$ T cells increased virus replication and HIV-1 viral proteins and mRNA, and they concluded that post-transcriptional m^6A editing and YTHDF1-3 proteins are positive regulators of HIV-1 infection (Kennedy *et al.* 2017). In contrast, Tirumuru *et al.* found that YTHDF1-3 were suppressors and decreased HIV-1 reverse transcription to inhibit HIV-1 infection in $CD4^+$ T cells upon overexpression (Tirumuru *et al.* 2016). The results of Lichinichi *et al.* were similar to those of Kennedy *et al.*, and these authors also discovered that m^6A methylation modulates Rev protein interaction with RREs in RNA, and that the methylation of an A7883 site modified by m^6A in the RRE bulge region perturbs HIV-1 replication and RNA nuclear export (Lichinichi *et al.* 2016a).

To explain the different roles of YTHDF1-3 based on the results of these different groups, Tirumuru *et al.* carried out further analysis using different cell lines, specifically HIV-1-target cell lines and viral processing cell lines. In the HIV-1-target cell lines such as the HeLa cells, YTHDF1-3 inhibited HIV-1 infection by decreasing levels of gRNA and preventing viral reverse transcription. In virus-processing cell lines such as HEK293T cells transfected with pNL4-3 or TZM-bl, YTHDF1-3 proteins promoted viral gag expression and virus production but suppressed HIV-1 infectivity (Fig. 2). Moreover, YTHDF1-3 proteins, which preferentially bind to m^6A RNA, formed complexes with gag in the presence of HIV-1 m^6A-RNA (Lu *et al.* 2018). Those results explain the different roles of YTHDF1-3 in different cell lines.

Moreover, in addition to different cells, the diverse reagents and experimental methods might explain the diametrically opposed roles of YTHDF proteins in virus replication. Kennedy *et al.* mainly focused on the transcription of viral RNA, whereas Tirumuru *et al.* paid more attention to the reverse transcription of viral RNA. The regulation of m^6A on viral RNA in cells might thus be complicated. Despite the inconsistent results regarding the roles of m^6A in regulating the replication of HIV virus and the expression of viral genes, all three publications indicated that m^6A methylation plays an important role in HIV life cycle, suggesting that the methylation of viral genes might be a potential treatment target for AIDS patients, although the effectiveness of this approach needs to be verified.

m^6A Modification in Herpesvirus

Latency is a trait of all herpesviruses, and these viruses can be induced to enter productive lytic replication in the presence of some agents such as 12-*O*-tetradecanoylphorbol-13-acetate (TPA), tumor necrosis factor alpha (TNF-α), sodium butyrate, and hydrogen peroxide (H_2O_2) (Ye *et al.* 2017). Kaposi's sarcoma-associated herpesvirus (KSHV), a herpesvirus, is an oncogenic human deoxyribonucleic acid (DNA) virus. Three recent publications revealed that KSHV mRNA contains m^6A modifications. In KSHV-infected cells, the levels of mRNAs modified by m^6A methylation were increased when latent KSHV was stimulated to undergo lytic replication (Hesser *et al.* 2018; Ye 2017; Ye *et al.* 2017). Further, Ye *et al.* and Hesser *et al.* discovered that suppressing m^6A by knocking down METTL3 inhibited splicing of the pre-mRNA encoding the replication transcription activator (RTA), which was important for the lytic replication of KSHV, with opposite results obtained after knocking down FTO (Hesser *et al.* 2018; Ye *et al.* 2017). Moreover, Ye *et al.* obtained a similar result by using the m^6A catalytic reaction inhibitor 3-deazaadenosine or the FTO-selective inhibitor meclofenamic acid (Ye *et al.* 2017). Therefore, m^6A methylation is favorable for lytic KSHV gene expression and replication. These two teams also revealed that the lytic switch protein RTA strongly induces m^6A methylation and enhances its own pre-mRNA splicing (Hesser *et al.* 2018; Ye 2017; Ye *et al.* 2017). In different cells, they found that several m^6A sites of open reading frame 50 (ORF50/RTA) are responsible for ORF50 (RTA) pre-mRNA splicing when KSHV was activated by TPA stimulation. They also found that the m^6A sites of ORF50 (RTA) pre-mRNA are bound by YTHDC1 and splicing factors SRSF3 and SRSF10 in the nucleus (Ye 2017; Ye *et al.* 2017), whereas those sites are bound by YTHDF1-3 in the cytoplasm (Hesser *et al.* 2018). Ye *et al.* demonstrated that RTA can increase its own expression through transcriptional and post-transcriptional mechanisms and that KSHV has two opposite mechanisms to operate the host m^6A machinery to regulate lytic

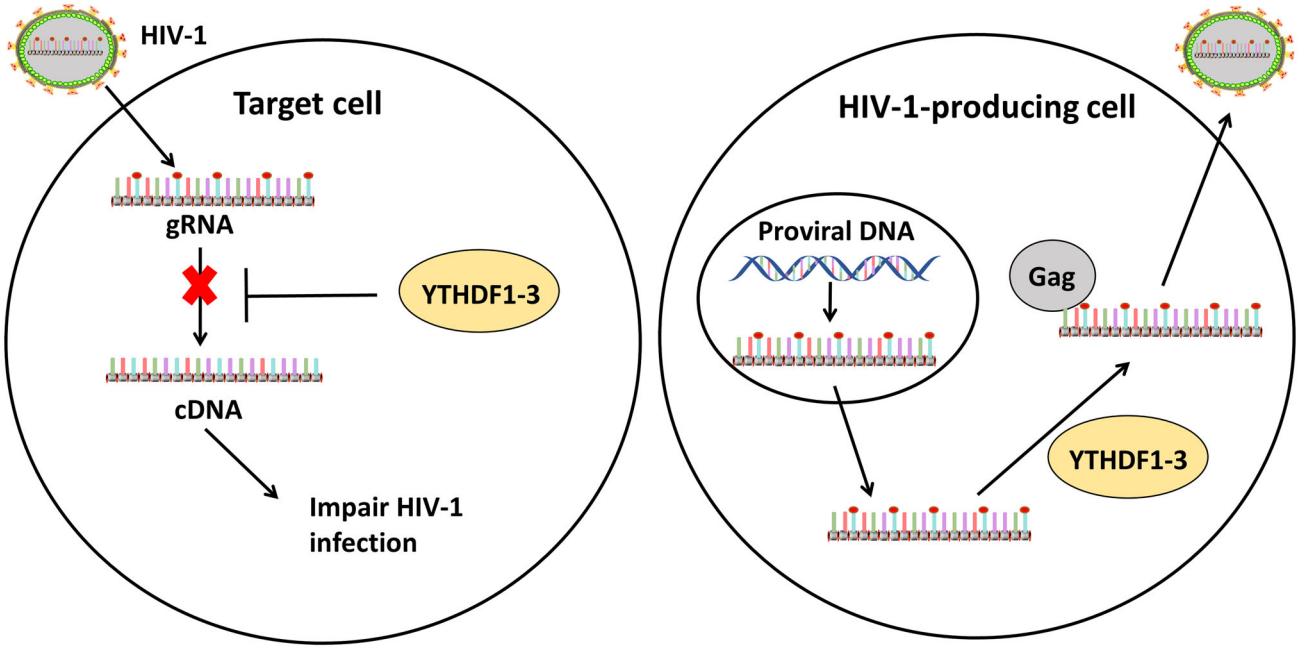

Fig. 2 The roles of m^{6}A methylation in HIV-1 life cycle. When HIV-1 viruses enter target cells, the exposed gRNAs, transcribed from the HIV-1 genome, are recognized by YTHDF1-3 proteins and degraded, which inhibits reverse transcription and virus infection (left). In HIV-1-processing cells, gRNAs are methylated and recognized by YTHDF1-3 proteins, which promotes HIV-1 Gag protein expression and virus production (right).

replication and latency, respectively (Ye 2017; Ye *et al.* 2017). Hesser *et al.* showed that the m^{6}A pathway controls ORF50 expression post-transcriptionally leading to a subsequent defect at the ORF50 promoter (Hesser *et al.* 2018).

The experiments of these two groups were performed using different cell lines, but they obtained similar results, specifically that m^{6}A has a positive effect on the expression and replication of KSHV genes by regulating the splicing of RTA pre-mRNA. These results indicate that this epitranscriptomic modification might play a similar role in other herpesviruses. Therefore, understanding the role and regulatory mechanism of m^{6}A modification in other herpesviruses could provide a new strategy to control herpesvirus infections.

m^{6}A Modification in *Flaviviridae*

Members of *Flaviviridae*, including Zika virus (ZIKV), dengue virus (DENV), West Nile virus, yellow fever virus (YFV), and hepatitis C virus (HCV), are positive single-stranded RNA viruses. Recent studies have shown that *Flaviviridae* viruses all contain m^{6}A-modified sites, which regulate their gene expression and replication (Gokhale *et al.* 2016; Lichinchi *et al.* 2016b).

Gokhale *et al.* reported that m^{6}A plays an important role in regulating the life cycle of HCV. Knocking out METTL3 and METTL14 increased the production of infectious HCV particles, and YTHDF1-3 proteins recognize and bind m^{6}A sites of HCV RNA to suppress viral infection, indicating that m^{6}A negatively regulates HCV. Then, this group mapped the m^{6}A sites of the HCV RNA genome and found that m^{6}A enhanced viral titer by increasing the interaction between the RNA and the core protein of HCV. Finally, they described several other *Flaviviridae* viral RNA m^{6}A epitranscriptomic maps, including those of ZIKV, DENV, and YFV. Taken together, their findings revealed that m^{6}A regulates viral infection and form the basis of future studies to explore the function of m^{6}A within the broader *Flaviviridae* family of viruses (Gokhale *et al.* 2016).

ZIKV was discovered in 1947 (Driggers *et al.* 2016), and it can induce severe neurological defects. Lichinchi *et al.* reported that ZIKV infection alters the N^6-methyladenosine topology and the function of viral and human RNAs. They identified 12 m^{6}A peaks by performing methylated RNA immunoprecipitation-sequencing (MeRIP-seq) experiments, and these peaks were abundant in ZIKV RNA. Knockdown of METTL3/METTL14 or ALKBH5/FTO increased or decreased viral mRNA levels, respectively. Moreover, silencing YTHDF1-3 proteins, which were found to bind ZIKV viral RNA, inhibited replication. The authors also discovered that ZIKV infection influences RNA methylation sites of host cell transcripts, and that new m^{6}A sites are preferentially deposited in the 5′UTR and coding sequence (Lichinchi *et al.* 2016b).

As described, these studies show that m^{6}A methylation plays an important role in regulating Flaviviruses. The methyltransferases METTL3 and METTL14 can modify mRNA and inhibit viral replication in ZIKV and HCV. However, the enzymes FTO and ALKBH5 can suppress this effect and thus exert an adverse effect on the lifecycle of these viruses. In addition, YTHDF1-3 recognize m^{6}A-modified sites to degrade viral mRNA. These studies showed that methylation-regulatory proteins in the host have inhibitory effects on *Flaviviridae* viruses, which provides a new direction for the clinical treatment of associated infections.

m^{6}A Modification in Polyomavirus

As early as the 1970s, m^{6}A methylation was discovered in SV40, which belongs to polyomaviruses, a family of small DNA tumor viruses (Canaani *et al.* 1979; Finkel and Groner 1983; Tsai *et al.* 2018). The sites of m^{6}A methylation in the mRNA of SV40 were found to occur in two sequences, specifically Gpm6ApC and (Ap)nm6ApC in late transcripts, which encode viral structural proteins (Canaani *et al.* 1979). In the 1980s, Finkel *et al.* confirmed that m^{6}A methylation in pre-mRNAs is important for the formation of late SV40 mRNAs (Finkel and Groner 1983). Recently, Tsai *et al.* found that the addition of m^{6}A to prototypic SV40 late mRNAs plays an important role in increasing viral gene expression and replication (Tsai *et al.* 2018). Tsai *et al.* mapped and identified 11 m^{6}A sites in late transcripts and discovered that overexpression of the m^{6}A-binding protein YTHDF2 led to more rapid viral replication and larger viral plaques, demonstrating that m^{6}A plays a positive role in SV40 gene expression. Intriguingly, alternative splicing of SV40 was not found to be regulated by m^{6}A. These results indicate that the addition of m^{6}A to SV40 late mRNAs enhances viral structural gene expression and replication (Tsai *et al.* 2018).

m^{6}A Modification in Influenza A Virus

IAV was first found to possess m^{6}A modification of mRNAs in 1976, and each influenza viral mRNA species contains an average of three m^{6}A residues (Krug *et al.* 1976). Narayan *et al.* determined that the distribution of m^{6}A modifications in different influenza virus mRNAs is different (Narayan *et al.* 1987). Recently, Courtney *et al.* examined the regulatory mechanism of m^{6}A modification with respect to IAV gene expression and replication. They demonstrated that IAV replication is inhibited by mutational inactivation of METTL3 in the human lung epithelial cell line A549, and that overexpression of YTHDF2 increases IAV replication and infectious particle production, which indicated that m^{6}A methylation is advantageous for the IAV life cycle. However, not all RNAs including mRNA/cRNA (plus) strands and vRNA (minus) strands exhibited elevated m^{6}A levels. Higher levels of m^{6}A methylation addition to viral mRNAs encoding the structural proteins HA, NA, M1/M2, and NP and a lower level on the mRNAs encoding the viral polymerase proteins PB2, PB1, and PA were identified. Meanwhile, lower levels of HA mRNA and protein were observed in mutated IAV, whereas other IAV mRNAs and proteins were not changed (Courtney *et al.* 2017). These results show that m^{6}A methylation regulates IAV gene replication and the expression of some proteins.

m^{6}A Modification in Hepatitis B Virus

Recently, m^{6}A modification to hepatitis B virus (HBV) RNA has been reported. Further, dual roles of m^{6}A modification in the life cycle of HBV were demonstrated, as this was found to lead to decreased expression of HBV-related proteins, but enhanced the reverse transcription process of HBV pgRNA (Imam *et al.* 2018).

Host Factors Involved in m^{6}A Modification

Meanwhile, some studies reported the function of m^{6}A methylation in virus–host interactions. Kariko *et al.* found that unmodified RNA can stimulate the innate immune system through the activation of Toll-like receptors (TLRs), but that modified RNA including those subject to m^{6}A methylation cannot active TLRs and trigger the immune defense system (Kariko *et al.* 2005). Similarly, retinoic acid-inducible gene I (RIG-I) can recognize RNA to activate innate immune signals, but it is not triggered by m^{6}A-modified viral RNA, mediating viral evasion of the innate immune system (Durbin *et al.* 2016). In addition, DEAD-box (DDX) 46, a member of DDX family of helicases, binds RNA encoded by the antiviral genes *Mavs*, *Traf3*, and *Traf6* in the nucleus and recruits the m^{6}A demethylase ALKBH5 to demethylate these RNAs and retain them in the nucleus, thereby suppressing antiviral innate immunity *in vivo* during viral infection (Zheng *et al.* 2017). These results indicate that the m^{6}A modification not only regulates the life cycle of the virus, but also exerts an effect on innate anti-viral immunity in the host. This provides a broader research direction for studying the effect of m^{6}A modification on antiviral immunity.

In summary, m^{6}A modification is important for the life cycle of the virus, and as m^{6}A modification is more and more widely studied in different viruses, m^{6}A modification could provide new therapeutic strategies to fight viral infection.

Regulation of m^{6}A in T Cells

N^6-methyladenosine on mRNA affects most post-transcriptional steps of gene expression and is associated with RNA metabolism including RNA stability, splicing, transport, translation, and localization (Brocard *et al.* 2017). m^{6}A regulates alternative splicing by recruiting hnRNPC, a nuclear RNA-binding protein involved in pre-mRNA processing (Liu *et al.* 2015). The "readers" can increase the stability of mRNA by reducing Hu antigen R binding (Brocard *et al.* 2017). Although m^{6}A methylation has similar effects on mRNAs in different cell types, it still has different functions in diverse cells.

Li *et al.* demonstrated that T cell homeostasis is controlled by m^{6}A mRNA methylation through the targeting of interleukin 7/signal transducer and activator of transcription 5/suppressor of cytokine signaling (IL-7/STAT5/SOCS) pathways, which regulate the homeostasis, differentiation, and proliferation of naïve T cells. STAT signaling can be inhibited by SOCS1, SOCS3, and CISH, members of the SOCS family, whose mRNAs are targeted by m^{6}A. In wild-type naïve T cells, *Socs1*, *Socs3*, and *Cish* m^{6}A mRNAs are recognized by YTHDF1-3 and degraded rapidly, and IL-7 signaling activated the Janus kinase (JAK)/STAT pathway to regulate the homeostasis, differentiation, and proliferation of naïve T cells (Fig. 3). In METTL3-deficient naïve T cells, *Socs1*, *Socs3*, and *Cish* mRNAs marked by m^{6}A exhibited slower decay, leading to higher levels of these mRNAs and increased protein expression. Increasing activity of the SOCS family was found to suppress the IL-7/STAT signaling pathway and inhibit T cell homeostasis, differentiation, and proliferation. Additionally, the authors found that T_H1 and T_H17 cells were reduced and T_H2 cells were increased in METTL3-deficient naïve T cell populations, but that the proportion of Treg cells was not changed compared to that in wild-type naïve T cells. However, m^{6}A modification had no role in T cell apoptosis or TCR-mediated proliferation (Li *et al.* 2017b).

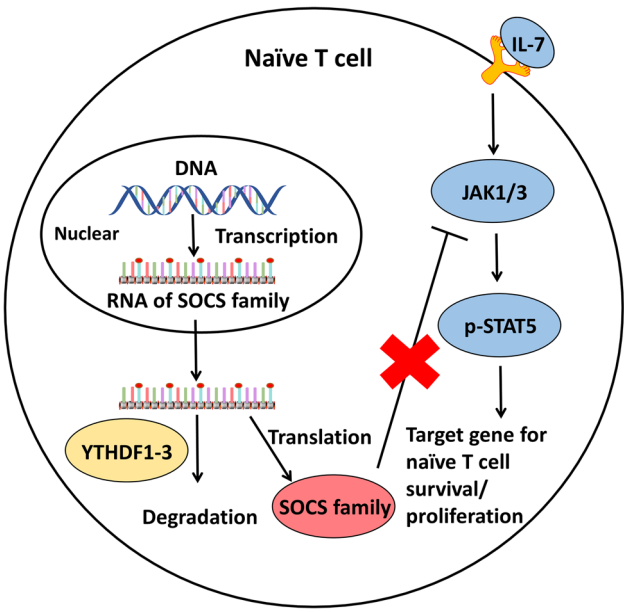

Fig. 3 Regulation of m^{6}A methylation in naïve T cells. The SOCS family inhibits the IL-7/STAT5/SOCS pathways to negatively regulate the homeostasis, differentiation, and proliferation of naïve T cells. However, RNAs of the SOCS family can be methylated in the nucleus, where they are recognized by YTHDF3 and degraded in the cytoplasm to sustain naïve T cell homeostasis, differentiation, and proliferation.

Meanwhile, Tong *et al.* revealed that m^{6}A mRNA methylation has Treg-suppressive functions. By mapping m^{6}A modifications, they found that these occur at a consensus sequence in the 3′UTR and 5′UTR in Treg cells. Depletion of METTL3 was found to increase mRNA levels of *SOCS* and suppress IL-2/STAT5 signaling, which is necessary for Treg functions and stability (Tong *et al.* 2018). These results showed that in different T cell subtypes, m^{6}A RNA modification targets genes that encode essential components of signaling pathways that regulate the differentiation of naïve T cells, in addition to suppressing the functions of Tregs.

In addition, some studies have found that T cells are associated with the anti-viral mechanism of the host, and this is especially true for HIV (Che *et al.* 2010; Smith *et al.* 2016). The primary expansion of naïve T cells cultured with HIV-1-exposed dendritic cells was decreased and consequently immune dysfunction was shown to occur *in vivo* in HIV-1-infected individuals (Che *et al.* 2010); further, naïve CD8$^+$ T cells were found to be required during persistent HIV-1 antiretroviral therapy (Smith *et al.* 2016). Moreover, T_H1 cells can secret interleukin-21 and interferon-γ, which are necessary for maintaining the anti-viral effect of IgG2 during IAV infection (Miyauchi *et al.* 2016). Thus, m^{6}A methylation might indirectly regulate the host anti-viral effect by regulating naïve T cell homeostasis, differentiation, and proliferation, which might also provide new strategies for antiviral therapy, specifically through the regulation of naïve T cells.

Conclusions and Perspectives

N^6-methyladenosine plays a vital role in virus replication and T cell homeostasis. During viral infection, m^{6}A-modified mRNA levels are significantly elevated and therefore promote the replication and expression of viral genes. However, the function of YTHDF2 is different with different viruses, and some results obtained by different teams are conflicting due to the different cell lines and experimental methods used. Further, T cell homeostasis is

controlled by m^6A mRNA methylation, which targets the IL-7/STAT5/SOCS pathways.

This m^6A methylation modification is present in viruses and regulates their life cycle. However, there are some questions that still need to be addressed. For viruses exhibiting latency, whether m^6A methylation plays a role in latency or activation and how the virus hijacks the m^6A machinery during infection are still unknown. Moreover, there are many viruses for which the roles of m^6A methylation are still unknown. With advances in technology and the continuous improvement of experimental methods, these problems might be resolved in the future.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no competing interests.

Animal and Human Rights Statement This article does not contain any studies with human or animal subjects performed by any of the authors.

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