Commentary

A novel role for Cish in the inhibition of TCR signaling

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Cancer immunotherapeutics focus primarily on stimulating the immune system to elicit endogenous immune responses to fight cancer or transferring primed immune effectors in adoptive cell transfer (ACT) paradigms (1,2). $CD8^+$ T cells are desirable immune effectors in cancer, however, their antitumor activity is often attenuated by tolerance mechanisms or the suppressive nature of the tumor microenvironment (3). As such, the goal of cancer immunotherapies is increasingly focusing on overcoming the tumor tolerance barrier by enhancing the antigen reactivity and effector function of tumor-specific T cells (4), however, the presence of T cells with high-affinity for their cognate antigen is often precluded by thymic selection (5,6). Therefore, developing methods of enhancing T cell receptor (TCR) signaling or functional avidity to maximize antitumor efficacy of tumor-specific T cells (7) can improve cancer therapies employing ACT, vaccines, or checkpoint inhibitors. Current approaches include the development of affinity-enhanced antigen receptors for ACT therapies, but these have been met with challenges (8,9), including treatment-related toxicities (10-12). In an effort to identify new druggable targets to increase $CD8^+$ T cell functional avidity for tumor-specific antigens and increase tumor killing, Palmer *et al*. identified a novel intrinsic pathway inhibiting TCR signaling cascades and limiting $CD8^+$ T cell activity in murine tumors (13).

There are eight existing members of the suppressor of cytokine signaling (SOCS) family of molecules (SOCS1-7 and Cish), which share a central SH2 domain and a C-terminal SOCS box. SOCS molecules are thought to negatively regulate cytokine signaling by sequestrating downstream signaling components (such as JAKs and STATs) and facilitating their proteasomal degradation using an E3 ligase-like mechanism (14). While it is known that Cish is induced upon TCR stimulation or the addition of cytokines (15,16), it's precise role in immune regulation has been elusive. Cish has been shown to interact with the IL-2, erythropoietin and growth hormone receptors and is implicated in the inhibition of STAT5 phosphorylation by direct competition for receptor binding sites (17,18). Palmer *et al*. challenge the current paradigm that Cish regulates immunity primarily by regulating cytokine signaling cascades. Instead, they focused on the role of Cish in TCR signaling in tumor-specific $CD8^+$ T cells.

Using a TCR transgenic model (pmel-1) targeting the melanoma antigen gp100, and a newly-created Cish-deficient mouse model, Palmer *et al*. explored the function of Cish in effector $CD8^+$ T cells. They detected basal levels of Cish in naïve T cells from wild-type mice that were rapidly induced upon TCR stimulation and enhanced in the hours to follow. They also demonstrated that Cish is up-regulated in an antigen-dependent manner in tumor-specific $CD8^+$ T cells infiltrating melanoma tumors. Deletion of Cish resulted in enhanced $CD8^+$ T cell expansion, functional avidity, and multi-functionality (production of multiple cytokines) in response to *in vitro* stimulation. Moreover, *in vivo* studies evaluating antitumor efficacy of Cish-deficient $CD8^+$ T cells demonstrated that the increased expansion and functional activity of these T cells resulted in superior therapeutic antitumor activity, eliminating established tumors. Importantly, to evaluate the therapeutic and translational applicability of their findings, Palmer *et al*. knocked down Cish expression in patient T cells using a retrovirus encoding a short hairpin microRNA (shmiR) and co-transduced patient T cells with a retrovirus encoding TCRs against various tumor-specific antigens. Analysis of

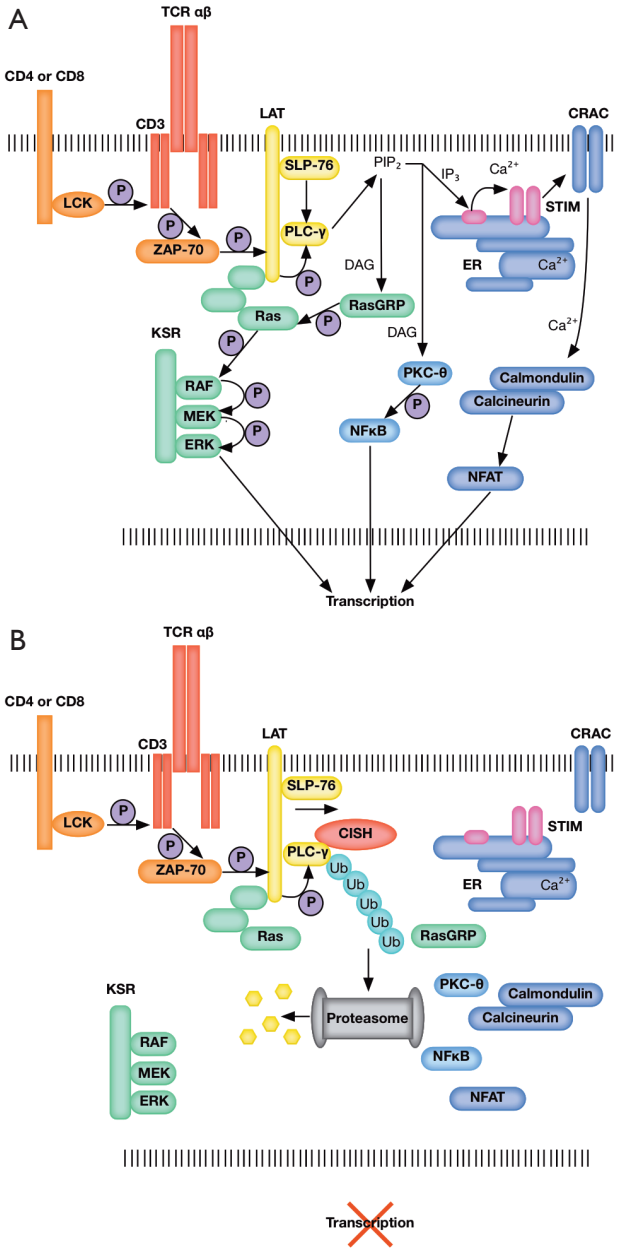

Figure 1 Cish regulation of TCR signaling. (A) Conventional TCR signaling pathway, in the absence of SOCS family member Cish. PLC-γ1 converts PIP_3 into IP_2 and DAG, which are responsible for increasing calcium flux and activation of enzymes such as PKC. These events are critical for the transcriptional activation of key T cell regulators such as NFAT and NFκB; (B) TCR signaling pathway, in the presence of the SOCS family member Cish. Cish physically interacts with the TCR signaling component PLC-γ1, targeting it for proteasomal degradation via polyubiquitination. The degradation of PLC-γ1 disrupts downstream signaling, ultimately reducing the activation of transcription factors such as NFAT and NFκB, blunting T cell activation.

effector function and tumor reactivity recapitulated the observations in Cish-deficient mice, suggesting that Cish negatively regulates both mouse and human TCR signaling and T cell-mediated antitumor immunity.

Interestingly, Palmer *et al*. observed no differences in STAT5 phosphorylation or activation levels between wild-type and Cish-deficient mice, contrary to existing reports. In search of an alternative mechanism by which Cish negatively regulates T cell activation and activity, gene expression profiles where compared between wild-type and $Cish^{-/-}$ $CD8^+$ T cells following TCR activation, revealing the up-regulation of critical pro-functional, proliferative and pro-survival genes (*Tbx21*, *Cmyc*, and *Bcl2l1*, respectively) in $Cish^{-/-}$ T cells. Further, an unexpected physical interaction between Cish and the critical TCR signaling component PLC-γ1 was identified. PLC-γ1 converts PIP_3 into IP_2 and DAG, increasing calcium flux and the activation of enzymes critical for the transcriptional activation of T cell regulators such as NFAT and NFκB (*Figure 1A*). Cish targets PLC-γ1 for proteasomal degradation via polyubiquitination, inhibiting downstream TCR signaling, preventing the activation of NFAT and NFκB, and inhibiting T cell activation (*Figure 1B*). Their findings implicate Cish as an intrinsic TCR checkpoint inhibitor, and position Cish as a novel cancer immunotherapy target to overcome tolerance and enhance T cell functional avidity, immune responses, and antitumor immunity.

However, a few aspects of this study should be addressed. The authors performed studies using T cells derived from germline knockouts. It has been observed previously, that aged, germline Cish knockout mice developed lung inflammation that did not occur in animals with T cell lineage-specific Cish deletion (19). Thus, it's possible that total-body deletion of Cish might impact T cell development/function in a T cell extrinsic manner. Further, the authors reported the absence of immunopathology in unmanipulated $Cish^{-/-}$ mice, however, the translational application of these findings is questionable. In a clinical setting, where cancer patients are likely immunocompromised, and infections are encountered at a frequency higher than those in the pathogen-free conditions in a mouse facility, autoimmunity might be a consequence of targeting Cish. The absence of Cish is also reported to induce TCR-dependent hyperactivity, which might cause spontaneous TCR signaling or T cell exhaustion, especially in the context of T cells with natural TCRs. The potential for autoimmunity and TCR hyperactivity by implementing Cish-depletion/inhibition to treat cancer will require

characterization in future studies.

In conclusion, Palmer *et al*. identified a novel role for Cish in immune regulation, and provided sufficient evidence supporting Cish as an inhibitor of TCR signaling with therapeutic potential. They demonstrated that Cish elimination enhanced T cell functionality and antitumor immunity. While, further studies are needed to develop Cish-targeted immunotherapeutic strategies, the current study suggests that Cish inhibitors may enhance the efficacy of many current cancer immunotherapies, including adoptive cell therapy with TCR or CAR-expressing T cells, cancer vaccines and checkpoint inhibitors, by improving TCR signaling within the tumor microenvironment.

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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