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Effect of M6A methylation on T cell homeostasis

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Abstract. The m6A methylation modification is a prevalent form of RNA modification in organisms, exerting its influence on gene expression. In recent years, there has been a gradual elucidation of the relationship between the physiological functions of m6A methylation modification and immune regulation, obesity, as well as malignant diseases such as tumors. Consequently, the study of m6A methylation modification has emerged as a focal point in current molecular biology research. This research focuses on the interaction between immune system homeostasis and m6A methylation alteration and the human immune system. It also describes the significant effects of three crucial regulatory proteins required for m6A methylation modification on the growth and differentiation of immune cells (T cells). This research provides a novel direction for future studies in immunology and disease pathology. However, there are still some research difficulties, for instance, a number of methyltransferases, demethylases, and recognition proteins control the alteration of m6A methylation, and the interaction and regulatory network among these proteins are not clear, so future research can focus on this direction.

Keywords: M6A methylation, T cell Homeostasis, IL-7/STAT5/SOCS signaling pathway, Th cell, Tregs.

1. Introduction

M6A methylation modification is one of the key internal modifications of RNA. Through altering mRNA binding and protein reading, it controls mRNA stability, translation efficiency, and variable splicing, which in turn modifies gene expression. In eukaryotes, it is a conserved post-transcriptional system that modifies and enhances genetic information. Interest in academic research on RNA modification has surged in recent years due to technological breakthroughs, such as the development of highly sensitive mass spectrometry for accurate detection of modifications and the invention of whole-transcriptome sequencing methods for mapping modification sites. It has been discovered that three crucial regulatory proteins, namely "Writer," "Eraser," and "Reader," are essential for m6A methylation modification and play a pivotal role in numerous post-transcriptional regulatory processes. Proteins like these play an important role in regulating processes including cell differentiation, embryonic development, and stress response in addition to speeding up cellular mRNA metabolism and translation. It has great potential research value in the fields of molecular biology, biochemistry and biomedicine. Studies have found that m6A methylation has a very important relationship with human immunity, The In 2017, scientists from Stanford University, Jinan University, and Yale University School of Medicine made a significant finding. They found that changes in m6A methylation can regulate T cell homeostasis. This discovery has ignited a heated global discussion among academics. This study examined the

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molecular mechanism by which the m6A methyltransferase Mettl3 system regulates T cell homeostasis by specifically inhibiting it in mice. It provides a new idea for the development of human immune drugs. Moreover, other studies have also shown that m6A methylation modification has guiding significance for cancer prevention and treatment. The main mechanism is that the low level of m6A caused by mutation or decreased expression of Mettl4 and Mettl3 can inhibit cancer through translation of regulatory factors that inhibit the activation of cancer signaling pathways [1]. Investigating the m6A methylation alteration can help us understand the development of many illnesses and develop targeted treatments at the molecular level. This paper provides a concise overview of the latest research findings on m6A methylation modification. It highlights the significant role of m6A in maintaining T cell homeostasis, and suggests that this knowledge could pave the way for new approaches in diagnosing, developing drugs for, and treating immune deficiency diseases, autoimmune diseases, and malignant tumors.

2. M6A mRNA methylation

2.1. The introduction of M^6A mRNA methylation

RNA methylation is the process of chemically modifying RNA methyladenine by selectively adding methyl groups with the help of methyltransferase enzymes. This alteration primarily occurs in the form of m6A methylation. Currently, m6A alteration has been detected on microRNA, circRNA, rRNA, tRNA, and snoRNA. The RRACH sequence is primarily influenced by m6A modification, which mostly targets adenine. The "Writer," "Eraser," and "Reader" proteins control how this change functions. Methyltransferase is responsible for adding methyl groups to molecules in this complex. Metatl3, mettl14, WTAP, and KIAA1429 are the building blocks of this complex. On the other hand, demethylases ALKBH5 and FTO have the ability to remove methyl groups and reverse methylation. A group of nuclear heterogeneous proteins called HNRNPA2B1 and HNRNPC, as well as YTH domain proteins such as YTHDF1, YTHDF2, YTHDF3, YTHDC1, and YTHDC2, have been recognized as m6A binding proteins that specifically interact with m6A. [2].

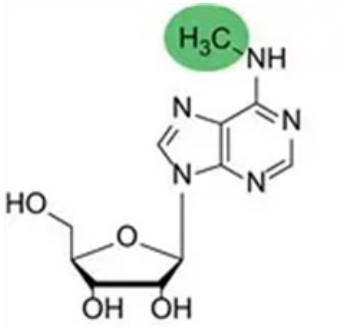

Figure 1. m6A mRNA methylation [2].

As shown in Fig 1, m6A inserts a methyl substituent into the N atom of adenosine 6. Note that m6A methylation occurs only at the head and tail of mRNA. Multiple steps in RNA metabolism include m6A methylation, including splicing, processing of microRNAs, translation, RNA degradation, nuclear export, and RNA processing. The whole process is shown below. Obviously the unmodified and modified RNA can add and remove methyl substituents under the action of methyltransferase "Writer" and demethylase "Eraser". Then the different functions of the extranuclear reading can recognize the methylated RNA and catalyze the mRNA nuclear output for subsequent translation.

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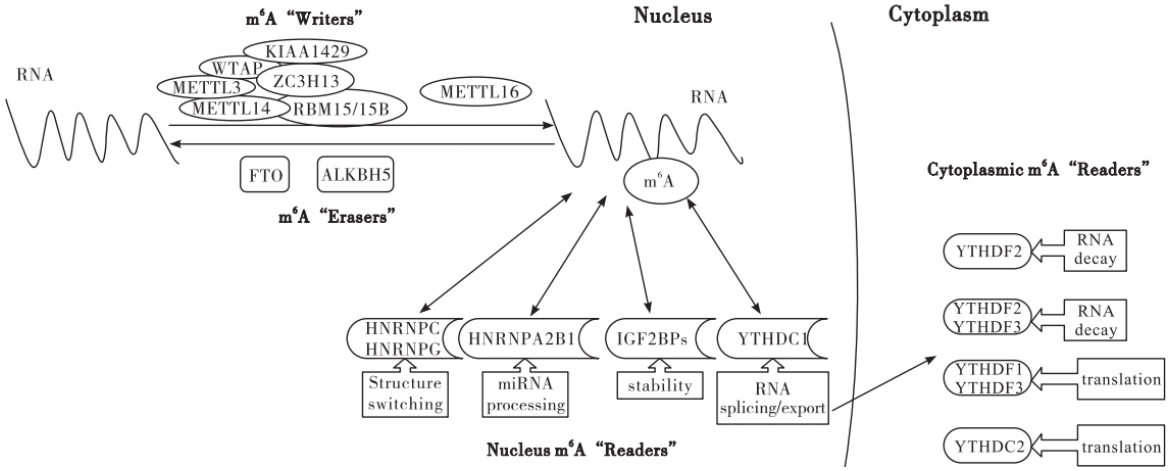

Figure 2. m6A mRNA methylation regulatory pattern diagram [11]

2.2. The importance of T cell homeostasis

T cells play a crucial role in the immune system by identifying and eliminating harmful infections and aberrant cells. Ensuring the equilibrium of T cells is crucial for the optimal performance of the immune system and the prevention of autoimmune conditions. The imbalance of T cell homeostasis may lead to diseases such as immune deficiency, autoimmune disease or malignant tumor.

2.3. The research significance

This study can reveal the mechanism of m6A methylation in T cell homeostasis and provide a new perspective for understanding T cell function and immune-related diseases. In order to develop innovative approaches for treating immune-related diseases, we aim to investigate the therapeutic potential of targeting m6A methylation. In addition, our objective is to further our comprehension of the influence of epigenetic control on T cell homeostasis by investigating the interaction between m6A methylation and other epigenetic changes.

3. mRNA methylation controls T cell homeostasis

T cell homeostasis is influenced by several variables, particularly various T cells. The purpose of this study is to investigate the effects of m6A methylation on several kinds of T cells, such as naive CD4+T cells, naive CD8+T cells, Th cells, and Treg cells. We discussed the mechanism behind the alteration of the m6A methylation process and its significance in relation to the maintenance of T cells.

3.1. M6A mRNA methylation and Naïve CD4+T cell homeostasis.

T cells derived from bone marrow stem cells, differentiate and mature in the thymus and migrate to the periphery to complete immune function. It possesses a diverse range of biological capabilities, including the direct elimination of target cells, the regulation or support of other immune cells in their duties, and the production of cytokines. These activities are crucial in exerting a dominating role in anti-tumor immunity. m6A methylation modification signals can control many aspects of T cell immunobiology, such as activation, proliferation, life, and death [3].

Studies have shown that m6A plays a crucial role in regulating the growth of immature CD4 T cells by selectively influencing the mRNA of the signaling protein involved in the first IL-7/STAT5/SOCS signaling pathway of CD4 T cells. This contributes to the preservation of the defense system's homeostasis [4]. METTL3, a crucial methyltransferase, has the ability to control the methylation modification of m6A RNA. Knocking down the Mettl3 gene specifically in CD4 T cells prevents the growth of Naive CD4+T cells. The variation in mRNA abundance of about 87% of genes is mainly determined by the rate of mRNA transcription, while a small number (about 13%) of genes are determined by the rate of degradation. This small number of genes are mainly early rapid induction

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genes, and most members of the SOCS family belong to early rapid induction genes induced by IL-7 stimulation. It has been shown that m6A in T cells can selectively target the SOCS protein family.

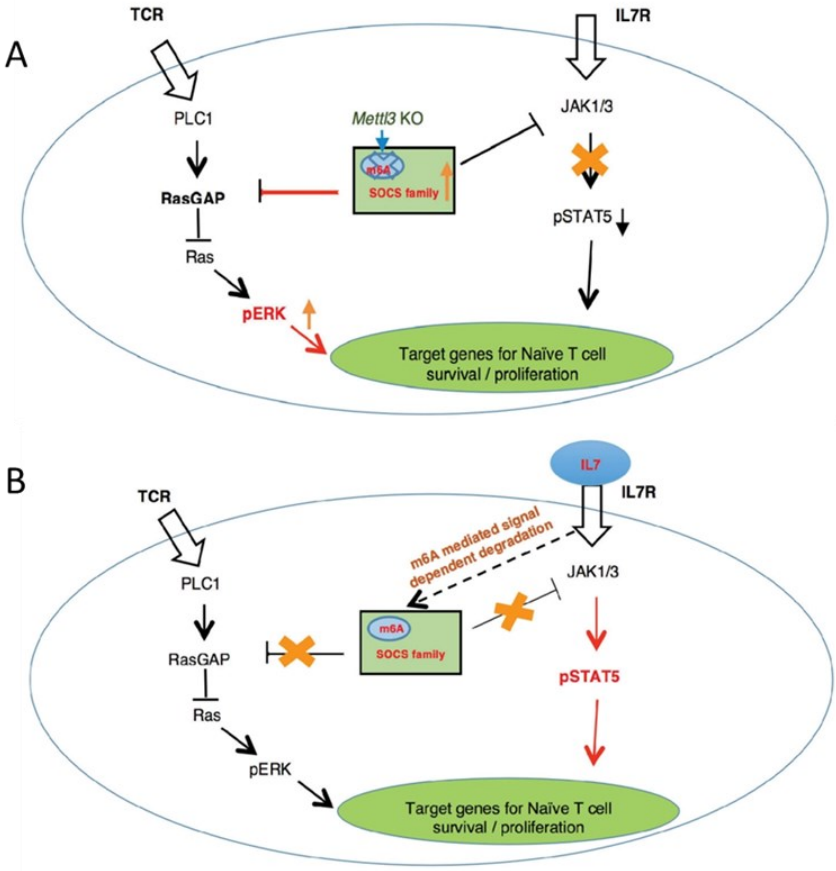

Figure 3. Modeling the regulation of Naive CD4+ T cell homeostasis by m6A [4]

Figure 3A illustrates the molecular mechanism of Mettl3 knockout in naive T cells. Lack of m6A led to a slowdown in the decomposition of SOCS mRNA and an increase in SOCS protein concentrations, eventually blocking the IL7 pathway. Figure 3B illustrates the revised model of T cell differentiation. m6A specifically breaks down the mRNA of SOCS1, SOCS3, and CISH when IL-7 stimulation occurs, which helps activate the downstream target STAT5 via IL-7-JAKs signaling. This process triggers the rewiring of immature T cells, stimulating their maturation and rapid increase in number [5].

3.2. M6A mRNA methylation and Naïve CD8+T cell homeostasis

CD8+T cells are cytotoxic T cells, which release perforin and granase to kill tumor cells after activation. Multiple studies have demonstrated a strong correlation between m6A methylation alteration and the invasion of CD8+T cells in tumors. There is no text provided. The expression of METTL14 in stromal cells of colon cancer exhibited a positive correlation with the amount of m6A and the degree of infiltration of CD8+T cells. In non-small cell lung cancer, the high expression of reading proteins YTHDF1 and YTHDF2 led to a significant increase in the degree of infiltration of lymphocyte subsets in tumor stroma, including CD8+T cells [6]. Similarly, in non-small cell lung cancer, LIU et al. found that the methyltransferase METTL3 can mediate the m6A modification of circ RNA circIGF2BP3 to promote its cyclization, and circIGF2BP3 can competitively up-regulate PKP3 through miR-328-3p and miR-3173-5p, reducing the infiltration of CD8+T cells. Inhibition of tumor immune response. Furthermore, mouse melanoma cells have the ability to hinder the activation of CD8+T cells by using

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the glycolytic route, which is facilitated by the demethylase FTO, in order to evade immune surveillance. Following the deletion of the FTO gene, there was a reduction in the glycolytic activity of tumor cells, and the function of CD8+T cells was restored [7].

3.3. M6A mRNA methylation and Helper T cell (Th)

Under the stimulation and regulation of various antigens and cytokines, the unactivated initial CD4+T cells differentiate into different types of Th cells, including Th1, Th2 and Th17 [8]. Among them, Th1 subtype can directly act on tumor cells by assisting cytotoxic CD8+T cells and B cells to exert anti-tumor function, and can also produce IFN-γ and TNF-α. Th2 subtype mainly secretes cytokines such as IL-4 and IL13, which can inhibit the killing effect of cytotoxic T cells. Moreover, m6A methylation can affect. The role of CD4+ T lymphocytes. Demethylase ALKBH5 can enhance the stability and translation of CXCL2 and IFN-γ mRNA by reducing the m6A level of CXCL2 and IFN-γ mRNA [9] and promote Th1 cell function. Below is the researchers' analysis of Th cells in mice with the specific methyltransferase knockout Mettl3. Figure 4A demonstrates the utilization of Western Blot analysis to assess The levels of expression of METTL3 and its related METTL14 in juvenile T cells and in vitro differentiated Th1, Th2, and Th17 cells. Figure 4B displays the proportion of CD4+ T cells in each T cell subtype, as determined by the researchers using FACS. The study findings revealed that the m6A alteration had an impact on the development of early CD4+ T cells. Specifically, it resulted in a decrease in Th1 and Th17 cells in MeTTL3-deficient early T cells, while simultaneously supporting an increase in Th2 cells. Elimination of the METTL3 gene in primary T cells led to decreased expression of SOCS1, SOCS3, and CISH proteins, thus, hindering the IL-7/STAT5 signaling pathway and impacting T cell homeostasis and differentiation.

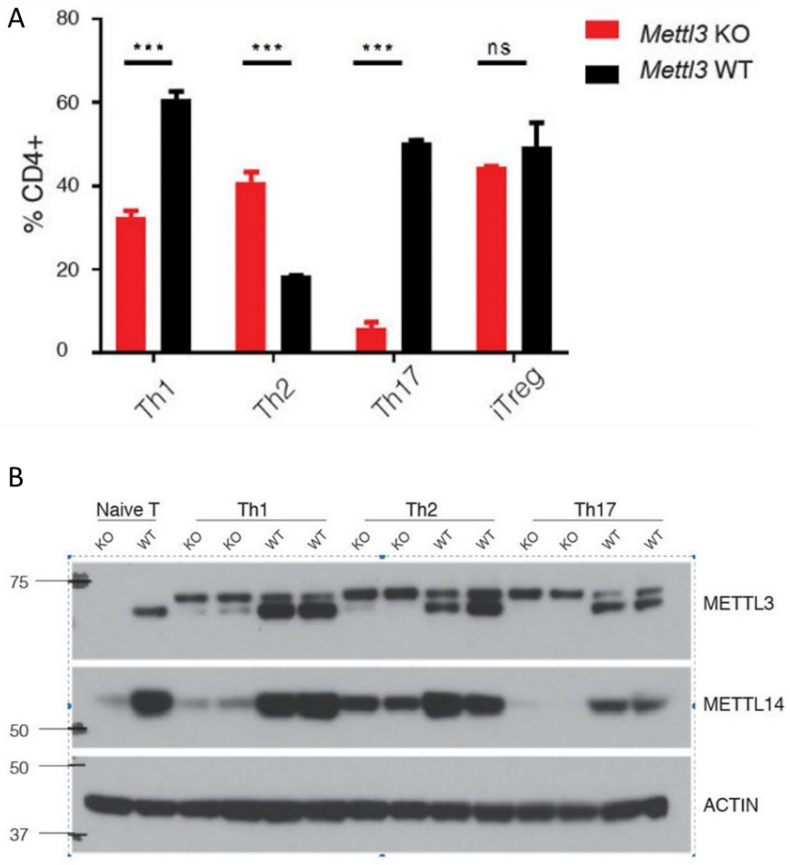

Figure 4. The analysis of Th cells in Mettl3 KO and Mettl3 WT mice [4]

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3.4. M6A mRNA methylation and Regulatory T cells (Tregs)

Tregs are a specific group of CD4+T cells that promote the suppression of immune cell function. m6A methylation is crucial for the differentiation and effector function of Tregs. When Mettl3 or Mettl14 is absent in initial T cells, it hinders T cell proliferation and the process of differentiating into effector T cells. Figure 5 illustrates that the removal of METTL14 impedes the continuous transformation of primary T cells into induced Tregs. The inhibitory activity of Tregs relies on Mettl3-mediated m6A methylation. Deletion of METTL3 in Tregs elevates Socs mRNA levels, leading to the inactivation of the IL-2/STAT5 signaling pathway and consequently impairing the role and durability of Treg cells [10].

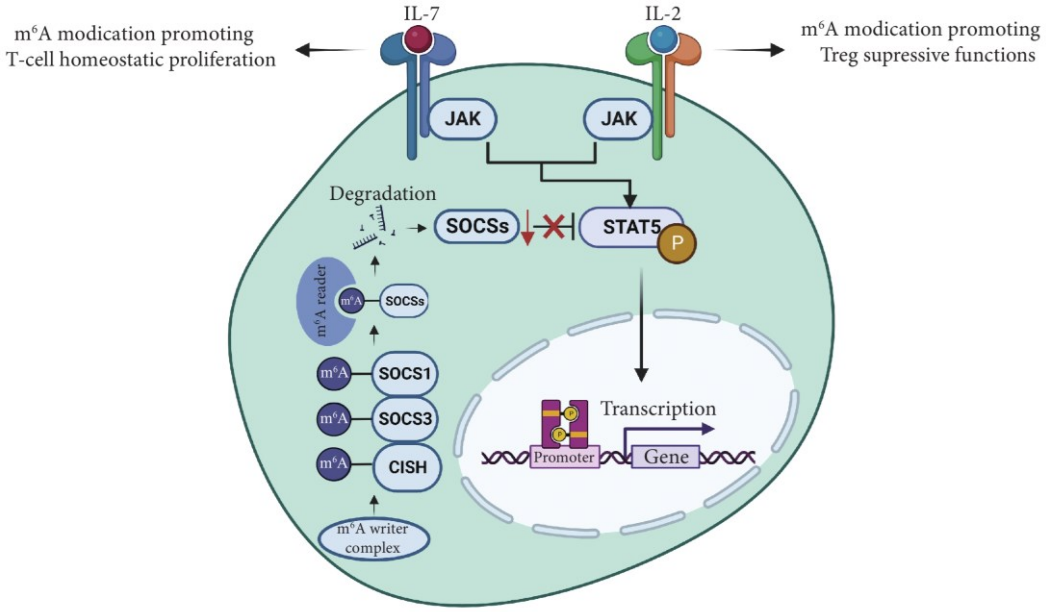

Figure 5. The function of m6A alteration in maintaining the balance of T-cells and enhancing the suppressive abilities of Treg cells has been investigated [12].

4. Conclusion

Although the mechanism of m6A methylation has been studied extensively, there are still some deficiencies in its complex details. The complexity of the regulatory mechanisms of m6A methylation is evident, as it is controlled by a range of different methyltransferases, demethylases, and recognition proteins. Therefore, the interactions and regulatory networks between these proteins require further study. In addition, the mechanism by which T cell homeostasis is regulated by transcription factors, epigenetic modification, metabolic reprogramming and other factors is still unclear. Further exploration is needed. In terms of research techniques, despite advances in molecular research techniques, there are still limitations in the study of m6A methylation, such as low resolution and limited sample size. Therefore, the continuous improvement and optimization of technical methods is necessary.

In this paper, the in-depth exploration of m6A methylation and T cell homeostasis provides a reference for the future study of m6A methylation and disease pathogenesis, and provides a new direction for the diagnosis, drug development and treatment of immune deficiency diseases, autoimmune diseases and malignant tumors. It is hoped that in the future, people can reveal the precise regulatory mechanism of m6A methylation in T cell homeostasis by systematically studying the function and interaction of m6A methyltransferase, demethylase and recognition protein, and combine m6A methylation with other regulatory factors of T cells to comprehensively analyze the regulatory network of T cell homeostasis. It offers novel concepts for the management of immune-related disorders. On the medical side, the role of m6A methylation in T cell homeostasis is utilized to develop new diagnostic and therapeutic techniques for immune-related diseases such as targeted drug design and immunotherapy based on m6A methylation.

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