Review

Foxp3 in the Immune System

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Abstract: Regulatory T cells (Tregs) play a central role in immune regulation and tolerance. The transcription factor FOXP3 is a master regulator of Tregs in both humans and mice. Mutations in FOXP3 lead to the development of IPEX syndrome in humans and the scurfy phenotype in mice, both of which are characterized by fatal systemic autoimmunity. Additionally, Treg dysfunction and FOXP3 expression instability have been implicated in non-genetic autoimmune diseases, including graft-versus-host disease, inflammatory bowel disease, rheumatoid arthritis, and multiple sclerosis. Recent investigations have explored FOXP3 expression in allergic diseases, revealing Treg alterations in food allergies, asthma, and atopic dermatitis. This review examines the multifaceted roles of FOXP3 and Tregs in health and various pathological states including autoimmune disorders, allergic diseases, and cancer. Additionally, this review focuses on the impact of recent technological advancements in facilitating Treg-mediated cell and gene therapy approaches, including CRISPR/Cas9-based gene editing. The critical function of FOXP3 in maintaining immune homeostasis and tolerance to both self-antigens and alloantigens has been emphasized. Considering the potential involvement of Tregs in allergic diseases, pharmacological interventions and cell-based immunomodulatory strategies may offer promising avenues for developing novel therapeutic approaches in this field.

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1. Introduction

The modulation of immune responses in vivo is orchestrated by regulatory T cells (Tregs) through their influence on immune reactions [1,2]. Forkhead box protein P3 (FOXP3) is the master regulator of Tregs. Following the initial discovery of Tregs in murine models, the significance of FOXP3 in the immune system has grown substantially owing to its pivotal role in immune homeostasis, tolerance induction, cancer, autoimmunity, and allergic diseases [1]. During thymic development, FOXP3 expression is subjected to epigenetic control, which determines the developmental trajectory of Tregs. This review explores the role of FOXP3 in the immune system and highlights recent advances in translational studies, including cell and gene therapy strategies.

2. Molecular Features of FOXP3

The Forkhead box protein family, a group of transcription factors, binds to specific genomic regions through forkhead domain [3] and is involved in cell growth, development, and differentiation. FOXP3 is a crucial transcription factor and a master regulator of Tregs in both humans and mice [1]. Structurally, FOXP3 is composed of a repressor, a zinc finger, a leucine zipper, and forkhead domains (**Figure.1**), forming a dimeric structure [4,5]. FOXP3 binds to IL2, CD25, and CTLA-4 loci, inducing a Treg-like gene expression profile. Corroborating these findings, RNA-seq analysis of human Tregs revealed a distinctive Treg-specific gene expression profile compared with effector T cells (Teffs) [6]. Additionally, Chip-on-chip analysis validated FOXP3 binding to its target site [7]. However, technical limitations, including the absence of a suitable antibody for Chip-seq, continue to impede the comprehensive mapping of genome-wide FOXP3 binding. ATAC-seq

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chromatin accessibility analysis of Tregs at both bulk cell and single-cell resolutions [8,9], revealed epigenetic, transcriptomic, and proteomic differences between Tregs and Teffs.

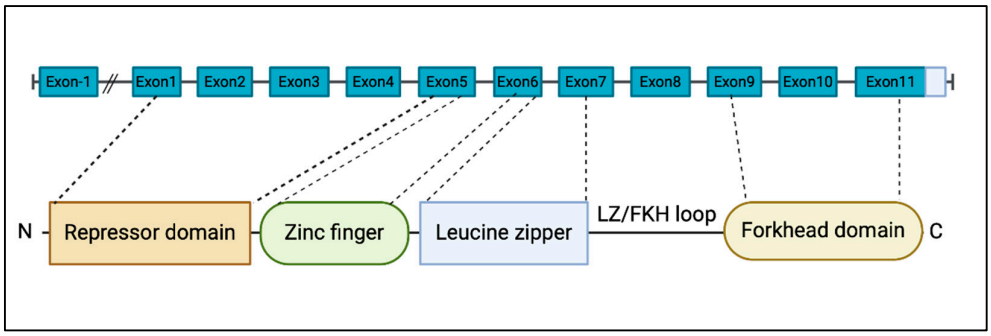

Figure 1. Molecular structure of FOXP3 gene.

3. FOXP3 expression in Tregs and Teffs

In humans, FOXP3 is expressed in both regulatory and effector T cells, albeit with differential regulation. The following section elucidates the functional and molecular differences between Tregs and Teffs.

3.1. FOXP3 Expression in Tregs

Tregs were initially identified as $CD4^+$ and $CD25^+$ cells in mice [10]. FOXP3 serves as a crucial regulator of Tregs[11,12], binding to target genes and influencing the gene expression profile. Sustained and robust FOXP3 expression in Tregs is subject to epigenetic regulation. The methylation status of conserved noncoding sequence 2 (CNS2), which is located between the promoter and enhancer regions, is determined during thymic development [13,14]. T cell progenitors with hypomethylated FOXP3 CNS2 that constitutively express FOXP3, differentiate into Tregs, whereas those with methylated FOXP3 CNS2, differentiate into Teffs (Figure.2). Additionally, the epigenetic profile of CNS2, also known as the Treg-specific demethylated region (TSDR), serves as a predictor of cell fate and aids in immunophenotyping diagnostics [15].

3.2. Activation-Induced FOXP3 Expression in T Cells

In contrast to murine T cells, human T cells express FOXP3 upon activation[16,17]. Although FOXP3 expression remains stable in Tregs, its activation-induced expression in T cells is transient and decreases following activation. Significantly, the methylation profile of FOXP3 CNS2 remained unaltered by activation, and activation-induced transient FOXP3 expression failed to trigger FOXP3 demethylation. Thus, activation-induced FOXP3 expression is transient and fails to differentiate Teffs into Tregs, a process that likely requires prolonged and enhanced FOXP3 expression.

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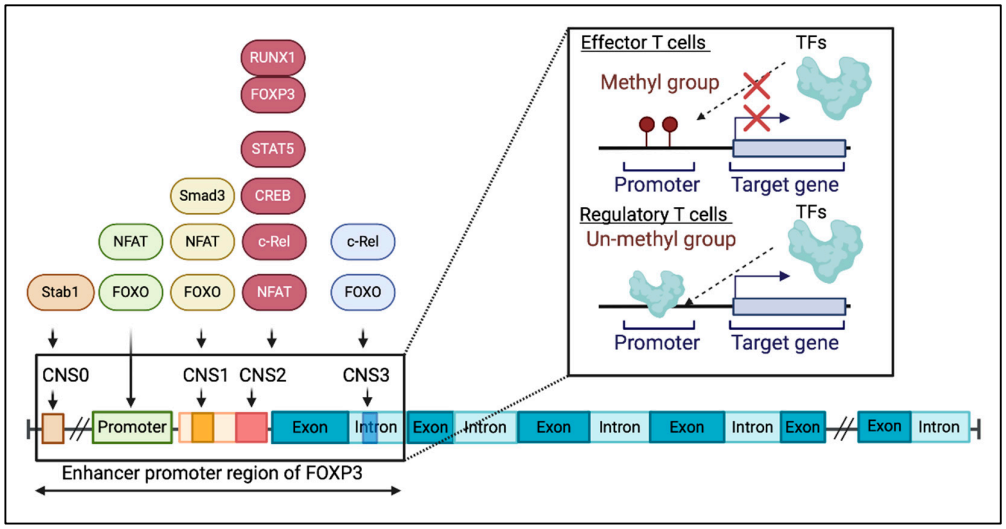

Figure 2. Promoter and enhancer regions of FOXP3 are controlled by methylation.

4. FOXP3 Gene Mutations Are Associated with IPEX Syndrome

Hemizygous FOXP3 mutations lead to immune dysregulation, polyendocrinopathy, enteropathy, and X-linked (IPEX) syndrome [18–21]. IPEX syndrome is characterized by severe eczema, type 1 diabetes (T1D), and inflammatory bowel disease (IBD), which manifests during the neonatal period. It is typically fatal in the absence of immunosuppressive therapy and/or stem cell transplantation [22,23]. Classified as an ultra-rare disease, the number of reported IPEX cases has increased, partly due to increased patient advocacy and awareness [24]. Analogous autoimmune manifestations are evident in mutations that affect diverse immunoregulatory molecules, including CD25, CTLA-4, LRBA, and BACH2. The concept of "Tregopathies" has emerged as a novel disease classification encompassing genetic autoimmune disorders caused by monogenic mutations in immunoregulatory molecules [25].

5. FOXP3, Implications in Autoimmune Disorders

Decreased FOXP3 expression has been documented in several autoimmune diseases including T1D, IBD, multiple sclerosis, rheumatoid arthritis, and systemic lupus erythematosus (SLE). The subsequent section explores the significance of FOXP3 in the pathogenesis of autoimmune diseases.

5.1. T1D

The presence of FOXP3 polymorphism was detected in T1D patients, although the polymorphism ratio did not exhibit a statistically significant increase within the studied cohort [26]. The critical role of Treg function in T1D has been emphasized by multiple researchers, with evidence pointing towards Treg dysfunction and instability. However, the relationship between Treg dysfunction and disease onset remains controversial [27]. The restoration of islet function following the onset of autoimmune reactions without resorting to conventional immunosuppressive therapies poses a substantial challenge. A promising therapeutic avenue may involve the utilization of adoptive or engineered Treg transfer in conjunction with transplantation of stem cell-derived islets.

5.2. IBD

Similar to T1D, Treg dysfunction or instability has been implicated in the pathogenesis of various immune-mediated disorders, including IBD, multiple sclerosis, rheumatoid arthritis, and SLE. A local imbalance between Teffs and Tregs has been reported in IBD. Experimental studies have demonstrated that adoptive Treg transfer significantly improves outcomes in murine colitis models, suggesting its potential therapeutic applications for IBD. However, the relationship between Treg

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dysfunction and IBD etiology remains controversial [28]. Further studies analyzing the immune cell profiles in patient samples are essential to elucidate gut tissue immunity for therapeutic discovery.

5.3. Multiple Sclerosis and Myasthenia Gravis

The role of Tregs in the pathogenesis of multiple sclerosis remains unclear despite multiple investigations demonstrating Treg dysfunction in both multiple sclerosis and myasthenia gravis [29,30]. A recent discovery revealed the presence of Tregs in the CNS under physiological conditions [31], suggesting their potential contribution to CNS immune tolerance. Similar to other types of autoimmune diseases, cell-based therapeutic approaches have been explored, with engineered T cells (chimeric autoantibody receptor T cells (CAAR-T cells)) showing promise in the treatment of autoimmune-mediated encephalitis [32,33]. Additional studies are warranted to understand disease mechanisms and explore alternative therapeutic strategies beyond conventional immunosuppressive approaches.

6. The Role of FOXP3 in Transplantation

6.1. The Role of FOXP3 in Hematopoietic Stem Cell Transplantation

In contrast to autoimmunity, the significant correlation between graft-versus-host disease (GvHD) and FOXP3 has been extensively investigated over the past years [34]. Because Treg dysfunction is a primary etiological factor in GvHD, adoptive Treg transfer has been explored as a potential therapeutic intervention [35]. Additionally, type-1-regulatory cells, another subset of Tregs, have been implicated in the pathogenesis of GvHD [36]. Recent research has focused on engineered type-1-regulatory cells as an alternative approach for adoptive transfer in GvHD treatment [37].

6.2. The Role of FOXP3 in Solid Organ Transplantation

FOXP3 plays a crucial role in maintaining tolerance in stem cell and organ transplantations, including liver, kidney, and islet transplantations, owing to the significant function of Tregs in preserving tolerance to self-antigens. In contrast to GvHD, the target antigen is an alloantigen stemming from the human leukocyte antigen (HLA) incompatibility between the donor and recipient. Consequently, adoptive Treg transfer using polyclonal or alloantigen-specific Tregs has been the subject of extensive research. Similar to GvHD, the potential of Tr1 infusion for kidney transplantation has been investigated [38].

7. The Role of FOXP3 in Allergic Disease

In FOXP3 mutant mice, the absence of Tregs resulted in allergic dysregulation and Th2 proliferation [39]. Tregs demonstrate a more pronounced suppressive effect on Th2 cells than on Th1 cells. Additionally, patients with IPEX syndrome and mice harboring specific FOXP3 mutations exhibit enhanced Th2 proliferation [40,41]. Th2 cells play a critical role in allergic reactions primarily through the production of IL-4 and IL-13. Dysfunctional Tregs and unstable FOXP3 expression may exacerbate allergen-initiated allergic reactions [42]. In addition to Th2 cells, Tregs potentially modulate various immune cells including B cells, mast cells, eosinophils, and basophils. Several studies have investigated the association between FOXP3 expression and allergic disease.

7.1. The Role of Tregs in Food Allergy

Tregs have been implicated in food allergies. The manifestation of severe food allergies as a clinical phenotype in IPEX syndrome [43], underscores the importance of FOXP3 in gut-associated food allergies. This was further supported by the induction of antigen-specific Tregs during oral immunotherapy for peanut allergies [44]. Additionally, FOXP3 hypomethylation was found to be associated with cow's milk allergy [45]. Thus, Tregs are crucial for modulating the gut immune system, indicating their potential involvement in food allergies [46]. Current research provides limited insights into the role of Tregs in allergic diseases beyond food allergies.

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7.2. The Role of Tregs in Various Allergic Disease (Asthma, Atopic Dermatitis and Urticaria)

Studies have demonstrated altered FOXP3 expression and Treg frequency under various allergic conditions. Patients with asthma exhibit decreased FOXP3 expression [47], whereas those with severe dermatitis show an increase in Tregs [48]. In an experimental murine model of atopic dermatitis, FOXP3-expressing Tregs regulated Th2 response[49]. Urticaria is associated with reduced Treg frequency [50]. Although current research does not conclusively establish Tregs as the primary etiological factor of allergic diseases, it is evident that allergic conditions, such as asthma and atopic dermatitis, impact both FOXP3 expression and Treg frequency. Thus, further clinical research is warranted to elucidate the underlying disease mechanisms and identify potential therapeutic targets, including FOXP3, in allergic diseases.

8. The Role of FOXP3 in Cancer

Recently, the role of FOXP3 in cancer has garnered significant attention, particularly in immunotherapy and immune checkpoint inhibition. Several studies have elucidated the function of Tregs in the tumor microenvironment [51]. Additionally, it has been postulated that CTLA-4 and PD-1/PD-L1 blockade could inhibit Treg function, thereby enhancing the ability of Teffs to eliminate tumor cells in the absence of Tregs.

8.1. The Role of FOXP3 Expression in Cancer Cell

FOXP3 expression is enhanced in several cancer cells, including breast cancer [52]. In breast cancer, FOXP3 functions as an oncogene suppressor and its decreased expression correlates with poor clinical outcomes [53]. These characteristics appear to be relatively specific to breast cancer, as they are rare in other types of cancer.

8.2. The Role of Tregs in the Tumor Microenvironment

Tregs infiltrate the tumor microenvironment in several types of cancers, including ovarian cancer, aiding cancer cells in evading the immune system [54]. In contrast, in a subset of cancers such as colon cancer, infiltrating Tregs in the tumor tissue correlate with favorable outcomes [55]. Consequently, the assumption that Tregs invariably suppress the host immune system to promote tumor growth may be an oversimplification of a complex biological process.

9. Treg Cell Therapy

Adoptive Treg transfer is a key experimental technique to validate Treg-mediated immune regulation. Technological innovations in cell therapy have enabled the initial trials of adoptive human Treg transfer in patients with T1D [56]. At present, Treg transfer is implemented in cases of autoimmunity, organ transplantation, and hematopoietic stem cell transplantation [57,58]. Incorporating rapamycin helps maintain Treg stability and augments FOXP3 expression [59]. Although the fundamental approach remains consistent, no severe adverse effects have been reported. Surprisingly, despite the immunosuppressive effects, the infection rates did not increase significantly [60]. During the early phase of the trial, Treg infusion led to a reduction in the infection frequency [61]. Clinical trials have not revealed any significant safety concerns. These findings suggest the potential application of adoptive transfer of Tregs in allergic diseases, although additional evidence is required before proceeding to clinical trials for allergies.

9.1. Engineered Tregs

The gene transfer approach was initially explored in engineered Tregs because of their plasticity [62–64]. Both retroviral and lentiviral vectors were used to transfer the FOXP3 gene, thereby conferring suppressive functions to Teffs. Engineered Tregs, produced using GMP-compatible methods, have demonstrated safety in preclinical studies [65,66]. Although initially investigated for IPEX syndrome, their potent suppressive properties suggest potential applications across a spectrum

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of genetic and nongenetic autoimmune disorders. Recent advancements in CRISPR/Cas9 technology have enabled site-specific editing of the FOXP3 locus, offering possibilities for enhancing or rectifying mutated FOXP3 in IPEX syndrome [67,68]. This approach may overcome several of the limitations associated with virus-mediated gene transfer.

Beyond its therapeutic implications, CRISPR/Cas9-mediated FOXP3 knockout has proven instrumental in elucidating the molecular function of Tregs [69–71]. This strategy has been extended to investigate other molecules, including PTEN, NFKB2, and RELC [72,73]. Collectively, CRISPR/Cas9 enables the investigation of Treg function through the selective deletion of genes involved in FOXP3 expression.

9.2. CAR-Treg

CAR-Treg, a novel approach in engineered Tregs, has been primarily investigated for alloantigens and autoantigens, including GAD and proinsulin, in T1D, with HLA-A2 antigen as the principal target [74–77] in ongoing clinical studies [78]. Additionally, CAR-Treg cells are being explored for GVHD, where adoptive Treg transfer has already been demonstrated in clinical trials. However, CAR-Tregs have therapeutic and possibly manufacturing advantages. In contrast to polyclonal Tregs, CAR-Tregs use their extracellular domains as homing molecule [79]. Beyond CAR transduction, additional modifications of immune molecules, such as OX40L transfer, may enhance therapeutic potential [80].

10. Conclusions

FOXP3, a critical transcription factor for Tregs, has been implicated in autoimmune disorders, allergies, and cancer. Th2 skewing observed in IPEX syndrome and scurfy mice indicates a potential correlation between FOXP3 and allergic diseases. Cell and gene therapies, as well as immunomodulatory strategies are potential therapeutic options for the treatment of allergic diseases.

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