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FoxP3 in T_{reg} cell biology: a molecular and structural perspective

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Summary

Regulatory T cells (T_{regs}) are specialized in immune suppression and play a dominant role in peripheral immune tolerance. T_{reg} cell lineage development and function maintenance is determined by the forkhead box protein 3 (FoxP3) transcriptional factor, whose activity is fine-tuned by its post-translational modifications (PTMs) and interaction partners. In this review, we summarize current studies in the crystal structures, the PTMs and interaction partners of FoxP3 protein, and discuss how these insights may provide a roadmap for new approaches to modulate T_{reg} suppression, and new therapies to enhance immune tolerance in autoimmune diseases.

Keywords: allosteric modifiers, FoxP3, protein interactions, post-translational modifications, regulatory T cells

Introduction

Regulatory T cells (T_{regs}) maintain peripheral immune homeostasis and limit inflammatory tissue damage during vigorous and deleterious immune responses. The forkhead box protein 3 (FoxP3) transcription factor is an important regulator of the T_{reg} cell lineage. FoxP3 mutations often leads to 'Scurfy' in rodents and to X-linked autoimmune disorders (XLAAD) in humans. FoxP3 protein contains multiple structural domains, interaction partners and various kinds of post-translational modifications. Studies of the regulatory mechanisms of T_{reg} cells have been partially elucidated by manipulating and studying changes in FoxP3 protein function.

In this review, we will first introduce current understanding of the FoxP3 protein's function in T_{reg} cell biology, and then review the known FoxP3 domain crystal structures, FoxP3 interaction partners and post-translational modifications. We then discuss ways to explore the complexity of FoxP3 by rationally designing compounds to modify FoxP3 protein function based on its structure or the structure of associated molecules that mediate a specific function. This review focuses on the regulation of T_{reg} cell function via targeting the molecule and associated modifiers as well as the atomic structure of the FoxP3 protein.

FoxP3 and T_{reg} cells

Immune tolerance processes enable our immune system to respond to non-self-induced damage while limiting collateral damage to self-tissues. The first studies to define T_{reg} cells as the basis for infectious tolerance were from the Waldmann laboratory [1]. $CD4^+CD25^+$ T_{regs} are immune suppressive T cells which play a predominant role in

peripheral immune tolerance [2]. T_{reg} cells can be classified into two main types: thymus T_{reg} cells (tT_{reg}) and peripheral T_{reg} cells (pT_{reg}), based on current understanding of their origins [3,4]. Although many factors contribute to the stable T_{reg} cell lineage, including surface protein markers [5–7] and genomic epigenetic modifications [8,9], accumulating evidence has demonstrated that FoxP3 transcription factor acts as a dominant regulator in T_{reg} cell development and function maintaining [10–12].

The role of FoxP3 in regulating immune tolerance was determined by analysis of lethal autoimmunity that developed in the Scurfy mice and in the immune dysregulation polyendocrinopathy enteropathy X-linked syndrome (IPEX) patients, a recessive immune disorder that appears at an early age [13–15]. Targeted deletion of FoxP3 in $CD4^+$ T cells of mice leads to severe autoimmunity [16], while ectopic expression of FoxP3 can re-program conventional $CD4^+$ T cells into cells which act as anti-inflammatory T_{reg} cells, and confers certain capacities to suppress autoinflammation *in vivo* [10,11]. Enforced expression of FoxP3 induces a T_{reg} phenotype in human leukemic Jurkat T cells, including increased expression of T_{reg}-associated cell surface markers as well as inhibition of cytokine production. FoxP3-transduced Jurkat T cells suppress the proliferation of human conventional T cells [17].

FoxP3 belongs to a forkhead transcription factor family which mainly localizes in the nucleus [18,19]. Genome-wide analyses of FoxP3 target genes has revealed that FoxP3 binds to perhaps as many as ~700 genes and miRNA, which are involved in the T cell receptor (TCR) signaling pathway, cell communication and transcriptional regulation [20]. This complexity highlights the important role for FoxP3 in modifying transcriptional and receptor signaling networks in T_{reg} cell development and maintenance. FoxP3 directly targeted genes comprise only a small portion of the entire program of Foxp3-dependent gene expression, suggesting that FoxP3 regulates a substantial part of the FoxP3-dependent transcriptional program indirectly through association with other transcription factors. FoxP3 plays a dual role as both a transcriptional activator and repressor of its target genes in T_{reg} cells [21]. FoxP3 heterodimerize with FoxP1, which enforces FoxP3-mediated regulation of gene expression and co-ordinates regulatory T cell function [22].

To maintain the T_{reg} lineage specificity under normal physiological conditions, FoxP3 has been suggested to reprogram certain T cell metabolic processes to modify cellular function in stressful environments. FoxP3 has been observed to suppress Myc expression and cell glycolysis. While lactate levels impair effector T cells through lactate dehydrogenase (LDH)-mediated nicotinamide adenine dinucleotide (NAD) depletion, FoxP3 expression may limit pyruvate to lactate reduction. Ectopic expression of FoxP3 expression increases oxygen consumption rates (OCR) and oxidative phosphorylation (OXPHOS) [23].

Structure of FoxP3 domains

Structurally, FoxP3 contains three major domains: a proline-rich N-terminal domain (1–97 aa) responsible for transcriptional activation and repression, a central zinc-finger leucine-zipper domain (98–260 aa) implicated in oligomer formation or association with other factors and a conserved C-terminal forkhead domain (337–423 aa) responsible for DNA binding. These domains operate together to modulate FoxP3 function [24].

A high-resolution crystal structure of a ternary complex containing the NFAT1 DNA-binding domain, the FoxP3 FKH domain and the ARRE2 site DNA of the interleukin (IL)-2 promoter has been both modeled and solved. Although the ternary complex of FoxP3, NFAT and DNA was observed

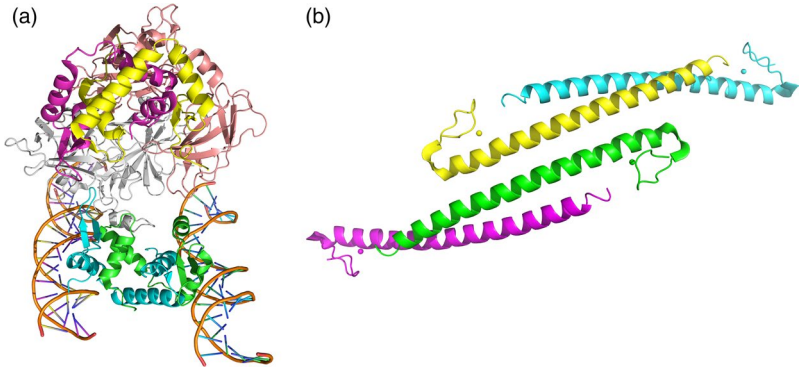

Fig. 1. (a) The FKH domain of forkhead box P3 (FoxP3) forms a domain-swapped dimer. One FKH monomer (green) forms a domain-swapped dimer with the other FKH monomer (cyan), thereby bridging two molecules of DNA. The picture was generated based on the crystal structure of FoxP3 forkhead domain complexed with DNA and NFAT (PDB code 3QRF) using Pymol. (b) The crystal structure of FoxP3 ZL domain, which tends to form oligomerization through a coiled-coil motif. The picture was generated based on the structure of FoxP3 ZL domain (PDB code 4I1L) using Pymol.

only in the presence of DNA containing the composite ARRE2 element, the FKH domain of FoxP3 tends to form a domain-swapped dimer through a DNA-independent process (Fig. 1a). An IPEX mutation (F373A) in the FKH domain may impair domain-swapping, which might abrogate FoxP3 suppressor function. Furthermore, the FoxP3 FKH dimer can simultaneously bind two distinct FoxP3-binding sites in solution and bring them into close approximation [25]. These structural and biochemical analyses suggest that a unique transcriptional function of FoxP3 is to mediate long-range interactions of FoxP3-targeted genes. In fact, another study revealed that FoxP3 is specialized for bridging long-distance genomic elements and reorganizes the three-dimensional structure of the T cell genome to stabilize cellular differentiation programs. The circular chromosome conformation capture sequencing (4C-seq) analysis demonstrated that FoxP3 could reorganize the chromosomal 'interactome' at the Ptpn22 locus by domain-swapping and/or through interactions with other factors [26].

The crystal structure of the mouse FoxP3 zinc finger and leucine zipper domain (FoxP3-ZL) from our laboratory provided details about the atomic features of homo- and hetero-association of FoxP3. The mFoxP3-ZL homodimer features an unusual two-stranded anti-parallel α-helical coiled coil with a twofold symmetry, and crystal packing analysis revealed that the mFoxP3-ZL coiled-coil dimer tends to form clusters due to the extended hydrophobic stretch on the coiled coil surface (Fig. 1b). Lysine 251 plays an indispensable role in FoxP3 coiled coil-mediated dimerization, and the delK251 mutant mFoxP3 fails to repress IL-2 production. Of note, K249 and K251 of mouse FoxP3, i.e. human FoxP3 K250 and K252 in the coiled coil region, were identified as acetylation sites, which implies that a structure-based regulation of the conformation and activity of FoxP3 protein by integration of post-translational modifications may modulate T_{reg} function [27].

The crystal structure of the FoxP3 N-terminal region and the full-length FoxP3 are still unavailable, because the N-terminal region displays intrinsic disorder. We have been successful in developing small crystals of an N-terminal region that extends to amino acid 262, and we postulate that partner binding and/or post-translational modification may stabilize this region and help to define the structural features of the FoxP3 N-terminal domain. The roles of binding partners and post-translation modifications in regulating FoxP3 function have been defined to some extent.

Importance of FoxP3 interactions

FoxP3 has a proline-rich N-terminal domain in comparison with other members of FoxP3 transcriptional factor family [28]. This unique N-terminal domain of the FoxP3 protein leads to interaction with many molecules, including FoxP1 [29], Eos [30], AML1/Runx1 [31], NFAT [32], RORα [33], TIP60 and HDAC7 [34], which are involved in various aspects of T_{reg} function and development, as reviewed elsewhere [35].

Studies have revealed that a green fluorescent protein (GFP) insertion at the FoxP3 N-terminal without a flexible linker leads to steric hindrance that blocks interaction with Eos, Tip60 and HDAC7, and thus to impaired iT_{reg} development and nT_{reg} stability in an autoimmune or inflammatory environment. Consequently, $FoxP3^{gfp}$ expression on the autoimmune-prone non-obese diabetic (NOD) background dramatically accelerated diabetes [36]. The N-terminal insertion of GFP of FoxP3 alleviates arthritis but exacerbates diabetes. Mechanistically, $Foxp3^{fgfp}$ T_{reg} cells display a significant over-representation of interferon regulatory factor 4 (IRF4)-dependent transcripts in which GFP insertion at FoxP3 N-terminal enhances the physical interaction with IRF4, but abrogates the interaction with hypoxia-inducible factor 1 (HIF-1). In contrast, the FoxP3 and FoxP3 heterodimer formation thought to occur via the leucine-zipper region distal from the GFP insertion site remains intact.

These observations imply that perturbations in T_{reg} cell phenotype and functional characteristics can affect autoimmune diseases in a unique but unfortunately unpredictable manner, and that specific modifications of FoxP3 and its interactions could serve as a basis for therapeutically modulating T_{reg} cell function in a qualitative manner [37].

T cells from rheumatoid arthritis patients display down-regulated TIP60 expression, which impairs T_{reg} development. Ectopic Tip60 expression appears to rescue FoxP3 function and prevents synovial inflammation and immune cell infiltration of the engrafted synovial tissue [38]. A missense mutation (A384T) in the FKH domain of FoxP3 impairs the suppressive function of T_{reg} cells while maintaining its ability to repress inflammatory cytokines.

A specific perturbation of FoxP3 interaction with TIP60 accounts for the impaired suppressive function of FoxP3A384T T_{reg} cells. The loss of function could be rescued by using an allosteric modifier to enhance FoxP3–TIP60 interaction pharmacologically (Fig. 2a). Our preclinical results have shown that the allosteric modification of TIP60 improves the FoxP3-mediated transcriptional program in FoxP3A384T T_{reg} cells, the therapeutic restoration of FoxP3–TIP60 interaction by this small molecule modifier promotes T_{reg} cell function without directly affecting T effector cell responses, and enhances disease protection in mouse models of colitis and collagen-induced arthritis [39].

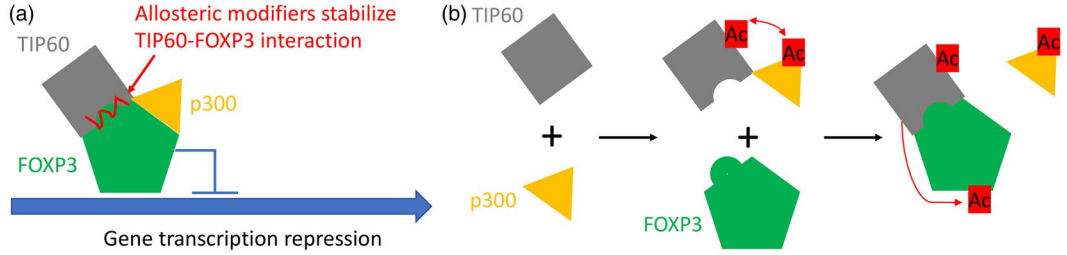

Fig. 2. Enhanced regulatory T cell (T_{reg}) suppressive function by targeting TIP60, p300 and forkhead box P3 (FoxP3) interaction. (a) A model of TIP60 allosteric modifiers help to stabilize TIP60–FoxP3 interactions by delaying the release of TIP60 from the TIP60–p300-FoxP3 complex. (b) TIP60 and p300 promote the acetylation of each other, and then promote FoxP3 acetylation co-operatively (gray square = TIP60; orange triangle = p300; green pentagon = FoxP3; red Ac = acetylation).

These studies imply that FoxP3 functions as a transcriptional complex defined by multiple FoxP3 partners. In fact, a single amino acid deletion of the zinc finger–leucine zipper domain region of FoxP3 (delE251) that defines an IPEX mutation disrupts the formation of the complex and heteromerization with FoxP1. This disruption limits repression of IL-2 transcription by FoxP3 upon T cell activation and compromises T_{reg} cell suppressive function, which explains some features of the pathogenesis of a disease syndrome developed in IPEX patients [29]. Certain aspects of the super-complex have also been comprehensively analyzed using biochemical and mass-spectrometric approaches [40]. The efforts focused on a T cell line TCli expressing biotin-tagged FoxP3 expression protein, and isolated the FoxP3 interaction complex by biotinylation-based pulldown, and then sequenced molecules by microliquid chromatography. Biotin-tagged FoxP3 forms multi-protein complexes of 400–800 kDa and associates with 361 partner proteins, among which ~30% are transcription-related. Of note, expression of many of these partners may also be influenced by FoxP3-related processes [40].

Post-translational modifications of FoxP3

Post-translational modifications (PTMs) represent a dynamic mechanism to co-ordinate environmental signals and the functional properties of proteins. There is emerging evidence indicating that the transcriptional activity of FoxP3 can be fine-tuned by its post-translational modifications.

Phosphorylation of FoxP3

Phosphorylation modification can occur on serine, threonine and tyrosine residues of protein, and these modifications are reversible. Phosphorylation regulates multiple aspects of protein function, including stability, localization and trafficking. Mouse and human FoxP3 sequences contain several conserved serine, threonine and tyrosine residues, which are potential interaction sites of certain kinases. FoxP3 can be phosphorylated on threonine residues [41].

The primary structure of FoxP3 contains four cyclin-dependent kinase (CDK) motifs (Ser/Thr-Pro) within the N-terminal repressor domain. CDK2 can partner with cyclin E to phosphorylate FoxP3 at these sites. Mutation of the N-terminal CDK motifs (Ser/Thr3>Ala) in FoxP3 elevated protein stability and increased FoxP3 transcriptional activity. Consistently, CD4 T cells expressing the Ser/Thr3>Ala mutant FoxP3 displayed significantly elevated suppressive capacity compared with cells expressing wild-type FoxP3 [42]. These results explain the finding that Cdk2-deficient T_{reg} are more suppressive than wild-type T_{reg}, as measured by the ability to suppress the proliferation of conventional $CD4^+$ T cells *in vitro* and to ameliorate colitis in an *in-vivo* mouse model of inflammatory bowel disease [43]. A role for CDK2 in influencing T_{reg} function through phosphorylation-dependent regulation of FoxP3 is apparent.

Pim-1 and Pim-2 kinases can act as oncogenic serine/threonine kinases, and their over-expression contributes to lymphoid transformation. Pim-1 and Pim-2 kinases promote the rapamycin-resistant survival, growth and proliferation of lymphocytes, including T_{reg} cells [44,45]. Both Pim-1 and Pim-2 kinases have been suggested to phosphorylate FoxP3 protein. Sequence alignment analysis predicts a potential Pim-1 interaction and phosphorylation motif within the FoxP3 forkhead domain. Pim-1 phosphorylates human FoxP3 at Ser422 of the forkhead domain and attenuates FoxP3 DNA binding activity in regulation of T_{reg} feature genes [46]. In contrast, Pim-2 kinase phosphorylates at multiple sites in the FoxP3 N-terminal domain, as identified by mass spectrum analyses. Pim-2-deficient mice are more resistant to dextran sulfate sodium (DSS)-induced acute colitis than their wild-type counterparts [47]. Phosphorylation of FoxP3 by Pim-1 and Pim-2 kinase negatively regulates T_{reg} suppressive function. Of note, TCR stimulation down-regulated Pim-1 expression but up-regulated FoxP3 expression. Therefore, at least some FoxP3 phosphorylation mediated by Pim-1 may be regulated by TCR stimulation.

FoxP3 phosphorylation is enhanced by TCR activation with incubation of anti-CD3/CD28 antibodies or pharmacological treatment with phorbol 12-myristate 13-acetate (PMA) and ionomycin. The TCR-mediated signaling pathway can regulate FoxP3 phosphorylation through the activation of Nemo-like kinase (NLK) in a transforming growth factor (TGF)-β TAK1-dependent manner. NLK-mediated phosphorylation of FoxP3 resulted in the stabilization of protein levels by preventing association with the STUB1 E3-ubiquitin protein ligase and proteasomal degradation. Conditional deletion of NLK in T_{reg} cells results in the loss of *in-vivo* suppressive capacity and autoinflammation in aged mice. NLK directly phosphorylates FoxP3 on multiple residues in the same manner as Pim-2 and CDK kinases [48].

One substantial caveat in these studies is that NLK or Pim-2 cannot be predicted to phosphorylate specific sites of FoxP3. There is incomplete information concerning the existence of other endogenous kinases involved in FoxP3 phosphorylation. It is clear, however, that FoxP3 undergoes phosphorylation modifications, and phosphorylation of FoxP3 can either positively or negatively modulate T_{reg} function.

Human and mouse FoxP3 possess four evolutionarily conserved tyrosine residues. Lymphocyte-specific protein tyrosine kinase (LCK), a Src family kinase that regulates tumor progression, co-localizes with FoxP3 in the MCF-7 cell nucleus and phosphorylates Tyr-342 of FoxP3. Tyr-342 phosphorylation of FoxP3 is involved in the inhibitory regulation of cancer malignancy by SKP2, vascular endothelial growth factor (VEGF)-A and matrix metallopeptidase 9 (MMP9) expression, while Y342F mutant blocks this inhibition [49]. Our preliminary data indicate that comparable FoxP3 tyrosine phosphorylation also occurs in human T cells, although its biological significance in modulating T cell function remains further investigation (unpublished data).

Tumor necrosis factor (TNF)-α may impair synovial T_{reg} cell function, a process that relates to the pathogenesis of active rheumatoid arthritis. TNF-α induces protein phosphatase 1 (PP1) to dephosphorylate Ser-418 in the C-terminal DNA-binding domain of FoxP3, and TNF-α antagonist therapy (for example, treatment with a TNF-α-specific antibody: infliximab) may restore T_{reg} cell function [50].

Acetylation/ubiquitination of FoxP3

Acetylation and ubiquitination are post-translational modifications of FoxP3, which have attracted extensive studies. Acetylation and ubiquitination are often mutually exclusive. Acetylation modifications may prevent polyubiquitination that occurs on the same lysine residue through a competition-based mechanism.

Histone acetyltransferases (HATs) and histone deacetylases (HDACs) are enzymes responsible for histone acetylation, but are also involved in acetylation of protein substrates. There are three major subsets of HATs: the Gcn5/PCAF family, the p300/CBP family and the MYST family [51]. TIP60 is a member of the MYST family, and was the first identified acetyltransferase that associates with and promotes FoxP3 acetylation. As discussed above, TIP60 functions as an essential subunit of the FoxP3 repression complex. The N-terminal 106–190 aa of FoxP3 is required for TIP60–FoxP3 interactions. Knock-down of endogenous TIP60 by targeted short hairpin RNAs (shRNA) relieves FoxP3-mediated repression [34]. p300, a member of p300/CBP family, was also suggested to acetylate FoxP3. Acetylation of FoxP3 by p300 prevents FoxP3 protein from proteasome-mediated degradation and treatment with histone deacetylase SIRT inhibitor (NAM) results in significantly increased numbers of functional T_{reg} cells [52].

TIP60 and p300 regulate FoxP3 acetylation and activity in a co-operative manner. p300 interacts with TIP60 to promote TIP60 autoacetylation and protein stability. The acetylation of TIP60 functions by acting with p300 as a molecular switch to allow TIP60 to change binding partners. Subsequently, p300 is released from this complex, and TIP60 interacts with and acetylates FoxP3. Reciprocally, TIP60 also promotes p300 acetylation that is critical for HAT activity of p300. Transcriptional activity of FoxP3 is thus promoted by TIP60 and p300 co-operative interactions (Fig. 2b). Conditional deletion of p300 ($p300^{fl/fl}$FoxP3YFP-Cre) in T_{reg} cells reveals that p300 has a modest effect on the suppressive function of T_{reg} cells. In contrast to $p300^{fl/fl}$FoxP3YFP-Cre mice, $Tip60^{fl/fl}$FoxP3YFP-Cre and $p300^{fl/fl}TIP60^{fl/fl}$FoxP3YFP-Cre mice developed severe and fatally autoimmune diseases at an early age, indicating that TIP60 plays a dominant role for the development and function of T_{reg} cells [53].

Loss of abundance of T_{reg} cells accounts for certain autoimmune diseases. SIRT1, a member of the lysine deacetylase Sirtuin (SIRT) family, may decrease FoxP3 acetylation. SIRT1 inhibition may affect FoxP3 acetylation and alter FoxP3 protein levels [52]. Sirt1-deficient iT_{reg} cells display increased FoxP3 stability and demonstrate restrained iT_{reg} conversion into pathogenic T cells. Administration of a pharmacological Sirt-1 inhibitor, Ex-527, can attenuate graft-*versus*-host disease (GVHD) while preserving the graft-*versus*-leukemia effect [54]. Treatment of mice with the pan-HDAC inhibitor trichostatin-A (TSA) was found to increase the proportions and absolute numbers of $FoxP3^+CD4^+$ T cells in peripheral lymphoid tissues. $HDAC9^{-/-}$ mice develop less DSS-induced colitis than wild-type mice. HDAC inhibitor therapy increases acetylation of both histone and the forkhead domain of FoxP3,

as well as FoxP3 protein expression and DNA binding. These changes lead to enhanced T_{reg} suppression and anergy by promoting the association of FoxP3 with target genes. HDAC6, HDAC9 and Sirt1 all deacetylated FoxP3. However, each protein mediates specific effects on discrete transcription factors that control expression of the gene encoding FoxP3, which suggests that combinations of HDAC inhibitors may be used to augment the therapeutic benefits for treating autoimmunity and organ transplantation [55].

Butyrate, a short-chain fatty acid (SCFA), produced by commensal microorganisms during starch fermentation, increases FoxP3 protein acetylation and facilitates extrathymic generation of T_{reg} cells [56]. Treatment with sodium butyrate up-regulates FoxP3 and IL-10 expression, and mitigates inflammatory skin reactions in part by induction of functionally active $FoxP3^+$ T_{reg} cells [57].

HDAC inhibitor therapy has been evaluated to modify T_{reg} function *in vivo* and shows promising effects on allograft survival and autoimmune diseases [58]. The administration of the HDAC inhibitor vorinostat in combination with standard GVHD prophylaxis is associated with a lower than expected incidence of severe acute GVHD. Vorinostat-treated patients who received allogeneic hematopoietic cell transplant (allo-HCT) experienced significant reductions in the plasma levels of proinflammatory cytokines and promotion of $CD25^+CD127^-$ T_{reg} frequency of peripheral blood mononuclear cells (PBMCs) with greater suppressive function [59].

FoxP3 protein levels and turnover rate may affect T_{reg} cell lineage stability. The Deubiquitinase USP7 was found to be up-regulated in T_{reg} cells. Ectopic expression of USP7 decreased FoxP3 polyubiquitination and increased FoxP3 expression. T_{reg} cells pretreated with deubiquitinase inhibitor altered their function in prevention of adoptive transfer-induced colitis [60]. It is thought that FoxP3 undergoes polyubiquitination and proteasome-based degradation in certain inflammatory conditions.

After lipopolysaccharide (LPS) stimulation, FoxP3 interacts with the stress-indicator protein heat shock protein (Hsp)70 and the E3 ubiquitin ligase Stub1. As a result, Stub1 mediates K48-linked polyubiquitination of multiple lysine residues of FoxP3, resulting in proteasome-based degradation. Knock-down of Stub1 by shRNA results in the dramatic accumulation of FoxP3 protein without changing the FoxP3 gene transcript, and promotes T_{reg} suppressive function *in vitro* and *in vivo* [61].

The E3 ubiquitin ligase Cbl-b was found to account for the defective development of thymic T_{reg} cells in *Cd28*-deficient mice. Upon TCR stimulation, Cbl-b, together with Stub1, targets FoxP3 for ubiquitination and subsequently degradation in the proteasome. The loss of Cbl-b rescues the defective development of tT_{reg} in *Cd28*-deficient mice [62]. Phosphorylation of FoxP3 by NLK increases FoxP3 protein stabilization by preventing association with the STUB1 E3-ubiquitin protein ligase [48]. A recent study reported that the E3 ligase TNF receptor-associated factor 6 (TRAF6) mediated FoxP3 K63-linked ubiquitination at the lysine 262 residue. K63-linked ubiquitination by TRAF6 ensures proper localization of FoxP3 and facilitates FoxP3 transcriptional activity in T_{regs} [63]. The E3 ubiquitin ligase ring finger protein 31 (RNF31) catalyzes the conjugation of atypical ubiquitin chains to the FoxP3 protein. This interaction positively regulates both FoxP3 stability and T_{reg} cell function. Moreover, RNF31 expression is correlated with intratumoral T_{reg} cells in gastric cancer, implying a potential role of RNF31 in mediating tumor immunity [64].

FoxP3 can methylate modification by the arginine methyl transferase protein PRMT5. Conditional knock-out of the PRMT5 gene in T_{regs} causes severe Scurfy-like autoimmunity. Consistently, pharmacological ablation of PRMT5 activity by DS-437 also reduces human T_{reg} functions, which enhance the anti-tumor effects of anti-erbB2/*neu* monoclonal antibody-targeted therapy in mice bearing CT26 human epidermal growth factor receptor (HER2) tumors [65].

FoxP3 acts as an important transcriptional factor for T_{reg} cell development and suppressive function in normal physiological and stressed biological environments. Understanding FoxP3 protein features and modulation mechanisms may help the design of rational therapies for immune disorders.

FoxP3 protein has various post-translational modifications, such as phosphorylation, acetylation, ubiquitination and methylation. Several relevant enzymes have been determined to involve in these modifications. In addition, accumulating evidence suggests that these post-translational modifications may affect FoxP3 protein conformation and interactions with other partners, which ultimately alters FoxP3 protein activity to modulate T_{reg} suppressive features. Based on the high-resolution crystal structure of FoxP3, combination therapy by targeting FoxP3 post-translational modifications with other therapies, may enhance therapeutic approaches in a variety of diseases.

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Disclosures

None.

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