BBA - Molecular Cell Research 1870 (2023) 119380

Contents lists available at ScienceDirect

BBA - Molecular Cell Research

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

Critical functions of N^6-adenosine methylation of mRNAs in T cells☆

Taku Ito-Kureha [a,*], Vigo Heissmeyer [a,b,*]

[a] Institute for Immunology, Biomedical Center, Faculty of Medicine, Ludwig-Maximilians-Universität in Munich, 82152 Planegg-Martinsried, Germany
[b] Research Unit Molecular Immune Regulation, Helmholtz Zentrum München, 81377 Munich, Germany

ARTICLE INFO

Keywords:
Epitranscriptomics
m6A
T cell
TCR signaling

ABSTRACT

The existence of N^6-adenosine methylation (m^6A) of mRNA has been known for a long time, but only recently its regulatory potential was uncovered. Current research deciphers the molecular determinants leading to the deposition of this modification and consequences for modified mRNAs. It also evaluates the importance of such modifications for specific cell types and programs. In this review, we summarize the current knowledge on m^6A modification of mRNAs in conventional and regulatory T cells and T-cell-driven immune responses and pathology. We discuss the impact of m^6A modification on T cell activation including cytokine and antigen receptor signaling or sensing of double-stranded RNAs (dsRNA).

1. Introduction

Several molecular mechanisms contribute to post-transcriptional regulation of gene expression in eukaryotic cells. These mechanisms of regulation can alter cell fate either through an impact on a broad range of target mRNAs or through very specific ones. For example, 3′ untranslated region (3′-UTR) shortening via alternative polyadenylation and alternative splicing of pre-mRNA may change the expression or activity of a plethora of targets at the same time [1,2]. In contrast, RNA-binding proteins (RBPs) and miRNAs may also regulate only few expressed mRNAs by recognizing specific mRNA-encoded *cis*-elements and selectively changing their stability or efficiency of translation [3,4].

2. Epitranscriptomics

In recent years, chemical modification of mRNA has been in the limelight as a new post-transcriptional mechanism of gene regulation. Studies over the past few years in this new field called “epitranscriptomics” have shown that N^6-methyladenosine (m^6A), which is the most prevalent modification in mRNAs, controls splicing, mRNA stability and translation [5]. On the one hand m^6A modification can alter the ability of mRNAs to adopt specific secondary structures, since the modification itself interferes with base pairing [6–8]. On the other hand, it is clear that m^6A creates unique binding sites on mRNAs for specific *trans*-acting factors i.e. the m^6A-binding proteins [9,10]. Currently, several m^6A-binding proteins have been identified and specific functions of different m^6A-binding proteins become apparent, however controversies remain. In each cell type and activation state we need to consider a unique regulatory network defined by the expressed mRNAs, deposition of m^6A marks and expression of m^6A-binding proteins. Here, we focus on the emerging prominent role of m^6A in T cells discussing current findings as well as challenges.

2.1. m^6A writers

The m^6A modification occurs in the nucleus, where m^6A marks are deposited on pre-mRNAs co-transcriptionally [11,12]. It is catalyzed by a methyltransferase complex of METTL3 and METTL14 proteins [13], which further includes the Wilms tumor 1 associated protein (WTAP) [14], RNA binding protein 15 (RBM15/15B) [15], KIAA1429 (VIRMA) [16], ZC3H13 [15] and HAKAI [17] (Fig. 1). WTAP and ZC3H13 are required for the activity and nuclear localization of the m^6A methyltransferase complex [13,15]. VIRMA and RBM15/RBM15B are similarly part of the complex and also essential for efficient m^6A modification of mRNAs [16]. Although METTL3, as a METTL3/METTL14 heterodimer, is the only component with catalytic activity [18,19], it is so far unclear how the other components enable or direct the METTL3-contained activity within the multiprotein complex. The modification occurs on selected mRNAs often positioned in a DRACH motif (D = G/A/U, R = G/A, H = A/U/C) [20]. The modification is enriched at the stop codon or at

☆ This article is part of a Special Issue entitled: Emerging Concepts & Non-Canonical Pathways in Cellular Signaling edited by Lienhard Schmitz, Geert Bultynck and Shazia Khan.

* Corresponding authors.
E-mail addresses: Taku.Kureha@med.uni-muenchen.de (T. Ito-Kureha), Vigo.Heissmeyer@med.uni-muenchen.de (V. Heissmeyer).

https://doi.org/10.1016/j.bbamcr.2022.119380
Received 28 July 2022; Received in revised form 22 September 2022; Accepted 28 September 2022
Available online 11 October 2022

the 5′ end of the terminal exons that often contain the stop codon [21]. Despite many potential DRACH motifs in most transcripts, the deposition of m^{6}A is selective for some mRNAs. It is so far believed that most modified mRNAs contain few m^{6}A modified sites which may be clustered, and only some mRNAs contain many [20,22]. Recent research revealed that deposition of m^{6}A in a context of ACA motifs is primarily explained by the extended sequence surrounding the modified site [23]. Despite great progress investigating this molecular program, the criteria by which specific sites are selected for m^{6}A modification are still not understood.

PCIF1 is yet another m^{6}A methyltransferase that produce *N*^{6},2′-*O*-dimethyladenosine (m^{6}Am) [24,25]. Importantly, m^{6}A modifications detected by m^{6}A-specific antibodies in the 5′-UTR of mRNAs often reflect m^{6}Am modifications because this modification is frequently found at the first transcribed nucleotide of mRNAs adjacent to the m^{7}G cap [26].

It is currently unclear how individual component of the methyltransferase complex respond to external signals, and how components of the complex are expressed or regulated in different cell types and during activation and differentiation of cells. Furthermore, it is unsolved whether and how regulation impacts on the activity of the complex causing differential activity or differential m^{6}A methylation of specific sites and targets. A recent analysis revealed that METTL3 and WTAP are phosphorylated by ERK [27], and suggested that the ERK-METTL3/WTAP signaling axis promotes stem cell differentiation and tumorigenesis. SUMOylation of METTL3 has also been detected and was shown to influence tumor growth in a cancer cell line [28]. However, many aspects of post-translational modification and regulation of writer proteins remain to be elucidated.

2.2. m^{6}A readers

Once the nuclear pre-mRNAs or nuclear mRNAs are m^{6}A-modified, these marks can be recognized by reader proteins. Prototypic m^{6}A reader proteins contain a YTH domain, which are specialized to directly bind to m^{6}A. Alternatively, m^{6}A changes the mRNA structure, which, by altering accessibility, can facilitate or impair interactions of other RNA-binding proteins. There are five YTH domain–containing proteins encoded in the mammalian genome. The YTHDF1, YTHDF2, and YTHDF3 paralogs are localized in the cytoplasm and share a highly conserved YTH domain as their only recognizable domain mediating interactions with m^{6}A-modified mRNA targets. Initial studies discriminated different regulatory functions and different targets for these proteins. YTHDF2 selectively induced mRNA decay [9,29], YTHDF1 enhanced translation through binding to the eukaryotic translation initiation factor eIF3 [30], and YTHDF3 promoted protein synthesis in synergy with YTHDF1, whereas the protein also affected mRNA decay by cooperation with YTHDF2 [31,32]. However, a more recent study demonstrated overlapping binding characteristics and redundant functions for all three YTHDF1, YTHDF2, YTHDF3 proteins [33]. Interestingly, upon heat-shock stress, YTHDF2 was shown to translocate into the nucleus to prevent mRNA demethylation [34]. Contrasting these findings, a subsequent study was unable to recapitulate relocalization to the nucleus of YTHDF2 in response to heat-shock [35]. Apparently, all three YTHDF proteins which comprise extended regions of low-complexity undergo liquid-liquid phase separation in vitro as well as in cells, which is enhanced by mRNAs containing multiple m^{6}A residues. Furthermore, the YTHDF–complex formation with mRNA is required to partition into P-bodies or stress granules [35]. In addition to the direct m^{6}A-binding reader proteins, several other RNA-binding proteins can be indirectly recruited to m^{6}A sites. IGF2BP1, IGF2BP2 and IGF2BP3 paralogs, which enhance mRNA stability, show themselves weak binding affinities for m^{6}A containing RNA [36,37]. However, these IGF2BP1-3 proteins can interact with YTHDF proteins, as determined by proximity-labeling of proteins [38].

YTHDC1, alternative name YT521-B, is localized in the nucleus and linked to the regulation of mRNA splicing and nuclear export of mRNAs [39,40]. The nuclear localization and splicing functions of YTHDC1 were found to be regulated by tyrosine phosphorylation [41]. YTHDC1 was also shown to promote exon inclusion of target mRNAs by binding to SRSF3, thereby outcompeting SRSF10 binding to the mRNAs. However, several other studies attributed a smaller role of m^{6}A-dependent YTHDC1-mediated regulation to splicing [42,43]. Unexpectedly, YTHDC1 contributed to epigenetic regulation of gene expression mediated by histone modification. As shown in a recent study, YTHDC1 co-transcriptionally alters the chromatin via H3K9me2 demethylation by recruiting KDM3B [44]. YTHDC1 is also required for *Xist* noncoding RNA-mediated inactivation of the X chromosome, which might be achieved by recruitment of transcriptional silencing proteins or interactions with Polycomb repressive complex 1 (PRC1) and 2 (PRC2) [45].

YTHDC2, unlike the other ubiquitously expressed YTH-domain containing proteins, is highly expressed only in testes and YTHDC2 knockout mice show defects mainly in spermatogenesis [46,47].

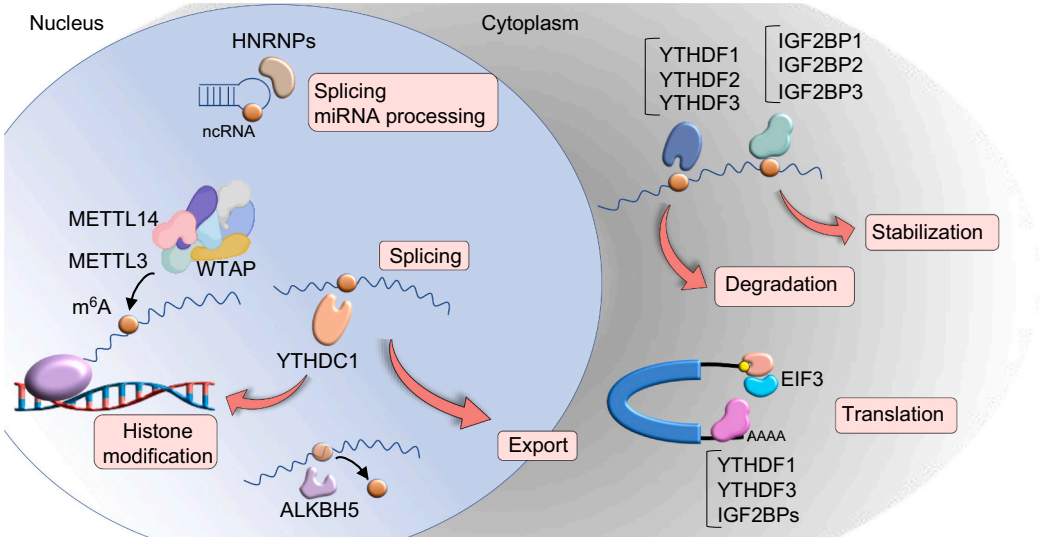

Fig. 1. m^{6}A methylation of mRNAs and regulatory mechanisms involving m^{6}A binding proteins.
Schematic representation showing how m^{6}A methylation of mRNAs is co-transcriptionally deposited, which is mediated by the methyltransferase complex. In this complex METTL3 and METTL14 form a heterodimer, which further include the accessory proteins, WTAP, RBM15/RBM15B, VIRMA, ZC3H13 and HAKAI. ALKBH5 is the nuclear eraser protein that can remove m^{6}A marks from mRNAs. In nucleus, the reader proteins, HNRNPs and YTHDC1, can change gene expression through splicing, histone modification or export of mRNAs to cytoplasm. Cytoplasmic reader proteins YTHDF family member can induce degradation of mRNAs or regulate translation efficiency, whereas IGF2BP family members can affect stabilization of mRNAs. YTHDF1, YTHDF3 and IGF2BP family members can potentially modulate translation.

Compared to the other YTH domain containing proteins, the m^{6}A-binding affinity of YTHDC2 has been shown to be lower [48]. Further experiments seem to be required to fully establish the molecular function of YTHDC2.

m^{6}A unfolds secondary structures and provides increased accessibility for HNRNPC, HNRNPG and HNRNPA2B1 to binding sites that are found near to m^{6}A sites [6–8]. Although these proteins were reported to contribute to pre-miRNA processing and splicing, the functional importance of this structure/switch model remains to be determined.

Overall it has not been solved yet how these reader proteins can discriminate between targets and whether or which protein/protein interactions contribute to target recognition or enable them to either trigger different post-transcriptional or epigenetic mechanisms of gene regulation.

2.3. *m^{6}A erasers*

There are two enzymes in mammalian cells, FTO and ALKBH5, that can erase m^{6}A marks which has been established through measurement of in vitro demethylating activity [49,50]. ALKBH5 showed higher affinity and processivity for m^{6}A, while FTO appeared to be less efficient on m^{6}A instead more selective to demethylate N^6,2′-*O*-dimethyladenosine (m^{6}Am). This suggests that ALKBH5 may be a physiologic m^{6}A demethylase for the internal m^{6}A modifications in mRNAs [51]. However, the extent to which demethylation of mRNAs by ALKBH5 during the short residence time of methylated mRNAs in the nucleus creates a dynamic methylated transcriptome and alters expression of specific genes is currently unclear.

3. Importance of m^{6}A modification in T cells

A few reports have already investigated the impact of the m^{6}A system in T cells, mainly focusing on mouse loss–of–function models of writer proteins, including Mettl3, Mettl14, Wtap and Virma, in which they studied T cell activation, regulation, differentiation and survival. In this section we will review the emerging m^{6}A functions in T cell biology and underlying molecular mechanisms and discuss how they shape T cell-mediated immune responses.

4. Loss of m^{6}A causes aberrant T cell functions and T cell-driven pathology

Several studies have demonstrated that m^{6}A depletion by genetic inactivation of *Mettl3*[52], *Mettl14*[53] or *Wtap*[54] specifically in regulatory T cells (Treg) causes systemic autoinflammatory disease and colitis. Under normal conditions, inflammation of the colon is prevented through a tight balance in which conventional CD4$^+$ T cells that are reactive to microbial antigens of the gut are suppressed by Treg cells. However, once an imbalance allows the conventional T cells to become more activated, the production and release of inflammatory cytokines occurs and colitis can develop (Fig. 2). An early report suggested that m^{6}A mRNA methylation may sustain suppressive functions of Treg cells through destabilization of mRNAs encoding for Socs gene family members, including Cish, Socs1, Socs3, Asb2 [52], which were marked by m^{6}A [55]. In this mechanistic explanation the elevated expression of Socs family proteins after Mettl3 depletion inhibits the interleukin (IL)-2/STAT5 signaling pathway, which weakens Treg cell functions as these strongly depend on IL-2 signaling. Interestingly, the very same molecular mechanism served as explanation for the reported observation that *Mettl3*-deficient conventional T cells appeared to be locked into a naive state [55], although the activation of conventional T cells is much less cytokine-dependent.

Also mice lacking Mettl14 [53] or Wtap [54] in all CD4$^+$ T cells develop autoinflammation and colitis at young age. Interestingly, these mice exhibit a reduced abundance of induced Treg cells in the gut, which are defined by Rorγt$^+$Helios$^-$ marker expression and are critical players in the prevention of gut inflammation. Accordingly, these mice showed spontaneous activation of the conventional CD4$^+$ T cells (Fig. 2). These results underscore the importance of m^{6}A for Treg cell function and their ability to suppress the activation of conventional T cells.

The development of colitis requires the combination of reduced Treg cell function and the ability of conventional CD4$^+$ T cells to become activated in peripheral tissues, since colitis is caused by activated conventional CD4$^+$ T cells [56]. It was therefore unclear how conventional CD4$^+$ T cells lacking m^{6}A modifications could contribute to the colitis phenotype, when the methyltransferase complex components were conditionally inactivated in regulatory as well as conventional T cells, since the mentioned results suggested a functional inactivation of both cell types at the same time [55]. This question has been puzzling researchers, and is still not entirely solved, since conventional T cells are strongly affected by m^{6}A deficiency and cannot be easily studied in experimental disease models [55,57].

Importantly, in the short term, the transfer of the *Mettl3*-knockout CD4$^+$CD45Rb^{hi} T cells into *Rag2*-deficient mice was not able to cause colitis because the few cells that could be re-isolated from these lymphopenic hosts still exhibited the CD45Rb^{hi} phenotype, which was interpreted as an impairment to become activated [55]. Based on this absence of activated T cells after adoptive transfer into lymphopenic

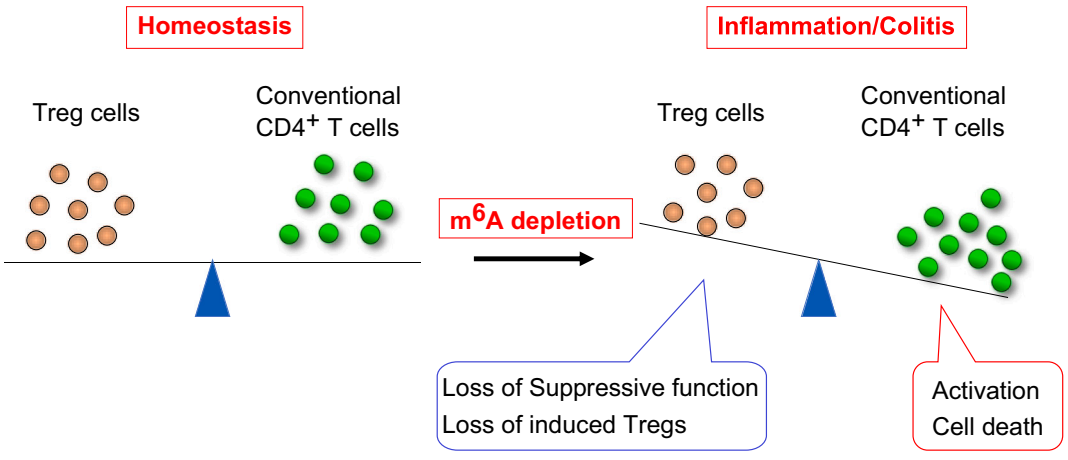

Fig. 2. m^{6}A is required for the balance of conventional T cells and Treg cells and in the prevention an inflammation and colitis.
Under normal conditions, gene regulation through m^{6}A in Treg cells is required for the prevention of an inflammation. m^{6}A depletion in Treg cells impairs their suppressive function and the peripheral differentiation of induced Treg cells (iTreg) in the gut, thereby causing an inflammation and colitis. Conversely, m^{6}A depletion in peripheral T cells causes activation of conventional T cells and TCR-activation induced cell death.

hosts and an increase in frequencies of naive T cells at steady state in mice lacking Mettl3 expression in peripheral T cells, the early work concluded that there was a block of activation in conventional CD4$^+$ T cells. However, the quantification of naive T cells lacking m^{6}A marks is complicated due to an apparent downregulation of the CD44 marker only in CD62^{hi} T cells in the secondary lymphoid organs, which can be seen in the published analyses [54,55].

Most recently, our group has provided evidence that m^{6}A has a crucial role in antigen receptor (TCR) signaling and survival of T cells [54]. Furthermore, works from other groups showed an importance of m^{6}A for follicular helper T cell (T_{FH}) differentiation [58,59]. In these studies of mice with m^{6}A-depletion, either caused by *Wtap*- or *Mettl3*-deficiency in peripheral T cells, the conventional CD4$^+$ T cells became activated upon TCR stimulation, while their differentiation and survival was severely impaired [54,58]. In contrast, another study shows that shRNA-mediated depletion of METTL3 or METTL14 in SMARTA TCR transgenic CD4$^+$ T cells enhances T_{FH} development when these cells are adoptively transferred into B6 host mice followed by LCMV infection [59]. This difference in phenotype may relate to the different experimental systems by ablating or reducing the targets at different developmental stages in a conditional knockout mouse model or in an shRNA knockdown approach. Indeed, for *Wtap*-deficient T cells evidence was presented that the m^{6}A methyltransferase complex is dispensable for the persistence of naive T cells but becomes essential for the survival of activated T cells stimulated through their TCR.

A major question was why m^{6}A-depleted CD4$^+$ T cells do not expand after adoptive transfers into *Rag*-deficient mice? In these lymphopenic hosts adoptively transferred T cells exhibit two distinct proliferation patterns, one pool of cells shows slow homeostatic expansion in response to IL-7 and the other one proliferates fast and progressively after TCR-dependent recognition of microbial antigens in the gut [60]. Proliferation-dye-labelled transfers of wild-type and Wtap-deficient CD4$^+$ T cells into *Rag1*-KO mice showed that both populations of proliferating cells, either induced by IL-7 or TCR signaling, were present and even displayed equal numbers of cell divisions for m^{6}A-sufficient and deficient T cells. Unexpectedly, the IL-7 induced proliferating cells were not reduced for the *Wtap*-knockout genotype. Instead these mice showed a strongly diminished pool of cells responding to TCR signaling. Further experiments led to the interpretation that TCR signaling in *Wtap*-deficient T cells caused strong activation induced death. For example, T cell activation by anti-CD3/anti-CD28 antibodies caused cell death of *Wtap*-deficient CD4$^+$ T cells, although neither Wtap nor Mettl3 depletion exerted a strong impact on proliferation. Consistently, the cell numbers of Mettl3-depleted CD4$^+$ T cells expressing SMARTA TCR transgene were shown to be decreased upon lymphocytic choriomeningitis virus (LCMV) infection [58]. These data suggest a role for m^{6}A in TCR signaling and activation-induced cell death. Of note, there is the difficulty to detect dead cells in vivo, since these are rapidly cleared by macrophages. Nevertheless, the m^{6}A-depleted CD4$^+$ T cells persist as naive cells, respond to IL-7, are initially able to proliferate upon TCR stimulation, but eventually die and disappear. In conclusion, the limited life-span and too low frequency is therefore one possible explanation for the failure of a single adoptive transfer of m^{6}A-deficient CD4$^+$ T cells into lymphopenic hosts to recapitulate the colitis observed in adult mice with genetic inactivation of m^{6}A methyltransferase complex components in all T cells.

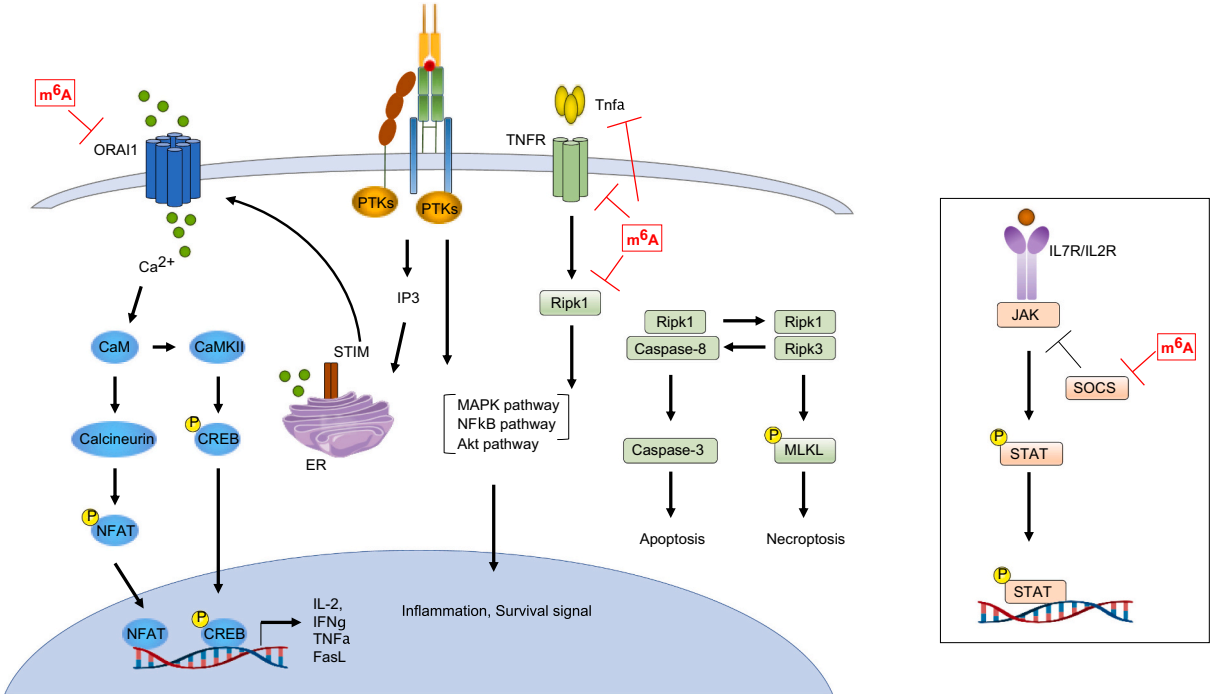

Fig. 3. m^{6}A mediated regulation of TCR and cytokine signaling.
Schematic representation of m^{6}A regulated signaling pathways in T cells. Upon TCR stimulation Ca^{2+} release from the endoplasmic reticulum induces the activation of stromal interaction molecules (STIM), which in turn activates ORAI1, the main plasma membrane channel for Ca^{2+} influx into lymphocytes. Uptake of extracellular Ca^{2+} ultimately induces gene expression of cytokines and FasL. In the humoral context of the T cell TNF tumor necrosis factor (TNF) binds to the tumor necrosis factor receptor superfamily (TNFRSF) and leads to the assembly of the TNFR-associated signaling complexes. This complex triggers the activation of MAPK/AP1 and NF-κB cascade, which regulates gene expression of genes involved in inflammation and survival. Dynamic changes of TNFR-associated signaling complexes, including Ripk1, Ripk3 and Caspase-8, can trigger a switch from inflammation and survival to cell death via apoptosis or necroptosis. Additional cytokine signals from IL-7 or IL-2 by binding to the cytokine receptors, IL-7R or IL-2R, result in dimerization of the receptors and activation of Janus kinases (JAKs), which in turn phosphorylate signal transducer and activator of transcription proteins (STATs). The induced translocation of STATs to the nucleus initiates the transcription of target genes. Suppressor of cytokine signaling (SOCS) family proteins can inhibit the activation of JAK. Inhibitory (blunt) arrows indicate that transcripts encoding for Orai1, Ripk1, Tnfα, Tnfr-II and SOCS proteins are m^{6}A-modified, suggesting a reduced expression and impaired function.

5. m^{6}A-regulated gene expression in T cells

T cell differentiation, effector function and survival depend on regulated gene expression, which is induced due to T cell activation via the antigen receptor and cytokine receptors. These pathways involve mitogen-activated protein kinase MAPK/AP1, NF-κB and Ca^{2+}/NFAT or JAK-STAT signaling cascades, respectively (Fig. 3) [61]. Several of these signaling pathways have already been shown to be regulated by m^{6}A modification. mRNA-sequencing of Mettl3- or Wtap-depleted CD4$^+$ T cells showed an enrichment of cytokine, TCR, MAPK and NF-κB signaling GO terms [54,55,58]. In Mettl3- or Wtap-depleted T cells, Stat5 phosphorylation after IL-7 stimulation was reduced. This impairment correlated with upregulation of inhibitors of cytokine signaling, the m^{6}A-modified *Socs* gene family member mRNAs *Cish*, *Socs1* and *Socs3* (Fig. 3) [55]. However, this inhibition did not significantly impact on IL-7 induced proliferation in vitro or *in vivo*[54]. In addition, phosphorylation of ERK and NF-κB was upregulated in the Mettl3-depleted CD4$^+$ T cells [55]. Wtap depletion in CD4$^+$ T cells enhanced and sustained Ca^{2+} signaling when the cells were stimulated through their TCR or different pharmacologic stimuli that cause store-operated calcium entry (SOCE) [54]. Importantly the transcript encoding for Orai1, the main plasma membrane channel for Ca^{2+} influx in lymphocytes was m^{6}A-modified and stabilized, resulting in upregulation of the protein expression upon ablation of Wtap (Fig. 3). Overload of Ca^{2+} influx can lead to cell death [62], while ORAI1 deficiency in CD4$^+$ T cells showed reduced activation-induced cell death (AICD) and increased survival [63]. Wtap depletion changes the phosphorylation status of NFAT and CREB downstream of Ca^{2+} signaling, and induces expression of transcription factors of Egr family members and c-Fos. Ultimately the elevated Ca^{2+}/NFAT/Egr activation in CD4$^+$ T cells with Wtap depletion correlated with increased FasL expression after anti-CD3/anti-CD28 stimulation. Indeed Egr2 and/or Egr3 were shown to directly induce expression of SOCS1 and SOCS3 [64], suggesting a Ca^{2+}-dependent indirect contribution to the increased expression of SOCS genes, which are also m^{6}A-modified [55]. Although the increased FasL may be responsible for causing AICD, an apoptosis inhibitor could not rescue the survival of Wtap-depletion CD4$^+$ T cells. This pointed at alternative pathways contributing to cell death in TCR–stimulated Wtap-depleted CD4$^+$ T cells.

Ripk1 is a key mediator of cell death and inflammation, which is activated through TNF-TNFR signaling (Fig. 3) [65]. While Ripk1 functions as a scaffold protein to induce NF-κB signaling, it can also trigger either apoptosis or necroptosis depending on Caspase-8 activity [66]. The mRNAs encoding for Ripk1, Tnfα and Tnfr-II (Tnfrsf1b) are m^{6}A-modified and Ripk1 expression is upregulated after Wtap depletion in CD4$^+$ T cells [54], which correlated with enhanced activation of NF-κB in Mettl3-depleted CD4$^+$ T cells [55]. On the other hand, a specific inhibitor of Ripk1 (Nec-1) partially rescued cell death of TCR-stimulated Wtap-deficient CD4$^+$ T cells. Importantly, combinatorial treatment with an apoptosis inhibitor (zVAD-fmk) and Nec-1 synergized to increase viability of Wtap-deficient CD4$^+$ T cells. Moreover, overexpression of Ripk1 and Orai1 reduced the viability of T cells in the context of TCR stimulation [54]. These findings suggest that m^{6}A ablation invokes AICD which results from dysregulated signaling pathways that are related to the upregulation of Orai1 and Ripk1. How these and other m^{6}A targets work together in the control of T cell biology requires much further investigation. However, these findings already highlight a decisive role of m^{6}A in the TCR response.

Interestingly, GO terms of anti-viral, innate immune response and response to interferon (IFN)-β are also appreciably enriched in the upregulated genes when m^{6}A-mediated regulation is disrupted [54,58]. It has also become clear that cells of the adaptive immune system also have sensing functions for pathogen-associated molecular patterns which have been more intensively studied in innate immune cells. For example, MDA5 [67] or LGP2 [68] deficiency is associated with functional defects in CD8$^+$ T cells, resulting in a failure to clear West Nile virus. TCR stimulation of CD4$^+$ T cells increases TLR-3 expression and treatment of CD4$^+$ T cells with the dsRNA synthetic analog poly(I:C) activates NF-κB signaling [69]. These evidences suggest that dsRNA can be sensed by T cells, and dsRNAs often accumulate during viral infection as a result of viral RNA replication. Several studies have reported that viral infection triggers a massive increase in m^{6}A in both host and viral mRNAs [70,71]. Indeed infection with the RNA virus Vesicular Stomatitis Virus (VSV) has been shown to increase m^{6}A modification in virus-derived transcripts and decrease viral dsRNA formation [72]. Genetic ablation of METTL3 in monocytes or hepatocytes causes enhanced type I IFN expression and accelerates VSV clearance. A recent study shows a role of m^{6}A modification in the prevention of aberrant endogenous dsRNA formation [73]. Loss of METTL3 activated innate immune responses that were mediated through endogenous RNAs engaging in extensive base-pairing and dsRNA formation, which was partially rescued by Mavs and RNaseI inactivation. These findings suggest that m^{6}A modification in general counteracts the ability of cellular RNAs to form endogenous dsRNAs, which is essential to prevent spontaneous and deleterious innate immune responses. It will be interesting to investigate whether or to which extent endogenous dsRNAs sensing and the activation of innate signaling contributes to physiologic T cell responses and effector functions.

Altogether, the current data sets have revealed an involvement of m^{6}A in TCR, cytokine and innate immune pathways, and their coinciding deregulation profoundly alters T cell biology. Future studies with selective targeting of specific reader proteins or m^{6}A-regulated *cis*-elements are now required to dissect how m^{6}A impacts on individual targets and pathways to control specific T cell fate decisions.

6. Concluding remarks

In recent years, the field of epitranscriptomic has been advancing at an enormous speed, and already several studies have reported the importance of m^{6}A in T cells. However, current concepts and regulatory mechanisms are still immature and require further confirmation, adjustment or clarification.

One very important question that has already been answered for CD4$^+$ T cells is: Where are m^{6}A modifications positioned on the cellular transcripts? This issue has been addressed via m^{6}A RNA-immunoprecipitation or m^{6}A antibody crosslink to mRNA in vitro or Ythdf2 protein crosslink to RNA in cells, combined with immunoprecipitation and RNA sequencing. Bioinformatic analyses revealed a good overlap for the identified targets, and the binding sites have been defined at near-nucleotide resolution. These data now serve as a valuable resource to inspire new hypotheses and uncover important post-transcriptional regulation.

Similar to studying the function of miRNAs by blunting their entire biogenesis via genetic inactivation of Dicer in T cells [74], the inactivation of m^{6}A writer complex components yields in strong and compound effects. Future analyses need to deconvolute contributions from specific mRNAs, readers and regulatory mechanisms.

Future research should also solve controversies and open questions: Why does the targeting of different writer complex components agree on many phenotypes and targets, but also display inconsistent or even opposite phenotypes. For example, why is *Cd4-cre*–mediated inactivation of *Mettl3* not leading to the profound spontaneous activation of CD4$^+$ and CD8$^+$ T cells, as observed for the comparable genetic inactivation of *Wtap* or *Virma*. Can these differences be explained by different deletion efficiencies and residual m^{6}A levels?

A recent study also involved a role for the m^{6}A eraser protein Alkbh5 in αβ and γδT cells [75,76]. Genetic deletion of *Alkbh5* rendered CD4$^+$ T cells unable to cause colitis and mice with Alkbh5 deficiency in T cells or lymphocytes were resistant to experimental induction of autoimmune encephalomyelitis or gastrointestinal *Salmonella typhimurium* infection, respectively. Alkbh5 deficiency in T cells was shown to affect the homing of CD4$^+$ T cells as well as the recruitment of neutrophils into

organs by altering the expression of the transcripts encoding IFNγ and Cxcl10 as well as Cxcl2. In addition, Alkbh5 depletion in lymphocytes specifically induces an expansion of γδT cells following downregulation of *Jagged1* and *Notch2*. Although these are intriguing findings, it is currently unclear whether the nuclear Alkbh5 protein affects quantitively or qualitatively the cytoplasmic m^6A marks, and whether and how it controls transcript splicing, half-life or translation.

Additional open questions include: How does posttranslational modification of writers and readers or even of the erasers contribute to m^6A function as, for example, Ythdf proteins showed strong upregulation in $CD4^+$ T cells after TCR or PMA stimulation. It is interesting that *Ythdf1*-deficient mice show an elevation of antigen-specific $CD8^+$ T cells and anti-tumor responses [77]. These antitumour responses were $CD8^+$ T cells extrinsic and explained by increased cross-priming from *Ythdf1*-deficient dendritic cells. Further investigations will have to address T cell intrinsic Ythdf1, Ythdf2 and Ythdf3 functions and elucidate possible redundant roles. These issues need to be considered and further assessed by future research. Moreover, which reader proteins work redundantly, cooperate or serve compartmentalized functions, and which environmental cues regulate them to cause differential expression or activity? Overall, future investigations of m^6A-mediated gene regulation need to carefully consider and experimentally reflect the context including cell type, differentiation stage, activation status and the involved stimulus or stress response. Furthermore, to discriminate acute from developmental effects the use of sophisticated inducible conditional gene inactivation may be crucial.

Although RNA modification and epitranscriptomics is a young field, it has already evidenced a tremendous impact on T cells. Many more findings and discoveries can be expected in the future, since these cells make sophisticated use of all layers of gene regulation to integrate the multitude of signals in their development and the generation of context-dependent appropriate immune responses, while avoiding over-activation and self-reactivity.

CRediT authorship contribution statement

Taku Ito-Kureha: Conceptualization, Writing-original draft, Preparing Figures, Writing – review & editing. Vigo Heissmeyer: review & editing. All authors read and approved the final version of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

Acknowledgements

This work was supported by the German Research Foundation grants HE3359/8-1 (#444891219), HE3359/7-1 (#432656284) and SFB1054 (project A03, #210592381), SPP 1935 (#313381103) and TRR338 (project C02, #452881907) to V.H.

References

[1] R. Elkon, A.P. Ugalde, R. Agami, Alternative cleavage and polyadenylation: extent, regulation and function, Nat. Rev. Genet. 14 (2013) 496–506.
[2] N.H. Gehring, J.Y. Roignant, Anything but ordinary - emerging splicing mechanisms in eukaryotic gene regulation, Trends Genet. 37 (2021) 355–372.
[3] M.W. Hentze, A. Castello, T. Schwarzl, T. Preiss, A brave new world of RNA-binding proteins, Nat. Rev. Mol. Cell Biol. 19 (2018) 327–341.
[4] L.F.R. Gebert, I.J. MacRae, Regulation of microRNA function in animals, Nat. Rev. Mol. Cell Biol. 20 (2019) 21–37.
[5] S. Zaccara, R.J. Ries, S.R. Jaffrey, Reading, writing and erasing mRNA methylation, Nat. Rev. Mol. Cell Biol. 20 (2019) 608–624.
[6] N. Liu, et al., N(6)-methyladenosine-dependent RNA structural switches regulate RNA-protein interactions, Nature 518 (2015) 560–564.
[7] N. Liu, et al., N6-methyladenosine alters RNA structure to regulate binding of a low-complexity protein, Nucleic Acids Res. 45 (2017) 6051–6063.
[8] B. Wu, et al., Molecular basis for the specific and multivariant recognitions of RNA substrates by human hnRNP A2/B1, Nat. Commun. 9 (2018) 420.
[9] X. Wang, et al., N6-methyladenosine-dependent regulation of messenger RNA stability, Nature 505 (2014) 117–120.
[10] D. Theler, C. Dominguez, M. Blatter, J. Boudet, F.H. Allain, Solution structure of the YTH domain in complex with N6-methyladenosine RNA: a reader of methylated RNA, Nucleic Acids Res. 42 (2014) 13911–13919.
[11] B. Slobodin, et al., Transcription impacts the efficiency of mRNA translation via co-transcriptional N6-adenosine methylation, Cell 169 (2017) 326–337, e312.
[12] R.B. Darnell, S. Ke, J.E. Darnell Jr., Pre-mRNA processing includes N(6) methylation of adenosine residues that are retained in mRNA exons and the fallacy of "RNA epigenetics", RNA 24 (2018) 262–267.
[13] J. Liu, et al., A METTL3-METTL14 complex mediates mammalian nuclear RNA N6-adenosine methylation, Nat. Chem. Biol. 10 (2014) 93–95.
[14] X.L. Ping, et al., Mammalian WTAP is a regulatory subunit of the RNA N6-methyladenosine methyltransferase, Cell Res. 24 (2014) 177–189.
[15] P. Knuckles, et al., Zc3h13/Flacc is required for adenosine methylation by bridging the mRNA-binding factor Rbm15/Spenito to the m(6)A machinery component Wtap/Fl(2)d, Genes Dev. 32 (2018) 415–429.
[16] Y. Yue, et al., VIRMA mediates preferential m(6)A mRNA methylation in 3'UTR and near stop codon and associates with alternative polyadenylation, Cell Discov. 4 (2018) 10.
[17] P. Bawankar, et al., Hakai is required for stabilization of core components of the m(6)A mRNA methylation machinery, Nat. Commun. 12 (2021) 3778.
[18] P. Wang, K.A. Doxtader, Y. Nam, Structural basis for cooperative function of Mettl3 and Mettl14 methyltransferases, Mol. Cell 63 (2016) 306–317.
[19] X. Wang, et al., Structural basis of N(6)-adenosine methylation by the METTL3-METTL14 complex, Nature 534 (2016) 575–578.
[20] D. Dominissini, et al., Topology of the human and mouse m6A RNA methylomes revealed by m6A-seq, Nature 485 (2012) 201–206.
[21] Y. Fu, D. Dominissini, G. Rechavi, C. He, Gene expression regulation mediated through reversible m6A RNA methylation, Nat. Rev. Genet. 15 (2014) 293–306.
[22] K.D. Meyer, et al., Comprehensive analysis of mRNA methylation reveals enrichment in 3' UTRs and near stop codons, Cell 149 (2012) 1635–1646.
[23] M.A. Garcia-Campos, et al., Deciphering the "m(6)A Code" via antibody-independent quantitative profiling, Cell 178 (2019) 731–747, e716.
[24] K. Boulias, et al., Identification of the m(6)Am methyltransferase PCIF1 reveals the location and functions of m(6)Am in the transcriptome, Mol. Cell 75 (2019) 631–643, e638.
[25] E. Sendinc, et al., PCIF1 catalyzes m6Am mRNA methylation to regulate gene expression, Mol. Cell 75 (2019) 620–630, e629.
[26] A.V. Grozhik, S.R. Jaffrey, Distinguishing RNA modifications from noise in epitranscriptome maps, Nat. Chem. Biol. 14 (2018) 215–225.
[27] H.L. Sun, et al., Stabilization of ERK-phosphorylated METTL3 by USP5 increases m(6)A methylation, Mol. Cell 80 (2020) 633–647, e637.
[28] Y. Du, et al., SUMOylation of the m6A-RNA methyltransferase METTL3 modulates its function, Nucleic Acids Res. 46 (2018) 5195–5208.
[29] H. Du, et al., YTHDF2 destabilizes m(6)A-containing RNA through direct recruitment of the CCR4-NOT deadenylase complex, Nat. Commun. 7 (2016) 12626.
[30] X. Wang, et al., N(6)-methyladenosine modulates messenger RNA translation efficiency, Cell 161 (2015) 1388–1399.
[31] H. Shi, et al., YTHDF3 facilitates translation and decay of N(6)-methyladenosine-modified RNA, Cell Res. 27 (2017) 315–328.
[32] A. Li, et al., Cytoplasmic m(6)A reader YTHDF3 promotes mRNA translation, Cell Res. 27 (2017) 444–447.
[33] S. Zaccara, S.R. Jaffrey, A unified model for the function of YTHDF proteins in regulating m(6)A-modified mRNA, Cell 181 (2020) 1582–1595, e1518.
[34] J. Zhou, et al., Dynamic m(6)A mRNA methylation directs translational control of heat shock response, Nature 526 (2015) 591–594.
[35] R.J. Ries, et al., m(6)A enhances the phase separation potential of mRNA, Nature 571 (2019) 424–428.
[36] R.R. Edupuganti, et al., N(6)-methyladenosine (m(6)A) recruits and repels proteins to regulate mRNA homeostasis, Nat. Struct. Mol. Biol. 24 (2017) 870–878.
[37] H. Huang, et al., Recognition of RNA N(6)-methyladenosine by IGF2BP proteins enhances mRNA stability and translation, Nat. Cell Biol. 20 (2018) 285–295.
[38] J.Y. Youn, et al., High-density proximity mapping reveals the subcellular organization of mRNA-associated granules and bodies, Mol. Cell 69 (2018) 517–532, e511.
[39] W. Xiao, et al., Nuclear m(6)A reader YTHDC1 regulates mRNA splicing, Mol. Cell 61 (2016) 507–519.
[40] I.A. Roundtree, et al., YTHDC1 mediates nuclear export of N(6)-methyladenosine methylated mRNAs, elife 6 (2017).
[41] I. Rafalska, et al., The intranuclear localization and function of YT521-B is regulated by tyrosine phosphorylation, Hum. Mol. Genet. 13 (2004) 1535–1549.
[42] S. Ke, et al., m(6)A mRNA modifications are deposited in nascent pre-mRNA and are not required for splicing but do specify cytoplasmic turnover, Genes Dev. 31 (2017) 990–1006.

[43] S. Geula, et al., Stem cells. m6A mRNA methylation facilitates resolution of naïve pluripotency toward differentiation, Science 347 (2015) 1002–1006.
[44] Y. Li, et al., N(6)-methyladenosine co-transcriptionally directs the demethylation of histone H3K9me2, Nat. Genet. 52 (2020) 870–877.
[45] D.P. Patil, et al., m(6)A RNA methylation promotes XIST-mediated transcriptional repression, Nature 537 (2016) 369–373.
[46] P.J. Hsu, et al., Ythdc2 is an N(6)-methyladenosine binding protein that regulates mammalian spermatogenesis, Cell Res. 27 (2017) 1115–1127.
[47] M.N. Wojtas, et al., Regulation of m(6)A transcripts by the 3'→5' RNA helicase YTHDC2 is essential for a successful meiotic program in the mammalian germline, Mol. Cell 68 (2017) 374–387, e312.
[48] C. Xu, et al., Structural basis for selective binding of m6A RNA by the YTHDC1 YTH domain, Nat. Chem. Biol. 10 (2014) 927–929.
[49] G. Jia, et al., N6-methyladenosine in nuclear RNA is a major substrate of the obesity-associated FTO, Nat. Chem. Biol. 7 (2011) 885–887.
[50] G. Zheng, et al., ALKBH5 is a mammalian RNA demethylase that impacts RNA metabolism and mouse fertility, Mol. Cell 49 (2013) 18–29.
[51] J. Mauer, et al., Reversible methylation of m(6)A(m) in the 5' cap controls mRNA stability, Nature 541 (2017) 371–375.
[52] J. Tong, et al., m(6)A mRNA methylation sustains treg suppressive functions, Cell Res. 28 (2018) 253–256.
[53] T.X. Lu, et al., A new model of spontaneous colitis in mice induced by deletion of an RNA m(6)A methyltransferase component METTL14 in T cells, Cell. Mol. Gastroenterol. Hepatol. 10 (2020) 747–761.
[54] T. Ito-Kureha, et al., The function of Wtap in N6-adenosine methylation of mRNAs controls T cell receptor signaling and survival of T cells, Nat. Immunol. 23 (2022) 1208–1221.
[55] H.B. Li, et al., m(6)A mRNA methylation controls T cell homeostasis by targeting the IL-7/STAT5/SOCS pathways, Nature 548 (2017) 338–342.
[56] T. Imam, S. Park, M.H. Kaplan, M.R. Olson, Effector T helper cell subsets in inflammatory bowel diseases, Front. Immunol. 9 (2018) 1212.
[57] J. Sprent, C.D. Surh, Writer's block: preventing m(6)A mRNA methylation promotes T cell naivety, Immunol. Cell Biol. 95 (2017) 855–856.
[58] Y. Yao, et al., METTL3-dependent m(6)A modification programs T follicular helper cell differentiation, Nat. Commun. 12 (2021) 1333.
[59] Y. Zhu, et al., The E3 ligase VHL promotes follicular helper T cell differentiation via glycolytic-epigenetic control, J. Exp. Med. 216 (2019) 1664–1681.
[60] W.C. Kieper, et al., Recent immune status determines the source of antigens that drive homeostatic T cell expansion, J. Immunol. 174 (2005) 3158–3163.
[61] O. Acuto, V. Di Bartolo, F. Michel, Tailoring T-cell receptor signals by proximal negative feedback mechanisms, Nat. Rev. Immunol. 8 (2008) 699–712.
[62] P. Pinton, C. Giorgi, R. Siviero, E. Zecchini, R. Rizzuto, Calcium and apoptosis: ER-mitochondria Ca2+ transfer in the control of apoptosis, Oncogene 27 (2008) 6407–6418.
[63] K.D. Kim, et al., ORAI1 deficiency impairs activated T cell death and enhances T cell survival, J. Immunol. 187 (2011) 3620–3630.
[64] S. Li, et al., The transcription factors Egr2 and Egr3 are essential for the control of inflammation and antigen-induced proliferation of B and T cells, Immunity 37 (2012) 685–696.
[65] M. Pasparakis, P. Vandenabeele, Necroptosis and its role in inflammation, Nature 517 (2015) 311–320.
[66] B. Tummers, D.R. Green, Caspase-8: regulating life and death, Immunol. Rev. 277 (2017) 76–89.
[67] H.M. Lazear, et al., Pattern recognition receptor MDA5 modulates CD8+ T cell-dependent clearance of West Nile virus from the central nervous system, J. Virol. 87 (2013) 11401–11415.
[68] M.S. Suthar, et al., The RIG-I-like receptor LGP2 controls CD8(+) T cell survival and fitness, Immunity 37 (2012) 235–248.
[69] A.E. Gelman, J. Zhang, Y. Choi, L.A. Turka, Toll-like receptor ligands directly promote activated CD4+ T cell survival, J. Immunol. 172 (2004) 6065–6073.
[70] G. Lichinchi, et al., Dynamics of the human and viral m(6)A RNA methylomes during HIV-1 infection of T cells, Nat. Microbiol. 1 (2016) 16011.
[71] N. Tirumuru, L. Wu, HIV-1 envelope proteins up-regulate N(6)-methyladenosine levels of cellular RNA independently of viral replication, J. Biol. Chem. 294 (2019) 3249–3260.
[72] W. Qiu, et al., N(6)-methyladenosine RNA modification suppresses antiviral innate sensing pathways via reshaping double-stranded RNA, Nat. Commun. 12 (2021) 1582.
[73] Y. Gao, et al., m(6)A modification prevents formation of endogenous double-stranded RNAs and deleterious innate immune responses during hematopoietic development, Immunity 52 (2020) 1007–1021, e1008.
[74] S.A. Muljo, et al., Aberrant T cell differentiation in the absence of dicer, J. Exp. Med. 202 (2005) 261–269.
[75] J. Zhou, et al., m(6)A demethylase ALKBH5 controls CD4(+) T cell pathogenicity and promotes autoimmunity, Sci. Adv. 7 (2021).
[76] C. Ding, et al., RNA m(6)A demethylase ALKBH5 regulates the development of γδ T cells, Proc. Natl. Acad. Sci. U. S. A. 119 (2022), e2203318119.
[77] D. Han, et al., Anti-tumour immunity controlled through mRNA m(6)A methylation and YTHDF1 in dendritic cells, Nature 566 (2019) 270–274.