HHS Public Access

Author manuscript

Sci Signal. Author manuscript; available in PMC 2015 November 25.

Published in final edited form as:

Sci Signal. ; 5(235): pe32. doi:10.1126/scisignal.2003364.

T Cell Signaling Targets for Enhancing Regulatory or Effector Function

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Abstract

To respond to infection, resting or naïve T cells must undergo activation, clonal expansion, and differentiation into specialized functional subsets of effector T cells. However, to prevent excessive or self-destructive immune responses, regulatory T cells (T_{regs}) are instrumental in suppressing the activation and function of effector cells, including effector T cells. The transcription factor Forkhead box P3 (Foxp3) regulates the expression of genes involved in the development and function of T_{regs}. Foxp3 interacts with other transcription factors and with epigenetic elements such as histone deacetylases (HDACs) and histone acetyltransferases. T_{reg} suppressive function can be increased by exposure to HDAC inhibitors. The individual contributions of different HDAC family members to T_{reg} function and their respective mechanisms of action, however, remain unclear. A study showed that HDAC6, HDAC9, and Sirtuin-1 had distinct effects on *Foxp3* expression and function, suggesting that selectively targeting HDACs individually or in combination may enhance T_{reg} stability and suppressive function. Another study showed that the receptor programmed death 1 (PD-1), a well-known inhibitor of T cell activation, halted cell cycle progression in effector T cells by inhibiting the transcription of the gene encoding the substrate-recognition component (Skp2) of the ubiquitin ligase SCF^{Skp2}. Together, these findings reveal new signaling targets for enhancing T_{reg} or effector T cell function that may be helpful in designing future therapies, either to increase T_{reg} suppressive function in transplantation and autoimmune diseases or to block PD-1 function, thus increasing the magnitude of antiviral or antitumor immune responses of effector T cells.

T cell activation requires two signals. Signal 1 arises from T cell receptor (TCR) engagement by the major histocompatability complex (MHC) and its cognate antigen. The critical element of signal 2 is provided by CD28 costimulation by the antigen-presenting cell (APC), which activates phosphatidylinositol 3-kinase (PI3K) and its downstream target Akt (also known as protein kinase B), leading to increased abundance of glucose transporters on the plasma membrane and an increase in glycolytic enzyme activity. Blocking PI3K activation would effectively prevent T cell activation. Programmed cell death protein 1 (PD-1), a cell-surface molecule serving as an inhibitory receptor, inhibits the CD28-

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mediated activation of PI3K upon engagement of PD-1 with its ligand (1–3). However, the molecular mechanism by which PD-1 affects cell cycle progression and T cell proliferation remains largely unknown. Patsoukis *et al*. demonstrated that PD-1 activation halted the cell cycle in the G_1 phase by inhibiting both PI3K and the Rasmitogen–activated and extracellular signal– regulated kinase kinase (MEK) pathway, as well as extracellular signal–regulated kinase (ERK) signaling pathways (4). This important signaling network is required for expression of the gene encoding interleukin 2 (IL-2) and T cell proliferation. Patsoukis *et al*. showed that a key aspect of PD-1–mediated suppression of T cell activation through the cell cycle machinery was the negative effect of PD-1 signaling on the expression of Skp2, the substrate-recognition component of ubiquitin ligase SCF^{Skp2}. By interrupting the SCF^{Skp2}-mediated activation of cyclin-dependent kinases (CDKs), PD-1–mediated suppressive signals prevented T cell proliferation by locking T cells in the G_1 phase (4) (Fig. 1A). This mechanistic insight may provide potential therapeutic opportunities to increase effector T cell immune responses against cancer and viral infection by targeting PD-1 signaling.

The engagement of PD-1 signaling leads to the inhibition of effector T cell activation. Regulatory T cells (T_{regs}), on the other hand, are dedicated suppressor cells, playing a pivotal role in the control of immunological self-tolerance and immune responses to pathogens and tumor antigens (5–13). The Forkhead family transcription factor Foxp3 is the master transcription factor for T_{reg} development and function (8–13). Scurfy mice, which lack *Foxp3*, are deficient in T_{regs} and develop severe lymphoproliferative autoimmune disease. Furthermore, mutations in the *Foxp3* gene in humans give rise to immune dysregulation and polyendocrinopathy enteropathy X-linked syndrome (IPEX), which is a life-threatening severe autoimmune disorder (8–13). T_{regs} can reverse or even cure established autoimmune diseases, and T_{reg} therapies can be efficacious in controlling autoimmune responses in organ and cell transplantation in animal models (5–13). The application of T_{regs} or the enhancement of their suppressive function to cure autoimmune diseases and prevent organ transplant rejection and graft-versus-host disease (GVHD) after bone marrow transplantation in humans is an active area of research (8–14). Recent phase I clinical trials have shown that T_{regs} are safe and well tolerated in patients and have potential efficacy in treating GVHD (15–18). However, one major problem associated with T_{reg} therapy is that the phenotype of the administered T_{regs} is unstable, and there is potential loss of suppressive activity over time in vivo (18). A better understanding of the factors governing T_{reg} function and stability will be crucial for the advancement of T_{reg} therapy to the clinic.

The methylation states of distinct regions of DNA in *Foxp3* contribute to the stability or relative instability of the T_{reg} phenotype. Repeated in vitro activation of human T_{regs} results in CpG island methylation within a conserved region of the Foxp3 gene, which precipitates a loss of its expression and leads to proinflammatory cytokine production by the conversion of T_{regs} into effector T cells (19). In contrast, pharmacological inhibition of DNA methylation in vivo increases the number of T_{regs} and enhances the suppression of diabetes in mice (20).

Adding another layer of complexity, another mechanism for the regulation of Foxp3 at the protein level integrates physiological cues from the microenvironment, such as hypoxia (21,

22). This ubiquitination-dependent pathway is likely to play a role in the newly appreciated potential for metabolic control of T_{reg} and effector T cell balance. Because ubiquitination-mediated removal of Foxp3 protein inhibits T_{reg} function, it is reasonable to argue that inhibiting Foxp3 degradation should have potential therapeutic implications.

Acetylation is another important posttranslational modification of Foxp3 that affects its stability and activity. Acetylation of Foxp3 is regulated by components of a Foxp3-associated supermolecular complex containing multiple histone acetyltransferases (HATs), histone deacetylases (HDACs), and other transcriptional co-regulators (23). HATs and HDACs play defining roles in the regulation of Foxp3 activity (Fig. 1B); thus, it is reasonable to expect that modulating their activity will correspondingly affect T_{reg} suppressive activity. For example, acetylation of Foxp3 by the HAT p300 can be reversed by the histone deacetylase Sirtuin-1 (Sirt1) (24). HDAC9, on the other hand, co-localizes with Foxp3 in resting T_{regs}. HDAC9 interaction with Foxp3 can be disrupted by TCR engagement because of the translocation of HDAC9 from the nucleus to the cytosol, which can be reversed by the pretreatment of T_{regs} with an HDAC inhibitor such as trichostatin A (25). HDAC9 interaction with and deacetylation of Foxp3 destabilizes T_{reg}-specific transcriptional programs. Hancock and colleagues previously demonstrated that either exposure to HDAC inhibitors (26) or genetic deletion of HDAC family members (27) increases the suppressive capacity of both murine and human $Foxp3^+$ T_{regs} by enhancing Foxp3 acetylation.

Beier *et al*. (28) further dissected the molecular mechanism by which isotype-specific inhibition or deletion of HDACs affects T_{reg} function. The authors demonstrated that T_{reg} function was significantly enhanced by inhibition of Sirt1. They also provided evidence to suggest that HDAC6 deacetylated Foxp3 in the nucleus. The loss of HDAC6 promoted the longevity of acetylated Foxp3, with a consequent increase in the resistance of Foxp3 to proteasomal degradation. In addition, they showed that HDAC6 was involved in promoting the activities of other transcriptional factors required for T_{reg} function, such as cyclic adenosine monophosphate response element–binding protein. They further showed that the loss of HDAC9, but not other HDACs, was associated with stabilization of the acetylated form of IL-2–mediated signal transducer and activator of transcription 5 (STAT5) and promoted its transcriptional activity in T_{regs} (28).

Given that HDAC6, HDAC9, and Sirt1 possess distinct mechanisms of enhancing T_{reg} suppressive activity, Beier *et al*. also explored the efficacy of combinatorial targeting of HDAC6, HDAC9, and Sirt1. The combined loss of HDAC6, HDAC9, and Sirt1 augmented T_{reg} function, and pharmacological inhibition of Sirt1 and HDAC6 was very effective in enhancing T_{reg} function in vivo (28). These studies provide a framework for further testing other small-molecule inhibitors of HDACs in relevant mouse models, as well as in clinical trials. A combined therapy using HDAC inhibitors in conjunction with small molecules targeting posttranslational pathways such as E3 ligase–mediated protein degradation warrants further investigation.

In summary, the dissection of the mechanisms underlying key processes of immune regulation—namely, PD-1 signaling in effector T cells and the activity of the transcription

factor Foxp3 in T_{regs}—revealed important roles for these signaling molecules in effector T cell and T_{reg} function. Furthermore, these studies provide new avenues to explore the potential benefits of modulating the activities of these molecules and their signaling pathways. Further investigation of these targets is likely to yield promising immunotherapies against autoimmune diseases or viral infection and cancer by enhancing T_{reg} or effector T cell function, respectively.

Acknowledgments

Work in the Pan laboratory is supported by an NIH grant. F.P. is a recipient of a Stewart Trust Scholar award and a young investigator award from the Melanoma Research Alliance.

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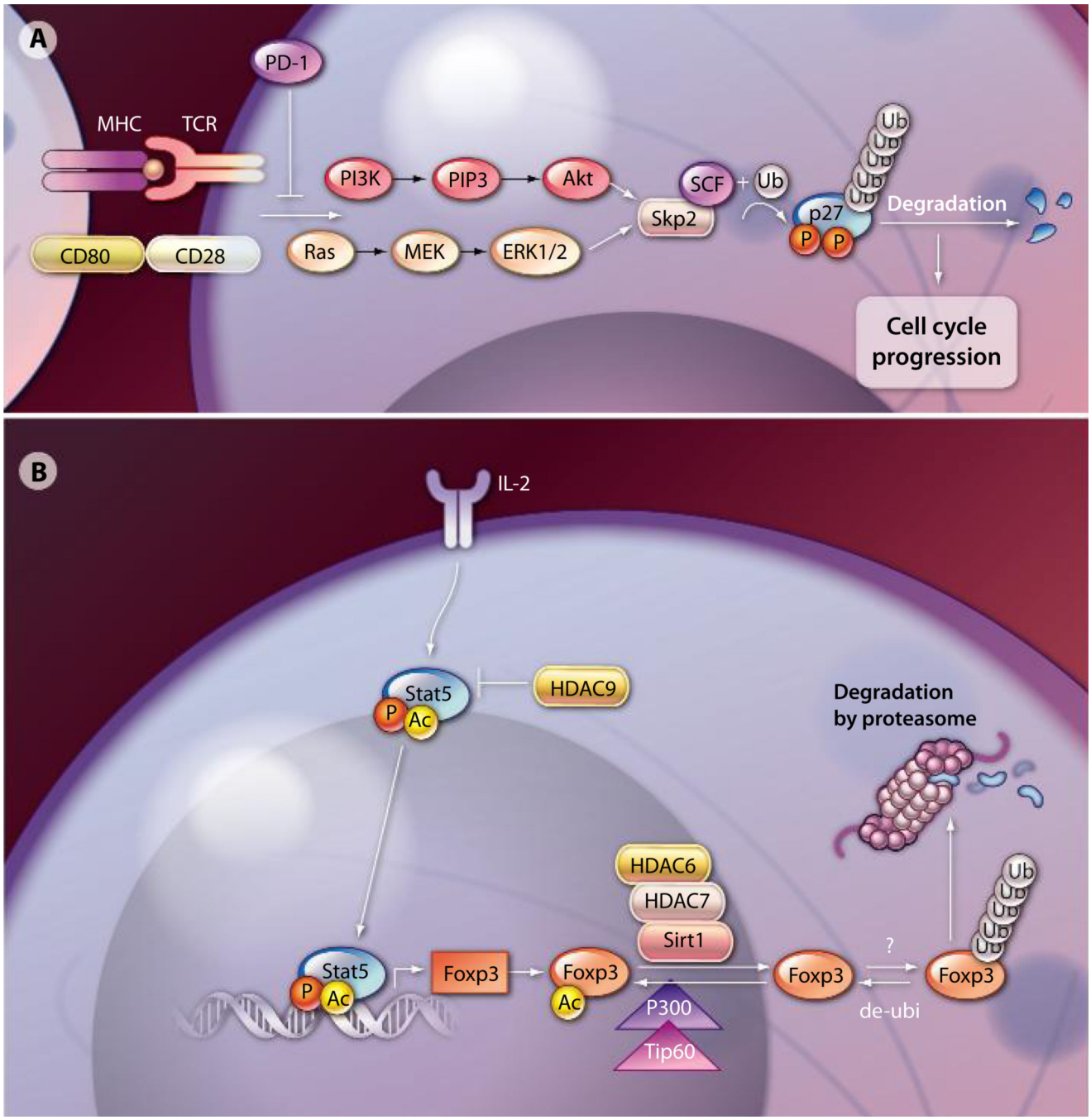

Fig. 1.

(**A**) PD-1 signaling in effector T cells. PD-1 inhibits the PI3K and Ras pathways resulting in decreased activity of the ubiquitin ligase SCF^{Skp2}. This ligase promotes the activation of CDKs and is involved in cell cycle progression. (**B**) Transcriptional and posttranslational regulation of Foxp3. HATs and HDACs have opposing roles in the modification of Foxp3 acetylation status. Acetylation of Foxp3 relies on the accessibility of HATs (Tip60 and p300) or HDACs (HDAC6, HDAC7, HDAC9, and Sirt1), as well as the activity of the

enzymes. Deacetylated Foxp3 can be further ubiquitinated by E3 ligases and is subject to the proteasome-dependent degradation pathway. AC inducates acetylation.