REVIEW ARTICLE

Epigenetic enzymes are the therapeutic targets for $CD4^+CD25^{+/high}Foxp3^+$ regulatory T cells

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$CD4^+CD25^{+/high}Foxp3^+$ regulatory T (Treg) cells are a subset of $CD4^+$ T cells that play an essential role in maintaining peripheral immune tolerance. Several transcriptional cofactors have been recently identified, which form complexes with transcription factor Foxp3 of Treg cells and contribute in the suppressive function of Treg cells. However, Foxp3 is still defined as a "master" (multiple pathway) regulator gene that controls the development and stability of Treg cells. Because of its importance, the regulatory mechanisms underlying Foxp3 expression have been a focus of intensive investigation. Recent progress suggests that the epigenetic mechanisms responsible for regulating the Foxp3 gene expression are key components of suppressive activity of Treg cells. This review not only discusses the basic concepts of biology and epigenetic modifications of Treg cells, but also analyzes the translational clinical aspect of epigenetic modifications of Treg cells, focusing on several ongoing clinical trials and the Food and Drugs administration–approved epigenetic-based drugs. The new progress in identifying epigenetic enzymes functional in Treg cells is a new target for the development of novel therapeutic approaches for autoimmune and inflammatory diseases, graft-vs-host disease and cancers. (Translational Research 2014;■:1–19)

Q2 **Abbreviations:** ■■ = ■■■

INTRODUCTION

$CD4^+CD25^{+/high}Foxp3^+$ regulatory T (Treg) cells are a subpopulation of $CD4^+$ T cells specialized in the suppression of pathogenic responses from the host immune system against self or foreign antigens.[1] The suppressive function of Treg cells in maintenance of self-tolerance and prevention of the development of autoimmune and chronic inflammatory diseases is mediated by different mechanisms such as cell-cell contact and/or secretion of anti-inflammatory cytokines such as Q3 interleukin 10 (IL-10), IL-35, and transforming growth factor β (TGF-β).[2,3]

One of the major milestones found in the studies of Treg cells was the identification of Foxp3. Foxp3 is a

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member of the forkhead/winged-helix family of transcription factors, which acts as a "master" (multiple pathway) regulator gene for the development and suppressive function of Treg cells.[4-6] The Foxp3 gene was identified by its significant mutations that cause fatal autoimmune diseases in early life, which is now termed immunodysregulation, polyendocrinopathy, enteropathy, and X-linked (IPEX) syndrome in mice and humans. From the time of the discovery of the Foxp3 gene, its role and modification have been one of the potential topics in translational medicine field because of the essential function of Foxp3 in maintaining immune tolerance and homeostasis.

In inflammatory environments, the suppressive function of Treg cells is perturbed and the development of TGF-β–induced Treg cells is reduced in an epigenetic manner,[7] suggesting that epigenetic regulation of Treg cell function and development is pathophysiologically relevant. In correlation with this finding, Treg cell suppression is also found to be disturbed in autoimmune type 1 diabetes, in which epigenetics is one of the pathologic mechanisms involved.[8] Epigenetics is defined by heritable changes that occur in gene expression without modification in the DNA sequence of the genome. These epigenetic mechanisms, which include DNA methylation/demethylation, histone modifications, and microRNAs (miRNAs) are the principal mechanisms involved in regulating chromosomal organization and gene expression via different and dynamic levels. More specifically, it has been demonstrated that epigenetic mechanisms play a significant role in regulating the expression of the Foxp3 gene and are leading to further regulations in Treg cell functions.[9-11] Emerging epigenetic therapies are providing new therapeutic agents for the control of various diseases.[12] In this review, we have focused on understanding the mechanisms of epigenetic modifications in the Foxp3 gene in the development of autoimmune and inflammatory diseases, graft-vs-host disease (GVHD), cancer, and therapeutic modalities to continue our long-term interest in identifying novel Treg cell therapy–related targets.[6,13-19] In addition, we have also analyzed the progress in identifying epigenetic enzymes as potential therapeutic targets for novel Treg cell–based therapy.

REGULATORY T CELLS

Originally termed suppressor T cells, the recognition of Treg cells as a cellular mechanism for immune tolerance resulted from experiments performed in the 1960s and 1970s by Gershon and Kondo,[20] which described the induction of suppressor T cells capable of downregulation of antigen-specific T-cell responses. Because of the lack of known molecular markers, research on suppressor T cells ceased. However, in 1995, Sakaguchi et al[21] identified CD25 as a surface phenotypic marker for suppressive CD4 cells in mice. Since then, suppressive T cells have been called Treg cells. Later, the discovery of Foxp3 as a specific transcription factor and marker of naturally occurring Treg (nTreg) cells and adaptive/induced Treg (iTreg) cells provided a molecular anchor to the population of Treg cells.[22] The identification of these molecular markers led to an increase in research interest in Treg cells during the last decade, which has identified Treg cells as a plausible therapeutic choice for several autoimmune diseases such as inflammatory bowel disease, systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), type 1 diabetes mellitus, and many other diseases.

Originally, the high expression of CD4 and CD25 surface markers was used to identify Treg cells. However, because $CD25^+$ has been found in other non–Treg T cells such as activated T cells, the measurement of the intracellular expression of Foxp3 transcription factor allowed for a more specific analysis of Treg cells. Because Foxp3 is also expressed in effector T cells, negative expression of CD127 is often used as an additional marker[23] owing to its inverse correlation with Foxp3 expression and suppressive function of human $CD4^+$ Treg cells. Although the functional significance of the expression of these markers remains to be defined, several additional markers have been described such as cytotoxic T lymphocyte–associated molecule-4 (CTLA-4), glucocorticoid-induced tumor necrosis factor receptor, CD39, and CD45RA.

Currently, several experimental systems are commercially available that simplify the identification, isolation, and characterization of Treg cells using fluorescent-conjugated antibodies for CD4, CD25, Foxp3, and CD127. Moreover, the isolation of mRNAs for cDNA synthesis is used to analyze Foxp3 expression in Treg cells using a quantitative real-time polymerase chain reaction (PCR).[24] Treg cells are characterized by the secretion of immunosuppressive/anti-inflammatory cytokines such as IL-10, IL-35, and TGF-β. Enzyme–linked immunosorbent assays and Western blots have been used for the detection of Treg cells, whereas cytokines have been measured using a cytokine secretion assay.[25] In addition, only in Treg cells a certain region within the Foxp3 gene (Treg-specific demethylated region [TSDR][26] or conserved noncoding sequence 2[27]) is found demethylated that allows for the monitoring of Treg cells through PCR or other DNA-based analysis methods.[26]

Treg cells have indispensable functions regarding maintaining immune homeostasis. They are essential in mediating peripheral immune tolerance, preventing autoimmune diseases, and suppressing inflammatory