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Analysis of the possibility and progress of FoxP3 in tumor treatment based on its effect on Tregs

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Abstract. Treg can inhibit immunity in cancer and a variety of autoimmune diseases. And foxp3 is a kind of spectrum specificity markers. It's also the main regulatory factor that can ensure the generation, maintenance and the immunosuppressive function of the Treg cells. At the same time, foxp3 can also promote the differentiation and function of Treg. In this paper, the interaction between foxp3 and various transcription factors (TFs) as well as their joint action are analyzed. These effects can have an impact on the suppressive effect of Treg cells, which in turn affects the status of the tumor. The post-translational modifications of foxp3 itself are also analyzed. foxp3 has been modified by methylation, acetylation, phosphorylation, ubiquitination, glycosylation and lactatation. Its own activity has been affected, and its binding ability to target genes has been changed. From these analyses, a conclusion can be drawn that the effect of CAR-T therapy can be enhanced by targeting multiple TFs and signaling pathways. At the same time, targeted inhibitors can be explored for various enzymes involved in post-translational modification. And these inhibitors can reduce the ability of foxp3. However, the research on TFs and post-translational modifications still has some limitations. Future studies can focus on these two aspects.

Keywords: Transcription factors, regulatory T cells, foxp3.

1. Introduction

With the development of cellular immunology, immunotherapy has gradually shown vigorous vitality in the treatment of tumors.CD4T cells play an important role in mediating adaptive immunity against a variety of pathogens, not only participating in tumor immunity, but also having a significant impact on autoimmunity, asthma and allergic reactions. Regulatory T cells (Treg), an immunosuppressive subpopulation of CD4+ T cells, are important cells for maintaining immune homeostasis and play an important role in maintaining autoimmune stability and suppressing inflammation. And foxp3 can mediate the enhancer promoter loop in three-dimensional space, affecting the role of Treg by influencing the distance between the two, and is specifically expressed in Treg, which is a core TF of Treg cells, affecting tumor proliferation, metastasis and invasion. Abnormalities in the function of foxp3 or Treg cells lead to the development of autoimmune diseases, tumors, and aging. Deficiency of foxp3 leads to pro-inflammatory and immune-promoting capacities of Treg, but also to a role in resistance to tumor

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invasion. Therefore, the mechanism of action of Treg and foxp3 is crucial in exploring the problem of tumor immune escape. CAR-T, a widely used immunotherapy method, is mainly used to transfect patients' T lymphocytes with chimeric antigen genes to form chimeric antigen receptor-modified T cells with tumor-killing properties. This is done by infusing them back into the patient's body to exert inhibition and targeted killing of tumor cells. This approach has been demonstrated in research and in the clinic, but is associated with a degree of tolerance, which reduces the effect of CAR-T therapy and causes relapse, and has not performed as well in solid tumors. Relapses have been reported in previous articles related to acute lymphoblastic leukemia (ALL) [1]. The published related research will be inevitable to discuss influence of Treg cells in the immune therapy. How to control Treg cells and make them play a positive role in tumor treatment is an urgent problem to be solved. foxp3 has become the center of attention due to its high effect on Treg cells. Studying foxp3 can effectively discuss how to increase the positive effect of Treg cells on tumor treatment and reduce the negative effect, which provides a premise for the development of new treatment. The therapeutic methods of Treg and the mechanism of foxp3 are discussed by studying the perspective of Treg cells and their stability, methylation, acetylation, phosphorylation, ubiquitination, lactatation and glycosylation of foxp3 protein post-translational modification, and the TF of foxp3. IL-2R/STAT pathway, TGF-β/SMAD pathway, hats, HDAC, PIM1 protein kinase, deubiquitinating kinase and ubiquitin-binding enzyme were explored. Therefore, how foxp3 and Treg cells are regulated in the tumor microenvironment can be understood. Therefore, how to remove the immunosuppression caused by Treg in existing immunotherapy may be explored. Clarifying the potential and progress of foxp3 in cancer therapy need to discuss the related sites that have been found to affect foxp3 in the process of exploration, and verify whether it may be a new target. The purpose of this study is to investigate the mechanism of foxp3 on Treg and the pathways involved in the process, and to investigate how Treg cells and foxp3 protein affect CAR-T immunotherapy and how to address this effect. and to investigate whether the proposed sites can be used as new target inhibitors. To explore the possibility and significance of foxp3 in immunotherapy. At the same time, studying whether the proposed site can be used as a new target inhibitor may be able to affect the possibility and significance of foxp3 in immunotherapy.

2. Treg

2.1. Treg cell classification and production

The regulatory T cell (Treg) is a type of cell sub -group with foxp3 as the core TF. The main function is to suppress immune response and make immune response not too strong, thereby preventing the occurrence of autoimmune diseases. foxp3 is a spectrometer -specific marker, and it is also the main regulatory gene to generate, maintain, and immunosuppressive functions for Treg cells. Treg is divided into natural Treg (nTreg) and induced adaptive Treg (iTreg) based on the source of development. nTreg cells mainly include CD4+Treg cells, while iTreg includes various subtypes such as TR1 cells, TH3 cells, and CD8+Treg cells. Part of the mature part of the thymus is called tTreg, and nTreg, which is induced by peripherals, is called pTreg. iTreg develops from the initial CD4+T cell differentiation after the cytokines such as TGF-β stimulation. Studies have found that compared with iTreg, pTreg 's immunosuppressive function and foxp3 expression are more stable [2].

2.2. Treg's action mechanism and impact on tumor

In the tumor microenvironment (TME), there is a large amount of Tregs infiltration phenomenon. Through their unique immunosuppressive mechanisms, these Tregs significantly impair the activity and efficacy of effector T cells in the antitumor immune response, thus becoming a key factor that hinders the effective antitumor immune response. Treg not only promoted the rapid proliferation of tumor cells, but also significantly enhanced their ability to infiltrate and spread to surrounding tissues. Tumor Immune Escape (TIE) is one of the important features of tumor formation, and Treg is the key factor of TIE. Clarifying the mechanism of Treg is essential for tumor immunotherapy.

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The mechanism of Treg inhibiting anti-tumor reactions includes contact-dependence mechanism and contact non-dependent mechanism. The former involves CTLA-4, lymphocyte activation gene 3 (LAG-3), and T cell immunoglobulin and ITIM domain (TIGIT). They exhibit contact-dependent inhibitory effects by suppressing the activation and maturation of dendritic cells (DCs), thereby weakening the immune response of anti-tumor T cells. The latter includes the secretion of IL-10, IL-35 and TGF-β which can prevent the surrounding effects from amplifying T cells and suppress its functions [3]. Furthermore, nTregs can also kill effect cells through granzyme A and perforated element to further weaken anti -tumor immune response [4]. These mechanisms help Tregs to play a role in promoting tumor progress in cancer.

Treg also affects the prognosis of tumors. It can be found in clinical data that the high infiltration of tumor tissues is related to poor prognosis of patients. The CD8+T cells are suppressed by Treg, so tumor tissue is also related to the poor prognosis of CD8+T cells in tumor tissue. According to studies, stigmasterol acts on Lactobacillus johnsonii, Lactobacillus murinus, and Lactobacillus reuteri, increasing the proportion of Treg and IFN-γ+ CD8+ T cells in intestinal mucosa and tumor tissue. Eventually, this increases apoptotic protein levels, which kills tumor cells [5]. This indicates that the lower the CD8+ T/Tregs ratio, the worse the prognosis of the patient. In addition, Treg cells can also exert immunosuppressive effects by expressing IL2RA (interleukin 2 receptor α, also known as CD25), foxp3, and TNFRSF18. The high expression of these factors is also associated with poor prognosis [6,7].

However, Treg cells also have a positive role in tumors. In some cases, they can also play a protective role. Based on the immunosuppressive properties of Treg cells, some researchers are exploring cancer treatment strategies targeting Treg cells to improve the therapeutic effect of tumors by eliminating or inhibiting the body's anti-tumor immune function.

2.3. Association between Treg and foxp3

Foxp3 is a core TF of Treg. When foxp3 is missing, Treg cells will overexpress IL-2, IL-4, IL-17 and IL-21, which will reduce the immunosuppressive function of Treg and acquire pro-inflammatory properties, aggravating the progression of the disease.

foxp3 recognizes and bridges DNA in a multimeric manner, which is a new DNA recognition and binding mode of foxp3. This mechanism reveals a new mechanism of foxp3 in regulating Treg function, which is of great significance for understanding how the immune system maintains self-tolerance and treats autoimmune diseases [8].

Foxp3 maintains its regulatory function by inhibiting Myc gene expression and Treg cell glycolysis, thereby conferring a metabolic advantage to Treg cells in a low-glycemic environment rich in lactate.

Treg cells with high expression of foxp3 exhibited significant immunosuppressive effects by targeting a wide range of natural and adaptive immune cells. These cells potentiate suppressor function through contact-dependent mechanisms or immunomodulatory cytokines. They can also act as immunosuppressors, through metabolic perturbation of target cells. Therefore, comprehending the immunosuppressive role of Treg cells and treating associated disorders require an understanding of the foxp3 regulatory mechanism.

3. Regulation of Tregs by the transcription factor foxp3

3.1. Foxp3's structure and how it affects Tregs

The transcription factor known as foxp3 (Forkhead box P3) is an essential transcription factor that controls the growth, operation, and maintenance of regulatory T cells, or Treg cells. For the immune system to remain in balance, autoimmunity must be avoided, and immune responses must be controlled by this kind of cell. The 431-amino acid protein that the foxp3 gene encodes is produced by its 12 exons. The central leucine zipper domain, the C-terminal forkhead (FKH) domain, and the C2H2 zinc finger domain are functional domains found in this protein. The nuclear localization, DNA binding, and interaction with other transcription factors are all significantly impacted by the FKH domain [9]. The regulation of the foxp3 gene's expression involves both transcriptional regulation and the involvement

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of transcriptional co-regulators. foxp3 is a transcription factor that is particular to Treg cells; it can bind to DNA and control the transcription of genes. foxp3 regulates the production of downstream target genes, including TGF-β, IL-10, and CTLA-4, which in turn dictates how Treg cells suppress the immune system. Simultaneously, foxp3 engages in complex regulatory network formation with other transcription factors and co-inhibitory molecules, augmenting Treg cells' inhibitory effect.

3.2. Transcriptional activation and promoter binding

The transcriptional activation and promoter binding of foxp3 is a highly complex process involving multi-level regulatory networks and multiple signaling pathways. The expression of the foxp3 gene is regulated by several transcription factors including STAT5, NFAT, and FOXO1. STAT5 is activated through the cytokine receptor-mediated JAK-STAT signaling pathway, translocates to the cell nucleus and directly binds to the foxp3 promoter, thereby initiating the transcription of its gene [10]. NFAT is dephosphorylated under T cell receptor (TCR) signaling and translocated to the cell nucleus, where it binds to the foxp3 promoter and jointly promotes the expression of foxp3. FOXO1, as another important transcription factor, enhances its transcriptional activity by binding to the foxp3 promoter region [11]. In addition, conserved non-coding sequences (CNS) within the foxp3 locus, such as CNS1, CNS2, and CNS3, play a key role in regulation. CNS1 enhances foxp3 transcription by binding to SMAD3 downstream of the TGF-β signaling pathway, while CNS2 contains binding sites for multiple transcription factors, including STAT5, NFAT, RUNX1, and CREB, thereby maintaining the sustained expression of foxp3. CNS3 contains a binding site for the NF-κB signaling molecule c-Rel, which plays an important role in the initial activation of foxp3 transcription. During transcriptional activation, the foxp3 promoter binds to a variety of transcription factors and cofactors to form a complex transcription complex, the formation and stability of which is essential for the effective transcription of foxp3. The interaction between NFAT and foxp3, which binds to the promoter region of the target gene, enhances transcriptional activity. In addition, foxp3 can interact with other proteins such as GATA3 and EZH2 to form a variety of transcriptional complexes, the composition and function of which may vary depending on the cell environment and stimulation conditions, thereby further fine-tuning the specific gene expression and function of Treg. Through this series of complex interactions and regulation, the expression and function of foxp3 are maintained, thus playing a key role in immune tolerance and preventing autoimmune diseases. This multi-level regulatory mechanism ensures the dynamic regulation of foxp3 under different physiological conditions, thereby effectively maintaining the function and stability of Treg cells.

3.3. Ikaros

The study found that the transcription factor Ikaros works closely with foxp3 to construct the main part of the epigenome and transcriptome of regulatory T cells (Tregs). Ikaros exerts its function in Tregs by relying on foxp3. This dependency relationship ensures their cooperative DNA binding in the Treg genome, thereby effectively regulating gene expression. Ikaros family members Eos and Helios can also cooperate with foxp3, among which Eos enhances the core Treg gene expression program ectopically co-expressed in conventional T cells through cooperation. This means that Eos not only plays a role in Tregs, but also can enhance the expression of Treg-related genes in other T cell types, highlighting its extensive role in immune regulation. The function of Helios has a key influence on the expression stability of foxp3. The loss of Helios function significantly destroys the expression stability of foxp3, thereby affecting the function and stability of Treg cells. This finding demonstrates the importance of Helios in maintaining foxp3 and its mediated inhibitory gene expression, emphasizing its unique role in Treg cell function [10]. Ikaros and its family members Eos and Helios play a key regulatory role in Treg cells by cooperating with foxp3. Eos supports the function of foxp3 by enhancing the expression of core Treg genes, while Helios ensures the functional integrity of Treg cells by maintaining the stability of foxp3. These findings reveal the synergistic role of Ikaros family transcription factors in the Treg cell regulatory network.

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3.4. Methylation

Transcription factors including NFAT, AP-1, and STAT5 attach to the promoter or enhancer regions of the foxp3 gene to control transcriptional activity, which in turn controls foxp3. The methylation of foxp3 DNA is linked to these actions. GATA binding protein 1 (GATA1) and GATA binding protein 3 (GATA3) can bind to the transcription factor binding site (CpG) in the Treg-specific demethylated region (CNS2). GATA3 can bind to foxp3 to maintain its activity, whereas GATA1 can collaborate with ATrich sequence binding protein 1 (SATB1) to maintain Treg cell expression. The binding of the foxp3 promoter to signal transducer and activator of transcription 1 (STAT1) can reduce methylation of CpG DNA at the STAT1 binding site, promoting foxp3 expression, whereas signal transducer and activator of transcription 3 (STAT3) negatively regulates foxp3, disrupting Treg homeostasis and reducing its inhibitory effect. This mechanism normally happens during inflammatory situations, and vitamin D can help to maintain the balance of the STAT1 and STAT3 pathways by modulating STAT3. Research has demonstrated that vitamin D can bind to the CNS2 area and, through decreasing the methylation level of the foxp3 gene promoter, boost the expression of foxp3. Therefore, while using vitamin D, special consideration should be given to the influence on Treg cells [12].

3.5. Other molecules that regulate Treg

Apart from the molecules (foxp3, CTLA-4, IL-10, STAT5, TGF-β) that were previously discussed, numerous other molecules play a crucial role in controlling the differentiation, upkeep, and functionality of Treg cells. These include PD-1 (programmed death protein 1), CD25 (IL-2Rα), NRP1 (neuropilil protein 1), ICOS (inducible T cell co-stimulatory molecule), and GITR (glucocorticoid-induced TNF receptor family-related gene). These elements control Treg function and activity via various signaling pathways. NRP1 may influence through VEGF and SEMA signaling routes, ICOS activates PI3K-AKT signaling pathways, GITR regulates through NF-κB and MAPK signaling pathways, and PD-1 and CD25 both boost and inhibit IL-2 signaling pathways.

PD-1 is an inhibitory receptor that binds to PD-L1/PD-L2, which prevents T cell activation. Treg cell PD-1 expression aids in immune response regulation, reduces excessive inflammation, and guards against tissue damage. To keep Treg cells' immunosuppressive ability intact, PD-1 signaling is crucial. The high-affinity IL-2 receptor's α chain, CD25, is extensively expressed in T cells. Treg cell survival and functionality depend on IL-2. Through its attachment to IL-2, CD25 stimulates the growth of Treg cells and suppresses the function of effector T cells. The production, upkeep, and immunosuppressive properties of Treg cells are collectively regulated by these substances via various signaling routes and activities. Research on immunotherapy has shown that controlling these molecules can lead to the creation of more potent treatments. Expression of PD-1 on Treg cells protects against tissue damage, lessens excessive inflammation, and helps regulate the immune response. PD-1 signaling plays an essential role in maintaining the immunosuppressive capacity of Treg cells. T cells have high levels of expression of CD25, the α chain of the high-affinity IL-2 receptor. IL-2 is necessary for Treg cell survival and functioning. By binding to IL-2, CD25 promotes the expansion of Treg cells and inhibits effector T cell activity. These compounds work together through a variety of signaling pathways and functions to control the development, maintenance, and immunosuppressive characteristics of Treg cells. Studies on immunotherapy have demonstrated that manipulating these molecules can result in the development of more effective therapies.

4. Regulation of Treg based on post-translational modification of foxp3

foxp3 protein is composed of four parts: an N-terminal domain, a zinc finger domain, a leucine zipper domain and a Forkhead domain at the C-terminus. Among them, the N-terminal domain is related to transcriptional repression. It is still unknown what the zinc finger domain can do. The dimerization of the foxp3 protein is associated with the leucine zipper domain, while nuclear import and DNA binding are associated with the Forkhead domain. When the structure of the different parts changes, the function of foxp3 changes accordingly (Figure 1).

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4.1. Foxp3 acetylated modification

Acetylation and acetylation are kinds of histone modifications after translation, mainly composed of histone acetyltransferase (HAT) and histone acetylation enzyme (HDAC) play a role.Hat acts as a coactivator of transcription to promote the acetylation of foxp3 protein, while HDAC acts as a corepressor to reduce the acetylation of foxp3. After being acetylated, foxp3 becomes more stable, which means that it can better combine with DNA and play a higher role. The function of foxp3 protein is determined by their interaction with the N-terminal lysine residues and the acetylation process. The structural changes of foxp3 protein, such as acetylation, determine the function of Treg cells [13].

There are few studies on the interaction between HATs and foxp3 protein. Through somewhat different and partially overlapping processes, the Hats family of the n-acetyl transferase (GNAT) family of gcn5 (the general control of inhibiting protein 5), and the p300/CBP associated factor Kat2b increase Treg function. PCAF can promote foxp3 by being combined with foxp3 protein acetylation and maintain Treg cell's stability and function. This was demonstrated by the conditional deletion of two HAT-related genes in mice.

The function of Treg was enhanced by the loss of HDAC6, HDAC9, and Sirtuin-1, while the foxp3+ Treg's function was diminished by the loss of HDAC3, HDAC5, or Sirtuin-3. In addition, it has been recently reported that HDAC10 can enhance the suppressive effect of foxp+ Treg cells [14]. It should be noted that during the process of transferring foxp3 protein from the nucleus to the cytoplasm, it may lose the transcriptional regulation of target genes, which leads to the disappearance of its inhibitory effect and changes in the cellular response to HDAC inhibitors (Figure 1A).

4.2. Foxp3 phosphorylated modification

Phosphorylation of foxp3 is a kind of post-translational modification, which can bi-directionally regulate the suppressive function of Treg. Studies have reported that under the condition of inflammation, PIM1 protein kinase combining ser422 foxp3 protein loci can change the current site space conformation. So that it can reduce foxp3 protein binding activity, thereby reducing the combination of foxp3 protein and DNA. Finally, a variety of target genes's (dc25 and ctla 4) expression can be cut down [15]. Phosphorylation of the Ser418 residue in the Forkhead domain at the c-terminus can promote the inhibitory effect of Treg cells. If dephosphorylation occurs at this site, it will cause the inactivation of foxp3 protein, thereby affecting the effect of Treg cells. PIM2 protein kinase can act on the N-terminal repressor domain of foxp3 protein, causing the difficulty of binding foxp3 protein to some cofactors and leading to the reduction in foxp3 function.

Cyclin E can cooperate with the kinase CDK2 to phosphorylate Ser-19 and Thr-175 by binding to the CDK motif within the four N-terminal domains, which may decrease the stability of foxp3 protein and impair the Treg cells' suppressive function. Phosphatase PP1 was able to maintain the inhibitory effect of foxp3 by mediating ser-418 dephosphorylation. In response to TCR stimulation, the kinase NLK phosphorylated seven sites such as Ser-19, Ser-156, Ser-189, Ser-273, Ser-278, Ser-295, and Thr-341 through the TAK1-NLK signaling pathway. Due to the phosphorylation of foxp3 protein, the Ubox in STUB1 cannot be recruited by HSP70 to bind to foxp3 protein; that is, the four lysine sites K227, K250, K263 and K268 cannot undergo K48-linked ubiquitination to maintain the stability of the protein (Figure 1B).

4.3. Foxp3 ubiquitin modification

foxp3 protein Ubiquitin is one of the types of modification after translation. Among them, E3 ubiquitin-conjugating enzymes can affect the function and stability of foxp3 protein through monubiquitination and polyubiquitination. Three E3 ubiquitin-conjugating enzymes, RNF31, Stub1 and TRAF6, are known to affect foxp3 protein. RNF31 mediates monubiquitination by regulating many signaling pathways such as TCR and BCR, and mediates polyubiquitination after forming complexes with HOIL-1 and SHARPIN. Stub1 mediates polyubiquitination. TRAF6 regulates nuclear transport to affect the polyubiquitination of foxp3 protein at Lys-262 using the K63-type polyubiquitination pathway.

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By removing the K48-type polyubiquitination tag of Lys-249 and Lys-251, the foxp3 can become more steady. USP21 modifies many residues such as Lys-206, Lys-216 and Lys-227, with k48 type modification, namely deubiquitination to prevent foxp3 degradation [15]. Deubiquitinating the K48-type polyubiquitin chain on foxp3 is how USP44 prevents its degradation when they are interact with each other. The interaction between USP22 and foxp3 prevents its degradation (Figure 1C).

4.4. Foxp3 methylation modification

Certain foxp3 sites' methylation has an effect on the function of Treg cells. The suppressive effect of Treg cells was affected by the methylation modification of the arginine and lysine residues of foxp3 protein. In methylation modification, protein arginine methyl transferase (PRMT) can transfer methyl groups and is connected to the foxp3 proteins. This process involves two members of the PRMT family: PRMT1 and PRMT5. PRMT1 catalyzes the methylation of foxp3 at arg48 and arg51, leading to dimethylarginine asymmetry.Inhibition of this process results in a decrease in the suppressive effect of Treg cells. Similarly, PRMT5 was able to keep the function of foxp3 steady by dimethylating foxp3 arg27, arg51 and arg146.The methylation status of arg51 is crucial in the suppressive function of Treg cells. If this site is mutated to lysine, Treg cells are less effective in suppressing. In the mice experiment, PRMT5 knockouted mice had a reduced number of Treg cells in the spleen and a reduced suppressor function of Treg cells that maintained a normal number in the peripheral lymphatic system [15]. This was presumed to be due to the lack of methylation at arg27, arg51 and arg146. In addition, Treg cells were found to have a better response to TGF-β by knocking down PRMT5, while TGF-β activates SMADs and STAT5 in cooperation with nuclear factor (NFAT) to induce foxp3 expression in Treg cells. Therefore, inhibition of PRMTs could reduce the function of foxp3. Therefore, the function of Treg cells can be suppressed. Thus, several PRMT5 inhibitors are currently in clinical trials or preclinical stages (Figure 1D).

4.5. Foxp3 glycosylation modification

The function of Treg cells is associated with the glycosylation of foxp3, which involves differentiation, growth, apoptosis and so on. Glycosylation modification of o-glycosylation in different extents influences foxp3 protein conformation, folding, expression and activity, etc. Most of the o-glycosylation occurs in the golgi apparatus, using glycosyl transferase to add a sugar base to the protein Ser or Thr residue. This process involves two enzymes, O-GlcNAc transferase (OGT) and O-GlcNAcase (OGA). O-GlcNAc is activated by TCR in Treg cells. It can perform O-linked N-acetylglucosaminylation (O-GlcNAc) to link foxp3 protein to the monosaccharide O-GlcNAc, which involves multiple sites of foxp3. OGT mediates glycosylation of O-GlcNA, whereas OGA mediates deglycosylation of O-GlcNA. Studies in mice have shown that the IL-2/STAT5 signaling pathway is weakened when O-GlcNAc modification is defective, which proves that glycosylation modification can activate STAT5 [16]. This can maintain the expression of foxp3 and then integrate the key signaling pathway, which means that the glycosylation can regulate the expression of foxp3 through the IL-2/STAT5 signaling pathway. In addition, hedgehog (Hh) signaling also promotes the O-GlcNAc modification of foxp3. Some molecules in the NκB pathway also interact with O-GlcNAc. The c-rel molecule can act as a cofactor of foxp3 and cooperate with p65, NFAT, Smad and CREB to promote the expression of foxp3. C-rel can be O-GlcNAc modified, which affects its binding to foxp3 promoter. This can decrease the expression of foxp3 and negatively regulates the immunosuppressive effect of Treg cells (Figure 1E).

4.6. Foxp3 lactic acid modification

Lactate, a small molecule, is crucial in cell growth and development. Because cancer cells far from blood vessels are in a condition of hypoxia, under hypoxic conditions glucose is able to produce ATP and pyruvate through glycolysis, which in turn is reduced to lactate by lactate dehydrogenase. Because of the Warburg effect, the lactate concentration in the center of cancer tissue can be as high as 50 mM, 5–20 times higher than the normal situation [17]. Due to the Warburg effect, excess lactate establishes an immunosuppressive environment conducive to the growth of cancer tissues, which means that

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immune cells are affected. At this point, lactate would, in turn, provide energy for tumor growth. In this case, the immunosuppressive function of Treg cells is enhanced. Lactate regulates the expression of foxp3 through the lactatation of MOESIN on the Lys72 residue, which interacts with TGF-β receptor I and the downstream SMAD3 signal. At the same time, there are still many breakthrough points for lactate modification in Treg. For example, most of the proteins involved in lactate modification are positively correlated proteins, and there is a lack of related research on the related proteins (Figure 1F).

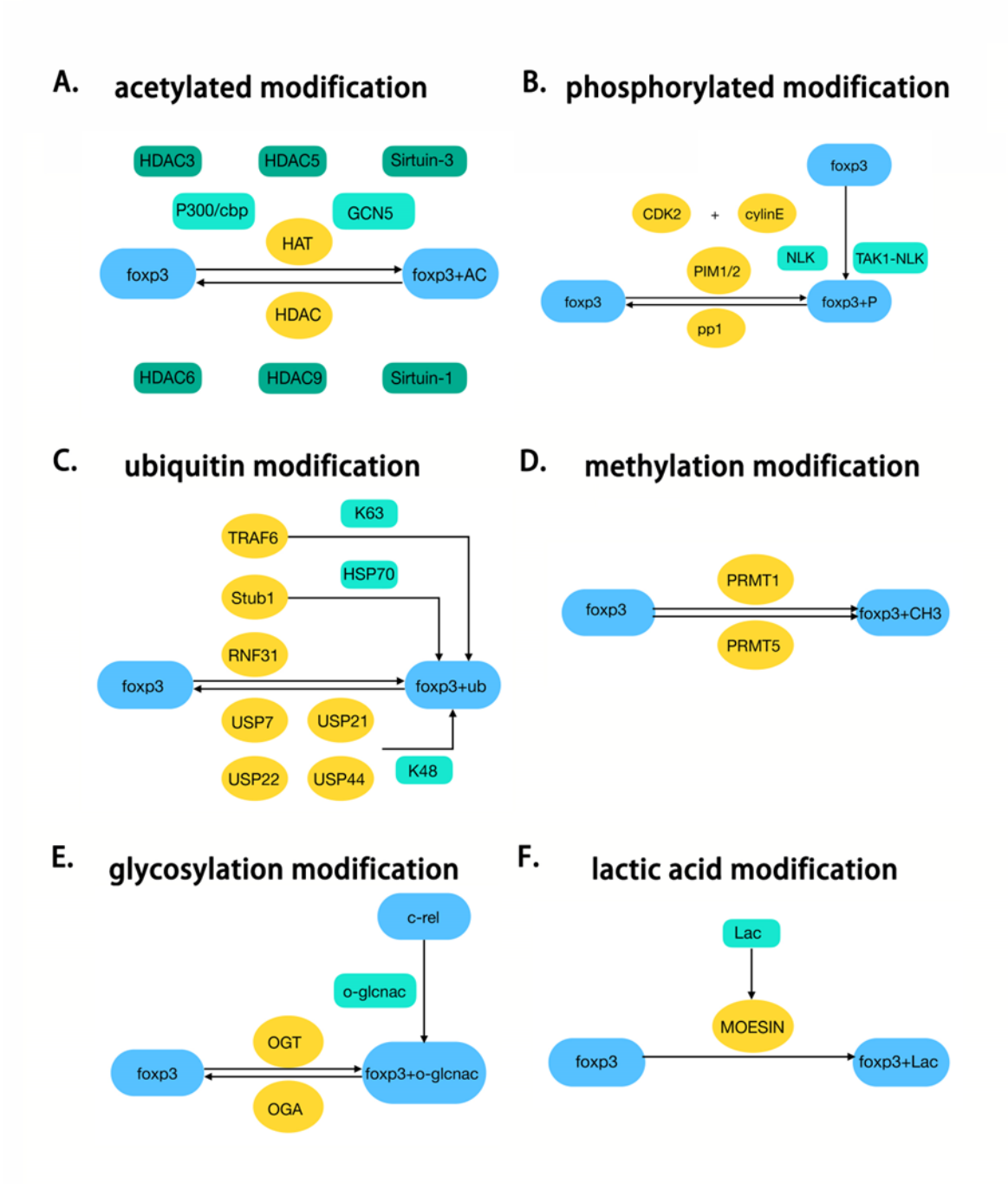

Figure 1. Six post-translational modifications of Foxp3.

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Foxp3 modification after translation, there are 6 ways were acetylation, (A) phosphorylation (B), ubiquitin (C), methylation (D), glycosylation (E), lactic acid (F). Some proteins involved in post-transcriptional modification have also been mentioned, namely (histone deacetylase 3) HDAC3, (histone deacetylase 5) HDAC5, (histone deacetylase 6) HDAC6, (histone deacetylase 9) HDAC9, To acetylation enzyme (3) mechanisms, mechanisms to acetylation enzyme (1) - 1, (acetylation enzyme) p300 / CBP, (histone acetyltransferase) GCN5, dependencies (cell cycle protein kinase) CDK2, cyclinE (cell cycle protein), pp1 (protein phosphatase 1), PIM1 (serine/threonine protein kinase 1), (serine/threonine protein kinase 2) PIM2, NLK kinase (nemo samples), (E3 ubiquitin ligase) TRAF6, (E3 ubiquitin ligase) Stub1, (E3 ubiquitin ligase ring finger protein 31) RNF31, (to ubiquitin 7) enzyme USP7, (to ubiquitin enzyme 21) USP21, 22) (to ubiquitin enzyme USP22, 44) (to ubiquitin enzyme USP44, arginine methyl transferase (1) PRMT1, PRMT5 arginine methyl transferase (5), (O - GlcNAc transferase) OGT, "OGA (β-N-acetylglucosaminidase), c-rel (NF-κB runner), MOESIN (cytoskeletal protein).

5. Immunotherapy resistance and modification of foxp3 in Treg mediated by pathway

5.1. CAR-T therapy and Treg

CAR-T cell therapy belongs to the adoptive cellular immunotherapy, which modifies and transforms T-cells through genetic engineering so that T-cells can specifically recognize tumor antigens and kill tumor cells. In the past decade, CAR-T therapy has made great progress and has successfully treated some patients with hematologic tumors, but CAR-T therapies have consistently failed to achieve the desired efficacy in targeting solid tumors, and how to deeply explore and eliminate those factors that hinder their potential is an urgent issue to be addressed.

The presence of Treg cells affects the effectiveness of CAR-T therapy and causes resistance to CAR-T therapy. foxp3, as a major transcription molecule of Treg cells and also a signature molecule, plays an important role in the inhibitory effect of Treg cells. Therefore, influencing the post-translational modification of foxp3 and reducing the function of foxp3 can effectively improve the function of CAR-T cells and enhance the anti-tumor response. Inhibition of phosphatase PP1 and deubiquitylated kinase can reduce the inhibitory effect of foxp3, and inhibitors of both have the potential to enhance the therapeutic ability of CAR-T therapy in combination with CAR-T therapy. However, there are fewer relevant studies on the effect of post-translational modification of foxp3 on CAR-T.

CTLA-4 (cytotoxic T-lymphocyte-associated protein 4) and PD-1 are known in preclinical studies to block the immune response and reduce the efficacy of CAR-T. To improve CAR-T efficacy, one of the extremely effective potential approaches is to block immune checkpoints such as CTLA-4 or PD-1 by applying specific antibodies or small molecule inhibitors. Blocking PD-1 immunosuppression enhances CAR-T immunotherapy and increases tumor elimination.

Studies have shown that the proportional balance between Treg and effector cells is regarded as an important indicator for evaluating the therapeutic efficacy. To further enhance the anti-tumor effect of CAR-T immunotherapy, researchers have explored a potential approach to modify the co-stimulatory structural domains of CAR-T cells through genetic modification and structural optimization, thereby reducing the inhibitory activity of Treg cells and enhancing the persistence and drug resistance of CAR-T cells. Classical co-stimulatory structural domains such as CD28 and 4-1BB can enhance cytokine secretion and CAR-T cell proliferation, resulting in a significant increase in overall anti-tumor efficacy. On this basis, combining mutant CD28 (mut06) with 4-1BB enhanced the in vivo anti-tumor activity and durability of CAR-T cells while reducing T-cell depletion. The combination of mut06 and 4-1BB improved the anti-tumor effect compared to mut06 and 4-1BB alone [18].

5.2. IL-2R/STAT pathway regulation of foxp3 for CAR-T therapy

IL-2, as a key immunomodulatory factor, has a signaling pathway that involves three major signaling systems, including the JAK-STAT signaling pathway. When IL-2 binds to the IL-2 receptor (IL-2R), it activates receptor tyrosine kinases (e.g., JAK1, JAK3, and TYK2), which further phosphorylate STAT5

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(Signal Transduction and Activation of TF 5) to form activated STAT5A and STAT5B. and activated STAT5A and STAT5B go to the nucleus to regulate expression of key TFs such as foxp3, affecting Treg development and function, and thus promoting CAR-T cell activity and proliferation.

In mouse knock-out studies, knockdown of STAT5B in CD4 T cells downregulated the expression of foxp3 mRNA by 50% within 24 hours, suggesting that the lack of STAT5B decreases the expression of foxp3 and also impairs Treg suppression [19]. In addition, modulation of foxp3 expression through the IL-2R/STAT pathway can also regulate the immunosuppressive function of CAR-T cells, thus improving their anti-tumor effects.

5.3. TGF-β/SMAD pathway regulation of foxp3 for CAR-T therapy

TGF-β is a cytokine that binds to its type II receptor (TGFBR2) and induces phosphorylation of the GS domain (glycine and serine rich residues) in the type I receptor. The phosphorylated type I receptor further activates the SMAD protein to become the SMAD complex and translocate into the nucleus. In the nucleus, the SMAD complex binds to the promoter of the foxp3 gene and controls the expression of foxp3 by regulating the activity of TFs, which in turn affects CAR-T cells.

The activated TGF-β/SMAD pathway can affect the activity and function of CAR-T cells. Specifically, TGF-β may inhibit the proliferation and activation of CAR-T cells and reduce their ability to kill tumor cells. Some studies have confirmed that M2-type macrophages can activate the TGF-β/SMAD signaling pathway, which promotes colorectal tumorigenesis and progression by increasing the expression of foxp3+ Tregs. By secreting TGF-β, M2-type macrophages can alter the tumor microenvironment, making it difficult for CAR-T cells to function effectively in this environment [20].

Conversely, inhibiting the TGF-β/SMAD pathway can improve CAR-T efficacy. The researchers used CRISPR/Cas9 technology to edit the TGFBR2 gene on the surface of CAR-T to block the TGF-β signaling pathway and prevent CAR-T cell depletion. Experiments in solid tumor model mice showed that the gene-edited CAR-T cells had stronger and longer-lasting tumor clearance in a TGF-β-enriched environment [21].

6. Foxp3 and targeted inhibitors

Histone acetylation enzyme inhibitors (HATi) are a new kind of epigenetic regulation of antitumor drugs. It inhibits the acetylation of foxp3 and reduces the stability of foxp3, leading to a decrease in the binding ability of foxp3 to DNA. Therefore, the role of foxp3 is reduced, which results in a decrease in Treg cell inhibition ability. At present, the study of HATi has made some progress in p300/cbp inhibitors. DCBP-1 and JET-209 are inhibitors directed against the bromodomain, whereas JQAD1 is an inhibitor directed against the HAT domain. All three of these inhibitors are under different conditions for different degrees of inhibition of p300/CBP. However, it is difficult to completely inhibit the carcinogenic ability of p300/cbp by inhibiting its HAT domain and bromodomain alone because multiple structural domain is essential in p300 / CBP work process. The protein degradation strategy based on protein degradation targeting chimeras can have a stronger anti-tumor effect. This could become the key to the development of new drugs.

Kinases USP21 and USP22 can induce the up-regulation of foxp3 protein expression, and the loss of these two kinases will lead to the weakening of foxp3 protein function, thereby reducing the inhibitory function of Treg cells. Therefore, USP22 can be used as a drug target to achieve anti-tumor therapy in the tumor microenvironment. According to studies, some research groups have developed small-molecule inhibitors of USP22 and found that the expression of foxp3 protein in Treg cells can be significantly reduced by them. So they can enhance tumor immunotherapy [22].

Human Treg cells display a high expression of the PIM1 protein kinase, which interacts with foxp3 at Ser422 and phosphorylates it. foxp3 at serine (418) is prevented from being phosphorylated by PIM1 at serine (422). The expression of target genes, including CD25, CTLA4, and GITR, was enhanced when PIM1 was knocked down. foxp3's inhibition of IL-2 gene expression could be lowered by this and Tregs' immunosuppressive activity could be increased.In addition, PIM1-specific inhibitors enhance the activity of the foxp3 binding with DNA, which is produced by Treg in vitro amplification of the original

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generation, and increase its effect on T cell proliferation inhibitory activity.In summary, the immunosuppressive activity of human Tregs can be made by PIM1 protein kinas , and therefore more attention needs to be paid to this target in cancer therapy.

The PRMTs family is able to keep Treg cells functional by maintaining foxp3 methylation. To lessen the inhibitory effect of Treg cells in tumor tissues, it is necessary to demethylate some sites of foxp3 to a certain extent. Thus, it is necessary to inhibit the PRMTs family. MRTX1719, as an inhibitor, can selectively inhibit PRMT5. It shows antitumor activity at a certain dose in methylthioadenosine phosphorylase-deficient tumors and inhibits prmt5 through SDMA modification [23].

Inhibition of foxp3 glycosylation also reduced the suppressive effect of Treg cells. The OGT inhibitor OSMI-1 alone can inhibit the glycosylation process of foxp3, and combining it with doxorubicin (DOX) can induce apoptosis of HepG2 cells [24]. Based on the role of lactate in tumor tissue, inhibition of lactate dehydrogenase can reduce the production of lactate, thereby reducing the inhibitory effect of Treg cells. The inhibitory effect of Treg cells may be enhanced due to the combination of MOESIN with TGF - bRII and its impact on its expression, thus enhancing the inhibitory effect of Treg cells. Therefore, MOESIN might be a potential drug target.

7. Conclusion

With the further development of immunology, oncology and molecular biology, the ways in which tumors escape, especially the post-translational modification of foxp3 protein and FOXP3-related TFs, have been revealed in recent years, and the study of the tumor microenvironment has gradually deepened. Targeted inhibitors, such as HATi and USP22, are specific small-molecule inhibitors that have been proposed. PIM1 protein kinase, MOESIN, etc. can be used as new drug targets for research. How to block inhibitory markers in immunotherapy is also discussed. Blockade of immune checkpoints with antibodies or small molecules and the combination of CAR-T costimulatory domains can make the immune efficacy of CAR-T improved. In addition, down-regulation of foxp3 expression through signaling pathways can also improve the anti-tumor effect of CAR-T. Numerous problems that require further exploration are still present due to the diversity and complexity of tumors. It is an essential task for tumor immunity to find more target and change the immunosuppressive state of the tumor microenvironment. The various molecules and structures involved in post-translational modification of foxp3 that affect the efficacy of CAR-T therapy need to be further explored. The transcription of foxp3 involves a variety of transcripts, each of which plays a different role, and the mechanism has not been fully elucidated. Inhibitors of the PRMTs family have side effects when used alone. At the same time, the role of the zinc finger domain in foxp3 protein is not clear, the research on lactate started late, and the mechanisms are not clear. These problems still need to be discussed, which may lead to new drug targets and new immunotherapy methods through the study of foxp3 in the future.

Authors Contribution

All the authors contributed equally and their names were listed in alphabetical order.

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