Dean&Francis
ISSN 2959-409X

The Role of m6A Modification in the PD-1/PD-L1 Pathway: from the Inherent Mechanism of Tumor Cells to the Regulation of Immune Microenvironment

Xu He

School of public health
Wuhan University
Wuhan, China
**Corresponding author: xuhnoo@whu.edu.cn*

Abstract:

The most common epigenome modification in eukaryotic mRNA is N6-methyladenosine modification, which is often referred to as m6A modification. It plays an important role in carcinogenesis, immunological evasion, and drug resistance. The programmed cell death protein 1/programmed death ligand 1(PD-1/PD-L1) pathway is critical for tumor immune evasion. Targeting PD-1/PD-L1 pathway has emerged as a crucial method for treating patients who were diagnosed with advanced tumors. Recent studies have found that: (1) m6A modification acts on this pathway by regulating intrinsic mechanisms of tumor cells, such as affecting the expression of PD-L1 to make it enhanced or decreased or activating the downstream signaling pathway; (2) m6A modification regulates this pathway by modifying the immune environment such as influencing T cell activity and macrophage polarization; (3) m6A modification plays multiple roles in this pathway through the bidirectional regulatory network, suggesting its important clinical translational potential as a possible therapeutic target and prognosis indicator. This article systematically reviewed the mechanism of molecular action of m6A modification in this PD-1/PD-L1 pathway, explored its joint utility and potential intervention strategies, aiming to provide new ideas for overcoming immunotherapy resistance.

Keywords:-N6-methyladenosine; PD-1/PD-L1; tumor microenvironment; apparent regulation

I. Introduction

One of the primary routes of tumor immune escape in physiological status is the immune checkpoint pathway, composed of PD-1 and its ligand PD-L1, the interaction of PD-1 and PD-L1 regulates T cell

ISSN 2959-409X

activity and maintains immune tolerance [1]. However, tumor cell development can inhibit T cell function by highly expressing PD-L1 and then combining with PD-1 located on the surface of T cells, resulting the failure of immune surveillance. PD-1/PD-L1 inhibitors have significantly improved the therapeutic effect of a variety of malignant tumors by blocking this pathway, but the clinical response rate is still limited, and the problem of primary or acquired drug resistance is increasingly prominent. Therefore, the key to improve the effect of immunotherapy is to explore the molecular regulatory mechanism of this pathway and find new therapeutic targets.

In recent years, epigenomic studies have exposed that RNA-related modification fulfils an irreplaceable role in tumor development and immune regulation. As the most common internal modification of eukaryotic mRNA, m6A modification is dynamically regulated by methyltransferases, demethylases and recognition proteins, and participates in the determination of cell fate by affecting RNA splicing, stability, translation and other metabolic processes. Recent studies have found that m6A RNA modification is a dynamic and reversible process that has a significant impact on numerous biological functions. The latest research shows that m6A methylation regulators have dual regulatory roles in a variety of malignancies, namely tumor promoting or tumor inhibiting effects [2]. In addition, abnormal m6A methylation levels may also greatly impact the immune response during anti-tumor immunotherapy [3].

The role of this PD-1/PD-L1 pathway involves not only the intrinsic mechanism of tumor cells but also the remodeling of immune microenvironment. Several investigations have demonstrated that m6A alteration contributes in the role via direct, indirect and other routes, and the interaction between the two has gradually become a research hotspot. This article focuses on how the m6A modification affects the PD-1/PD-L1 pathway, and then prospects its future in tumor immunotherapy based on relevant clinical data. All the researches target to improve the efficacy of relevant inhibitors and develop new biomarkers.

II. Biological Basis of m6A Modification

As one of the RNA methylation modifications, m6A modifications are dynamically distributed in multiple functional regions of transcripts, including key sites such as 5'-untranslated region (5'UTR), coding region and 3'-untranslated region(3'UTR) [4]. This important epigenetic modification is dynamically balanced by a precise regulatory network consisting of methyltransferases, demethylases and binding proteins, which are also often referred to as writers, erasers and readers.

A. m6A Methyltransferase

There are many kinds of methyltransferase complexes that use different substances as methyl donors. Its core components have methyltransferase like 3 (METTL3) and methyltransferase like 14 (METTL14), and they form functional heterodimers. The former provides catalytic activity, while the latter enhances catalytic efficiency and promotes RNA binding through allosteric regulation [5,6]. Wilms'tumor 1-associated protein (WTAP) is also one of the components of the 'writers'. Although it does not have catalytic ability, it can ensure the specificity of modification by mediating complex localization and substrate recognition [7].

B. m6A Demethylase

In terms of demethylation regulation, fat mass and obesity-associated protein (FTO) is involved in a reversible regulatory mechanism. As the first demethylase discovered, FTO not only participates in the removal of m6A modification, but also regulates m6A modification. This property decides it play an irreplaceable role in the regulation of organism energy metabolism [8]. And ALKB homolog 5 (ALKBH5) is also a kind of 'erasers', which shows specificity for m6A modification. The both cooperate to maintain the dynamic balance of RNA methylation modification in cells.

C. m6A Reading Protein

As a key effector molecule of RNA epigenetic regulation, m6A reading protein can uniquely detect and bind m6A modification sites on RNA, regulating all aspects of RNA metabolism, including post transcriptional processing, nucleocytoplasmic transport, stability maintenance and translation efficiency. According to the structural features and functional differences, these proteins can be broadly classified into three types: the first one is YTHDF1-3 and YTHDC1-2 protein families (YTHD) with characteristic YTH domains, the second is members of the heterogeneous ribonucleoprotein hnRNP family, and the last includes other proteins containing binding domains of RNA, such as the insulin-like growth factor mRNA reading proteins. The m6A reading proteins also have important biological significance in clinical transformation. Relevant studies found that the complete remission rate of R-CHOP treatment in patients with insulin-like growth factor binding protein 3 (IGFBP-3) positive expression reached 42.0%, which was substantially greater than 26.4% in the negative expression group [9]. Further research revealed

that increased IGFBP-3 expression was not only substantially linked with better treatment response, but also negatively correlated with tumor malignant progression. These data show that IGFBP-3 may serve as a new potential molecular marker for the prognosis of diffuse large B-cell lymphoma and has possible usefulness as a therapeutic target. Those m6A modified RNA relies on reading proteins to exert its subsequent functions, and the biological effects of the same m6A modified RNA after binding to different reading proteins may also be different. Therefore, regulating the interaction between m6A reading proteins and m6A modified RNAs may be a new strategy for disease treatment.

III. m6A Modification Regulates PD-L1 Expression and Downstream Signaling Pathways

The regulation of PD-L1 expression and the effects of its downstream signaling pathways involves the intrinsic mechanism of tumor cells. Recent investigations have indicated that m6A alteration has a considerable effect on tumor immune escape by directly influencing the expression of immunological checkpoint protein PD-L1 or indirectly controlling its expression through related signaling pathways.

It has been confirmed that m6A methylation modification has important regulatory functions on two types immunity, innate and adaptive [10], especially in the aspect of regulating the immunological checkpoint expression. In an investigation of oral squamous cell carcinoma, METTL3 mediated m6A modification can significantly improve the expression of PD-L1 as well as inhibiting the activation of $CD8^{+}$T cells [11]. METTL3 is the core catalytic subunit of m6A methyltransferase complex, which can recognize the mRNA specific sequences of PD-L1, such as 3'UTR or coding region, and add m6A methylation marks. The methylated PD-L1 mRNA is not easily recognized by RNA degrading enzymes, thus prolonging its half-life, resulting in the upregulation of its protein expression. In addition, the m6A reading protein YTHDF1 increases protein synthesis by promoting the translation efficiency of PD-L1 mRNA, and YTHDF2 inhibits its degradation by preventing mRNA deadenylation. Both maintain the high level expression of PD-L1 mRNA, resulting in the continuous high expression of PD-L1 protein on the surface of tumor cells.

Recent investigations have discovered that m6A methylation-related genes can also influence this pathway by influencing long non-coding RNA (lncRNA), thereby affecting the sensitivity of tumors to PD-1 treatment. For example, in hepatocellular carcinoma, METTL14 can regulate PD-L1 expression via another mir-223/STAT1 pathway by promoting m6A modification of lncRNA-MIR155HG [12]. METTL14 forms a methyltransferase complex with METTL3, specifically recognizing conserved sequences in lncRNA-MIR155HG transcripts, adding m6A modification at its specific adenylate sites. Methylated lncRNA-MIR155HG binds to m6A reading protein, protecting it from degradation by RNase, prolonging its half-life, resulting in high expression of lncRNA-MIR155HG in hepatocellular carcinoma. And lncRNA-MIR155HG contains a binding site complementary to mir-223, which can adsorb mir-223 to reduce the inhibitory effect of free mir-223 on the downstream target gene STAT1 mRNA. After STAT1 is activated, it forms a dimer and translocates into the nucleus, which directly promotes PD-L1 transcription combined with the activation sequence of PD-L1 gene promoter region, thus promoting immune escape. GATA3-AS1, a lncRNA highly expressed in breast cancer tissues, can also increase PD-L1 protein expression through the miR-676-3p/COPS5 (COP9 signalosome Subunit 5) pathway and promote immune escape [13]. GATA3-AS1 acts as a competitive endogenous RNA to adsorb mir-676-3p, inhibit its activity, and reduce its degradation or translational inhibition of downstream target gene COPS5 mRNA. That is, the downregulation of mir-676-3p leads to the increase of COPS5 expression. COPS5 prevents the PD-L1 proteasomal degradation through deubiquitination, this can increase the stability of PD-L1 protein.

IV. Effects of m6A Modification on Immune Cell Function

It has been convinced that m6A modification can regulate the expression of immunological checkpoint molecules, but it also can directly impact the activity of various immune cells such as T cells and macrophages, thereby remodel the immune microenvironment.

The latest study revealed that when ALKBH5 was deleted, the m6A methylation level of the mRNA 3'UTR region of PD-L1 increased, which accelerate the degradation process of PD-L1. However, in hepatocellular carcinoma, the expression of ALKBH5 may linked with PD-L1, which affects the recruitment of PD-L1-positive macrophages by regulating mitogen activated protein kinase kinase 8(MAP3K8) through its m6A methylation pathway [14]. ALKBH5 removes the m6A modification of MAP3K8 mRNA to protect it from YTHDF2 mediated degradation, thereby enhancing MAP3K8 mRNA stability and protein expression. MAP3K8 activates nuclear factor kappa B(NF-κB) and promotes the secretion of C-C mo-

ISSN 2959-409X

tif chemokine receptor 2(CCL2). CCL2 is an important chemokine, it can recruit $CCR2^+$ monocytes into the tumor microenvironment and differentiate into PD-$L1^+$ tumor associated macrophages (TAMs) which can inhibit $CD8^+$T cell function through signaling pathway and promote tumor immune escape. At the same time, TAMs secrete immunosuppressive factors such as IL-10 and TGF-β to further inhibit anti-tumor immunity.

Another study investigated the infiltration of twenty-two kinds of immune cells in patients with colon cancer using an algorithm, the study divided into two groups according to the exposure level of risk factors and found that the composition of immune cells between the two groups had substantial changes [15]. The infiltration level of $CD8^+$T cells was lower in the group with higher hazard risk, while the infiltration of memory $CD4^+$T cells in the resting state of memory was higher. It has confirmed a strong association between high infiltration of $CD4^+$T cells and lower overall survival, this may be because $CD4^+$T cells are dominated by immunosuppressive subtypes when they are in a state of high infiltration, such as regulatory T cells (Tregs). In addition, the proportion of Tregs in the group with high-risk was obviously increased, further supporting the formation of an immunosuppressive microenvironment. However, combined with other studies, m6A modification may affect M2 polarization of macrophages by regulating chemokines or inflammatory factors, thus promoting tumor progression. These results suggest that m6A related lncRNAs may promote T cell dysfunction by regulating part of exhaustion related genes of T cell, and the increase of Tregs divided in the high-risk group could be connected to the regulation of m6A modification on the stability of its major transcription factors. That is, m6A modification will influence both the functional status and infiltration situation of these immune cells.

The expression of methyltransferase KIAA1429 is positively correlated with macrophage infiltration [15], which may promote M2 type polarization through m6A dependent mechanism, m6A modification can regulate M2 macrophage related genes. The mRNA of M2 marker genes such as Arg1 (arginase 1) and CD206 (MRC1) may be stabilized by m6A modification. The m6A modification mediated by KIAA1429 recruits YTHDF1 and YTHDF2 to protect these mRNAs from degradation or promote their translation. In addition to the regulation of macrophage polarization related genes, KIAA1429 can also promote M2 polarization through m6A dependent signaling pathways. STAT3 and STAT6 are the core transcription factors of M2 polarization. And m6A modification can activate STAT3/STAT6 pathway and further upregulate M2 markers. M2 macrophages may promote tumor growth, metastasis and angiogenesis, and inhibit anti-tumor immune response, this is associated with bad prognosis.

V. Clinical Translational Potential of m6A Modification

The central role of m6A modification in tumor immune regulation makes it a potential clinical therapeutic target. The model of m6A prognostic has become a research hotspot in cancer precision medicine and is transitioning from basic research to precision therapy. For example, the risk score model based on eleven m6A related lncRNAs showed good predictive performance in both the training set and the validation set (3-year AUC>0.67), and was significantly correlated with clinicopathological characteristics such as T stage and metastatic status of tumors [16]. This model can help identify high-risk patients and guide individualized treatment. This shows the clinical translational potential of m6A prognostic model, but large-scale prospective studies are still needed to verify its reliability.

It can also be applied to immunotherapy response prediction. In the anti-PD-1 treatment cohort, patients in the KIAA1429 high expression group had a better remission rate, demonstrating that it can be employed as a predictive marker of immunotherapy response. High KIAA1429 expression is substantially related with poor prognosis of lung cancer patients, and it is universal in a variety of cancers such as adrenocortical carcinoma and hepatocellular carcinoma [15]. The study verified that KIAA1429 is an independent prognostic factor, suggesting that we can use this to judge the effect of immunotherapy.

The therapeutic potential of targeting m6A cannot be ignored. All of these showed that m6A alteration has an important role in PD-L1 expression and related signaling pathways, and related targeted therapies can achieve the downregulation of PD-L1 or PD-1 through direct or indirect ways, thus affecting the sensitivity of tumors to immune checkpoint inhibitors. Currently, two kinds of PD-1/PD-L1 inhibitors resistance have become a main impediment in clinical application, that is primary or acquired resistance. And m6A modification may become a key target to break through drug resistance. For example, METTL3 inhibitor can reduce PD-L1 levels and restore T cell function in a model about PD-1-resistant non-small cell lung cancer. In melanoma resistant patients, FTO inhibitors combined with anti-PD-1 therapy can significantly improve the remission rate and reduce T cell exhaustion.

VI. Conclusion

With the continuous development and in-depth study of epigenetics, scholars' understanding of the pathogenesis of various types of tumors has gradually become clear, and the clinical demand for appropriate treatment options has also been increasing. And m6A methylation influences the onset and progression of cancers via a multitude of

pathways. This article focuses on its series of molecular roles in the PD-1/PD-L1 pathway. Through discussion, a conclusion was drawn that m6A modification affects immune escape by affecting the intrinsic mechanism of tumor cells, that is, by regulating PD-L1 expression and downstream signaling pathways, and further influences the cancers by directly affecting the intrinsic biological molecular mechanisms of immune cells to shape the tumor immune microenvironment. And in the above process, m6A modification plays a dual role, that is, sometimes it can promote the immunological escape of tumor cells and sometimes it can inhibit the development and spread of tumor cells.

However, the regulatory mechanism of m6A modification on tumor cells is a complex, diverse process, involving the interaction of a variety of enzymes and proteins, which has not been fully explained in this paper. Future research will further reveal the specific mechanism of m6A modification, and its results are expected to provide a scientific basis for future researches of new therapeutic strategy, and ultimately bring more effective treatment to tumor patients. Clinically, targeted therapy for m6A methylated molecules has also become a major research hotspot, suggesting that we can develop a precision treatment scheme based on m6A methylation, and combine the strength of multiple disciplines to accelerate the transformation of relevant research results to clinical application.

References

[1] A. H. Sharpe and K. E. Pauken, "The diverse functions of the PD1 inhibitory pathway," *Nat. Rev. Immunol.*, vol. 18, no. 3, pp. 153–167, Mar. 2018.

[2] T. Wang, S. Kong, M. Tao, and S. Ju, "The potential role of RNA N6-methyladenosine in cancer progression," *Mol. Cancer*, vol. 19, no. 1, p. 88, 2020.

[3] D. Han *et al.*, "Anti-tumour immunity controlled through mRNA m(6)A methylation and YTHDF1 in dendritic cells," *Nature*, vol. 566, no. 7743, pp. 270–274, 2019.

[4] I. Barbieri and T. Kouzarides, "Role of RNA modifications in cancer," *Nat. Rev. Cancer*, vol. 20, no. 6, pp. 303–322, 2020.

[5] J. Z. Liu, Y. N. Yue, D. L. Han *et al.*, "A METTL3-METTL14 complex mediates mammalian nuclear RNA N6-adenosine methylation," *Nat. Chem. Biol.*, vol. 10, no. 2, pp. 93–95, 2014.

[6] S. Zaccara, R. J. Ries, and S. R. Jaffrey, "Reading, writing and erasing mRNA methylation," *Nat. Rev. Mol. Cell Biol.*, vol. 20, no. 10, pp. 608–624, 2019.

[7] Y. M. Wang *et al.*, "The emerging role of m6A modification in regulating the immune system and autoimmune diseases," *Front. Cell Dev. Biol.*, vol. 9, p. 755691, 2021.

[8] J. B. Wei *et al.*, "Differential m6A, m6Am, and m1A demethylation mediated by FTO in the cell nucleus and cytoplasm," *Mol. Cell*, vol. 71, no. 6, pp. 973–985, 2018.

[9] H. B. Li, D. Wang, Y. Zhang *et al.*, "IGFBP3 Enhances Treatment Outcome and Predicts Favorable Prognosis in ABC-DLBCL," *J. Oncol.*, p. 1388041, Oct. 2023.

[10] Y. Liu, R. Song, L. Zhao *et al.*, "m(6)A demethylase ALKBH5 is required for antibacterial innate defense by intrinsic motivation of neutrophil migration," *Signal Transduct. Target. Ther.*, vol. 7, no. 1, p. 194, 2022.

[11] Y. Al, S. Liu, H. Luo *et al.*, "METTL3 Intensifies the Progress of Oral Squamous Cell Carcinoma via Modulating the m6A Amount of PRMT5 and PD-L1," *J. Immunol. Res.*, p. 6149558, 2021.

[12] N. Tsuruta, K. Tsuchihashi, H. Ohmura *et al.*, "RNA N6-methyladenosine demethylase FTO regulates PD-L1 expression in colon cancer cells," *Biochem. Biophys. Res. Commun.*, vol. 530, no. 1, pp. 235–239, 2020.

[13] E. A. Salama, R. E. Adbeltawab, and H. M. El Tayebi, "XIST and TSIX: Novel Cancer Immune Biomarkers in PD-L1-Overexpressing Breast Cancer Patients," *Front. Oncol.*, vol. 9, p. 1459, 2019.

[14] Y. You, D. Wen, L. Zeng *et al.*, "ALKBH5/MAP3K8 axis regulates PD-L1+ macrophage infiltration and promotes hepatocellular carcinoma progression," *Int. J. Biol. Sci.*, vol. 18, no. 13, pp. 5001–5018, 2022.

[15] L. Guo, Q. Huai, B. Zhou, J. Ying, and W. Guo, "Comprehensive analysis of the prognostic impact and immune implication of KIAA1429 in lung adenocarcinoma," *Cancer Innov.*, vol. 1, pp. 328–343, 2022.

[16] L. Cao, S. Zhang, Y. Ba, and H. Zhang, "Identification of m6A-related lncRNAs as prognostic signature within colon tumor immune microenvironment," *Cancer Rep.*, vol. 6, no. 6, p. e1828, 2023.