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Review Article

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Phosphatase Ssu72 Is Essential for Homeostatic Balance Between CD4⁺ T Cell Lineages

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Conflict of Interest
The authors declare no potential conflicts of interest.

Abbreviations
APC, Ag-presenting cell; aUC, active ulcerative colitis; BAT, brown adipose tissue; CIA, collagen-induced arthritis; CTD, carboxyl-terminal domain; DSS, dextran sulfate sodium; DUSP2, dual specificity phosphatase 2; HCC, hepatocellular carcinoma; HNF4α, hepatocyte nuclear factor 4α; IBD, inflammatory bowel disease; IKBKE, IκB kinase epsilon; ITAM,

ABSTRACT

Ssu72, a dual-specificity protein phosphatase, not only participates in transcription biogenesis, but also affects pathophysiological functions in a tissue-specific manner. Recently, it has been shown that Ssu72 is required for T cell differentiation and function by controlling multiple immune receptor-mediated signals, including TCR and several cytokine receptor signaling pathways. Ssu72 deficiency in T cells is associated with impaired fine-tuning of receptor-mediated signaling and a defect in CD4⁺ T cell homeostasis, resulting in immune-mediated diseases. However, the mechanism by which Ssu72 in T cells integrates the pathophysiology of multiple immune-mediated diseases is still poorly elucidated. In this review, we will focus on the immunoregulatory mechanism of Ssu72 phosphatase in CD4⁺ T cell differentiation, activation, and phenotypic function. We will also discuss the current understanding of the correlation between Ssu72 in T cells and pathological functions which suggests that Ssu72 might be a therapeutic target in autoimmune disorders and other diseases.

Keywords: Ssu72 phosphatase; CD4-positive T-lymphocytes; Autoimmune diseases; T-cell receptors; Autoimmunity

INTRODUCTION

Ssu72 phosphatase is involved in a broad range of activities ranging from transcriptional biogenesis to physiological functions. Ssu72 regulates the transcription cycle and RNA processing by dephosphorylating Ser5P and Ser7P in the carboxyl-terminal domain (CTD) of RNA polymerase II (RNAPII) and participates in mRNA 3'-end processing and gene looping (1,2). The protein structure of Ssu72 phosphatase possesses a central five-stranded β-sheet (β1–β5) surrounded by helices on both sides (**Fig. 1**) (3). Ssu72 is highly conserved in eukaryotes, and has structural similarities to other low-molecular-mass phosphotyrosine protein phosphatases. Ssu72 includes three unique structural characteristics: two-stranded antiparallel β-sheets (β2A and β2B), αD helix, and an extra helix (αG) and a β-strand (β5) (**Fig. 1**) (3). A small β-sheet (β2A and β2B) comprises the distinct active site of Ssu72, and the αD helix in Ssu72 is involved in phosphopeptide binding (3). Furthermore, the structural features of αG and β5 at the C terminus of Ssu72 contribute to interactions with symplekin (3). Ssu72 also forms highly conserved residues with Cys12 in the N-terminal phosphatase domain of human Ssu72 conferring its phosphatase activity (**Fig. 1**) (3,4). Mutation of

immunoreceptor tyrosine-based activation motif; iTreg, induced Treg; IκB, inhibitor of κB; LCMV, lymphocytic choriomeningitis virus; NAFLD, non-alcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; PLCγ1, phospholipase C gamma 1; PTPN2, phosphatase non-receptor type 2; pTreg, peripherally-induced Treg; RA, rheumatoid arthritis; RNAPII, RNA polymerase II; SFK, SRC-family kinase; TBK1, TANK binding kinase 1; tTreg, thymus-derived Treg; UC, ulcerative colitis; ZAP70, zeta-chain-associated protein kinase 70.

Author Contributions

Conceptualization: Kim MH, Lee CW; Data curation: Kim MH, Lee CW; Formal analysis: Kim MH, Lee CW; Funding acquisition: Lee CW; Supervision: Lee CW; Writing - original draft: Kim MH, Lee CW; Writing - review & editing: Lee CW.

Cys12 with Ser almost completely abolishes its phosphatase activity, indicating that the phosphatase domain is essential for the protein configuration of phosphatase activity (4,5). Although the importance of the RNAPII-independent phosphatase activity of Ssu72 has not been fully understood, Ssu72 exerts an RNAPII-independent phosphatase activity in a tissue-specific manner, thereby affecting physiological functions and pathogenesis (6,7). For example, Ssu72 plays an important role as a cohesin-binding phosphatase by interacting with Aurora B kinase and regulating duplicated sister chromatid separation (4). The Aurora B-mediated phosphorylation of Ssu72 at Ser19 is associated with protein configuration and the phosphatase activity of Ssu72 (**Fig. 1**) (4). Additionally, recent studies have shown that the phosphatase activity of Ssu72 profoundly affects the maintenance of hepatic chromosome integrity and the monitoring of the development of liver diseases, including non-alcoholic fatty liver disease (NAFLD), fibrosis, and steatohepatitis-associated hepatocellular carcinoma (HCC) (8,9).

Functions of Ssu72 in immune cells have been expanded to include intervening in immune responses by regulating multiple signaling pathways, including TCR engagement and GM-CSF receptor signaling (10-12). Recent studies have provided evidence that Ssu72 contributes to the differentiation, activation, and function of $CD4^+$ T cell lineages by negatively controlling immune receptor signaling pathways (6,10,11,13). Given that the immune system is largely dependent on the phosphorylation of target proteins for recognition and transduction of extracellular signals, it is not surprising that Ssu72 is involved in regulating signaling cascades in a phosphatase activating-dependent manner. In addition, it has been reported that multiple protein tyrosine phosphatases in T cells are responsible for maintaining $CD4^+$ T cell homeostasis by mediating the regulation of intracellular signaling. Regulatory mechanisms of $CD4^+$ T cell differentiation include the strength of TCR signal, cytokine-related JAK-STAT pathways, and various cytosolic signaling (14,15). Phosphatase dysfunction or lack of phosphatase activity can result in an imbalance in $CD4^+$ T cell homeostasis, potentially leading to immune pathologies such as autoimmune diseases (14,15). Here, we review the current understanding of the physiological role of Ssu72 phosphatase in $CD4^+$ T cell and the immunoregulatory mechanisms of $CD4^+$ T cell homeostasis and functions. We highlight how Ssu72 dysfunction in T cells correlates with the development of autoimmunity and discuss the potential of Ssu72 as a therapeutic target.

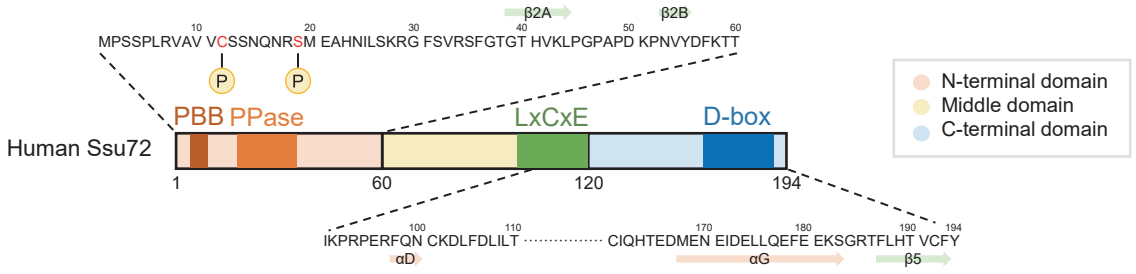

Figure 1. Schematic representation of human Ssu72 phosphatase domain structures.
The Ssu72 domain comprises N-terminal (1–60), middle (61–120) or C-terminal (129–194) domains. Ssu72 possesses three structural features that distinguish it from low-molecular-mass phosphotyrosine protein phosphatases: 1) β2A and β2B, 2) αD helix, and 3) an extra helix (αG) and a β-strand (β5). Additionally, the Ssu72 protein forms functional motifs, such as PBB, D-box, and LxCxE domains, and contains a PPase domain inside.
PBB, Polo-box binding; PPase, phosphatase.

IMPORTANCE OF PROTEIN PHOSPHORYLATION IN CD4$^+$ T CELL DIFFERENTIATION

Upon recognition of a specific Ag, naïve CD4$^+$ T cells differentiate into transcriptionally and functionally distinct CD4$^+$ T cell lineages, including Th1 cells, Th2 cells, Th17 cells, Tregs, and so on (16-18). In peripheral circulation, CD4$^+$ T cell differentiation begins with TCR recognizing Ags presented by Ag-presenting cells (APCs) in naïve T cells. The interaction of APCs with T cells induces the binding of costimulatory receptors such as CD28 and results in the recruitment of CD8 or CD4 co-receptors, respectively, that can bind to conserved regions of MHC class I or II (19). The CD4 internal domain binds to and activates the SRC-family kinase (SFK), lymphocyte-specific protein tyrosine kinase, to promote phosphorylation of tyrosine residues within the immunoreceptor tyrosine-based activation motif (ITAM) of TCR-associated CD3 and zeta chains. Phosphorylated ITAM activates zeta-chain-associated protein kinase 70 (ZAP70) and mediates TCR signaling downstream to direct cellular responses by cooperating with SFK. In addition to TCR signals, specific cytokines (such as IL-4, IL-12, and IFN-γ) play a dominant role in inducing polarization of CD4$^+$ T cell subsets (20). The importance for these polarizing cytokines results from their binding to their respective receptors and promoting phosphorylation cascades of JAK and STAT proteins. In the process of CD4$^+$ T cell differentiation, the binding of cytokines and receptors corresponding to the CD4$^+$ T cell subset leads to the activation of receptor-related JAK molecules. Activated JAK molecules are responsible for phosphorylation of STATs. Tyrosine phosphorylated STAT proteins can lead to the establishment of CD4$^+$ Th lineage-specific enhancer landscape and prompt the expression of distinct transcription factors (21). Regulatory programs of transcription factors such as T-bet (also called Tbx21), Gata3, RORγt, and Foxp3 can promote Th1, Th2, Th17, and Tregs polarization, respectively (22-25).

CD4$^+$ T cells belong to a major T cell lineage of the adaptive immune system and play crucial roles in the defense against pathogens, the regulation of inflammatory responses, and tumor surveillance (16,26). Naïve CD4$^+$ T cells are pluripotent precursors with defined Ag recognition specificities and substantial plasticity to develop into distinct effector or regulatory lineages according to signals from cells responsible for innate immunity (27). The development of CD4$^+$ effector T cells by cytokines derived from pathogen-activated cells of the innate immune system is a hallmark of adaptive immunity. The divergence of differentiation into CD4$^+$ effector T cells, including Th1 and Th2 cells, is due to cross-regulation of polarizing cytokines (such as IFN-γ and IL-4), and inappropriate or poorly controlled CD4$^+$ effector T cells affect immunopathological aspects.

As mentioned above, the polarization of CD4$^+$ T cell lineage is fine-tuned by TCR signaling and cytokine signaling cascades (20). Recent *in vitro* and *in vivo* studies have demonstrated that the intensity of TCR signal and costimulatory receptors can dictate the outcome of CD4$^+$ T cell polarization (28,29). High doses of peptides or potent agonistic ligands can promote the development of Th1 cells, whereas stimulation with low doses of peptides or small amounts of agonistic ligands can induce polarization of naïve CD4$^+$ T cells into Th2 cells (29). Foxp3$^+$ peripheral Tregs, which are mainly generated from CD4$^+$ Foxp3$^-$ T cells exposed to Ag during tolerant conditions or homeostatic proliferation, promote Foxp3 expression and induced Tregs (iTregs) differentiation more at moderate TCR signal intensities than weak or strong TCR signals (30-32). In addition to the strength of TCR signal, the cytokine environment is essential for CD4$^+$ T cell differentiation. Activation of the JAK-STAT signaling pathway in response to specific cytokines can lead to polarization of naïve CD4$^+$ T cells

into specific CD4⁺ T cell subsets. Several studies have provided evidence that a conditional knockout model lacking the gene encoding STAT3 is required for CD4⁺ T cell lineage differentiation (33,34). Furthermore, a mouse model deficient in STAT5 has confirmed its role as a regulator in Th2, Th17, and Tregs lineage differentiation (35-37). Tyrosine phosphorylation is considered essential for fine-tuning of the JAK-STAT signaling pathway (14,38). It has been revealed that phosphatases, including protein tyrosine phosphatase non-receptor type 2 (PTPN2), PTPN6, PTPN11, and dual specificity phosphatase 2 (DUSP2) known to hydrolyze phosphorylated tyrosine residues of JAK and STATs, are involved in CD4⁺ T cell activation and polarization regulation (39-42). Abnormal control of phosphorylation status in signaling pathways involved in CD4⁺ T cell differentiation can lead to failure of expression of specific transcription factors required for polarization into distinct CD4⁺ T cell subsets (14,15,43). Disruption of CD4⁺ T cell homeostasis can lead to an imbalance between effector and regulatory functions, disrupt the immune response, and eventually lead to the development of immune-mediated diseases (44,45). Therefore, the molecular regulatory mechanism for maintaining the balance of CD4⁺ T cell homeostasis requires regulation of tyrosine phosphorylation of complex intracellular molecules, suggesting a central role of phosphatases for the maintenance of CD4⁺ T cell lineages.

Ssu72 PHOSPHATASE IN DIFFERENTIATION AND MAINTENANCE OF PERIPHERAL CD4⁺ T CELLS

Ssu72 phosphatase in Th1, Th2, and Th17 cells

Recent studies have suggested that appropriate coordination of phosphorylation status of TCR-related target molecules by Ssu72 phosphatase can facilitate balanced differentiation between CD4⁺ T cell lineages (6,10,11,13). In conditional knock-out mice, in which the *Ssu72* gene was deleted from T cells, Ssu72 deficiency augmented the differentiation into Th1 and Th2 lineages, whereas Treg population was reduced (10,11). Depletion of Ssu72 in T cells can increase the production of distinct signature cytokines, IFN-γ and IL-4 under Th1- and Th2-polarizing conditions, respectively (11). Consistently, Ssu72-deleted CD4⁺ T cells show increased expression of IFN-γ, IL-4, IL-17, GM-CSF, and transcription factors, including *Tbx21*, *Gata3* and *Rorc* in response to TCR stimulation both *in vivo* and *in vitro* (10). These results indicate that Ssu72 might contribute to the differentiation of naive T cells to CD4⁺ Th cells and negatively regulate the polarization of naïve CD4⁺ T cells to effector CD4⁺ T cells. Interestingly, the regulatory mechanism by which Ssu72 controls the homeostatic balance between CD4⁺ T cell lineages is associated with fine-tuning of TCR signaling. The balance between the phosphorylation (hyperphosphorylation) and dephosphorylation (hypophosphorylation) of the target proteins in TCR signaling is required for signaling complex formation and the propagation of TCR signals (46,47). Multiple protein tyrosine phosphatases have been reported to be responsible for CD4⁺ T cell activation and differentiation by negatively regulating TCR-mediated signaling pathways (14). Interestingly, Ssu72 phosphatase has been newly identified as a fine-tuning regulator of TCR signal by modulating the phosphorylation status of TCR-mediated molecules including ZAP70 in a phosphorylation-dependent manner (**Fig. 2**) (10). Ssu72-deleted CD4⁺ T cells show increased phosphorylation of ZAP70 (Y292, Y319, and Y493) and various downstream molecules including ERK, MEK, and NF-κB after TCR-mediated stimulation (10). These results suggest that Ssu72 can act as a critical modifier for the maintenance of phosphorylation status of TCR-mediated molecules such as ZAP70 and regulate CD4⁺ T cell activation and differentiation during TCR-mediated stimulation.

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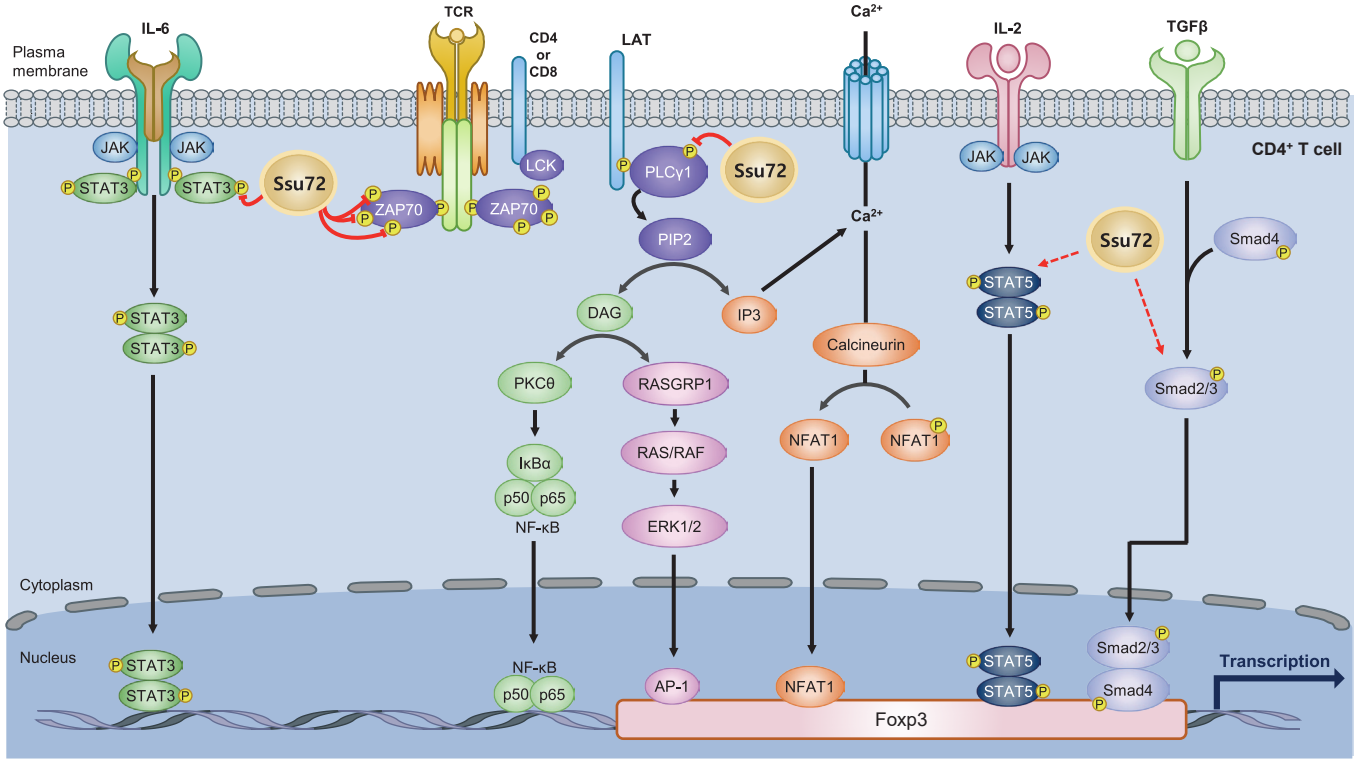

Figure 2. An overview of the immunomodulatory role of Ssu72 phosphatase in CD4⁺ T cells and immune receptor-mediated signaling pathways. Ssu72 phosphatase in peripheral T cell immunity maintains CD4⁺ T cell lineage homeostasis and physiological conditions by regulating multiple immune receptor signaling pathways, including TCR, IL-6-JAK-STAT3, and STAT5-Smad2/3 signaling pathways. Fine-tuning of immune receptor signaling pathways is essential for controlling appropriate signal strength, thereby affecting differentiation, activation, and function of T cells.

Another study has provided evidence that Ssu72 also controls Th17 cell differentiation by dephosphorylating phosphorylated-STAT3 (13). STAT3 is a critical transcription factor involved in Il17a gene expression, leading to Th17 cell differentiation (48). Ssu72 can directly bind STAT3 and dephosphorylate STAT3 Tyr705 and Ser727. Such functions of Ssu72 are mediated by various kinases including JAK, proto-oncogene tyrosine-protein kinase Src, protein kinase C, and other MAPKs both *in vitro* and *in vivo* (**Fig. 2**) (13,49,50). The loss of phosphatase activity of Ssu72 promotes excessive STAT3 activation, resulting in polarization of naïve CD4⁺ T cells toward Th17 cells. Moreover, the overexpression of Ssu72 in Th17 cells stimulated with IL-6 can reduce the phosphorylation status of STAT3 and inhibit inflammatory responses by regulating IL-1β, IL-17A, IL-21, and the inhibitor of κB (IκB) kinase family TANK binding kinase 1 (TBK1) and IκB kinase epsilon (IKBKE) (13,51,52). In response to IL-6, STAT3 is activated by homodimerization of the signaling β-receptor gp130 phosphorylation of STAT3 through activation of JAK (53). IL-6-dependent STAT3 activation negatively regulates TGF-β-induced Foxp3 expression and promotes differentiation of naive CD4⁺ T cells into Th17 cells (54). Thus, it has been suggested that Ssu72 can play a potential role as a modulator that downregulates IL-6-JAK-STAT3 signaling axis downstream, contributing to CD4⁺ T cell homeostasis (13). Taken together, these findings indicate that the phosphatase activity of Ssu72 is critical for regulating immune receptor-mediating signaling pathways, including TCR signaling and STAT3 signaling pathways, resulting in CD4⁺ T cell differentiation and maintenance.

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Ssu72 and Tregs

Downregulation of Treg signature genes in Ssu72-deficient T cells in the periphery supports the possibility that Ssu72 can contribute to the differentiation into Tregs in the periphery. Foxp3-expressing Tregs are a specialized subset of $CD25^+CD4^+$ T cells that engage in the maintenance of homeostatic tolerance and the control of pathological immune responses, including autoimmune disorders and immune responses to cancer (55). Tregs are divided into 2 different subsets: thymus-derived Tregs (tTregs) and peripherally-induced Tregs (pTregs) (56,57). tTregs, which are derived from the thymus, play essential roles in maintaining immune tolerance to self-Ag and major MHC complexes presented on thymic APCs. However, Foxp3 can be converted into conventional T cells after encountering foreign Ags under tolerogenic conditions. These so-called pTregs derived from extrathymic development are responsible for the control of locally peripheral tolerance at mucosal sites of inflammation such as the gut, lung, and skin known to directly encounter environmental Ags (58,59). Unlike tTregs, Tregs differentiating from $Foxp3^-CD4^+$ T cells in the presence of TGF-β and IL-2 are termed iTregs when generated *in vitro* or pTregs when generated *in vivo* (59,60). Interestingly, Ssu72-deleted $CD4^+$ T cells can impair the induction of Foxp3 expression in a phosphorylation-dependent manner, leading to inhibition of the polarization of naïve $CD4^+$ T cells into iTregs (11). The loss of Ssu72 phosphatase activity in T cells can lead to failure of Foxp3 induction (11). Foxp3 is essential for Treg development, differentiation, and function in both thymus and peripheral lymphoid organs (25). Foxp3 expression is induced by the presentation of peptides derived from autoantigens of host through TCR–MHC class II interactions (30,55). Recent findings have suggested that Ssu72 can regulate the induction of Foxp3, resulting in the maintenance of the differentiation into Tregs in the periphery by contributing to TCR signaling downstream cascades (11). Ssu72 deficiency in $CD4^+$ T cells exhibits upregulation of the phosphorylation status of phospholipase C gamma 1 (PLCγ1) after TCR stimulation. In response to TCR stimulation, hyperphosphorylated PLCγ1 by Ssu72 depletion can induce impaired translocation of nuclear factor of activated T-cells 1 into the nucleus, thereby inhibiting the induction of Foxp3 expression (**Fig. 2**). PLCγ1 performs a critical function in regulating TCR downstream signaling pathways via catalyzing phosphatidylinositol 4,5-bisphosphate to diacylglycerol and inositol (1,4,5)-trisphosphate (61). PLCγ1 deficiency can lead to peripheral T cell lymphopenia and interfere with TCR-mediated signaling and production of cytokines, including IL-2 and IFN-γ (62). The development of autoimmune diseases in PLCγ1-deficient mice is attributed to impaired Treg development and function (62). Surprisingly, T cell development and functional characteristics in PLCγ1-deficient mice are similar to those of Ssu72-deficient mice, revealing the potential role of Ssu72 in T cell development, activation, and pathophysiology.

In addition to TCR signaling downstream cascades, the differentiation of naïve T cells into Tregs is also promoted by IL-2 and TGF-β signaling (63,64). The extrathymic development of $Foxp3^+$ Tregs (pTreg and iTreg) depends on the presence of TGF-β and IL-2, which are activated by Smad2/3 and STAT5, respectively, leading to Foxp3 induction (65-67). Interestingly, Ssu72-deficient T cells show limited phosphorylation of Stat5 and Smad2/3 after IL-2 and TGF-β stimulation (**Fig. 2**), although a direct interaction of Ssu72 with Stat5 or Smad2/3 has not yet been evaluated (11). Moreover, these receptor-mediated signaling cascades can influence the expression level of Ssu72 protein (11). The expression of Ssu72 upregulated by TCR stimulation and/or IL-2 signaling can promote the differentiation of naïve $CD4^+$ T cells into peripheral Tregs. Peripherally differentiated Tregs can secrete autocrine TGF-β, allowing secreted TGF-β to expand the proportion of Tregs (68,69). Therefore, Ssu72 play an essential role in IL-2 and TGF-β signaling axes-mediated

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differentiation of peripheral Tregs. In summary, Ssu72 can serve as a regulator of Foxp3 induction and ultimately affect the differentiation of naïve CD4⁺ T cells into Tregs in the periphery by orchestrating receptor-mediated signaling pathways.

The role of Ssu72 as a regulator in the homeostatic balance between Th17 and Tregs

Several studies have established that reversible alterations of differentiation between Th17 and Tregs are associated with the development of autoimmune diseases (60,70,71). Th17 cells are classified as IL-17-releasing cells that exhibit a high expression of RORγt. They are critical for host defense against microbial pathogens such as fungi, viruses, and bacteria, and the development of autoimmune diseases (72). After TGF-β signaling for initial differentiation, Th17 cells contribute to inflammatory responses and autoimmunity, whereas Tregs maintain immune homeostasis by downregulating this phenomenon (73). Therefore, unraveling the mechanisms affecting the balance between Th17 and Treg provides a pathophysiological understanding of autoimmunity and tolerance. Recently, it has been reported that Ssu72 can orchestrate Th17/Treg plasticity and balance (**Fig. 3**) (11,13). Ssu72-overexpressing splenocytes show reduced proportion of Th17 cells with inhibited Treg differentiation (13). Multiple factors including TCR signaling, cytokine signaling, and Foxp3 stability are essential for appropriate regulation of Th17/Treg balance (73). In particular, cytokine receptor signaling pathways are required for homeostatic regulation of CD4⁺ T cell lineages. They are closely related to the pathogenesis of autoimmune diseases. At an early stage, TGF-β required for Th17 differentiation in order to express IL-17 and RORγt can induce differentiation of both Th17 and Tregs (74,75). However, the presence of IL-6 leads to Th17 cell differentiation by phosphorylating and activating STAT3, whereas IL-2 signaling induces the expression of Foxp3 via upregulating the phosphorylation status of STAT5 and ultimately contributes to polarization toward Tregs (76,77). Recently, in response to TGF-β and IL-2, Ssu72-deficient CD4⁺ T cells exhibit limited phosphorylation of Smad2/3 and STAT5, which bind to the Foxp3 locus, promote expression of the Foxp3 gene, and markedly abrogate Foxp3 expression.

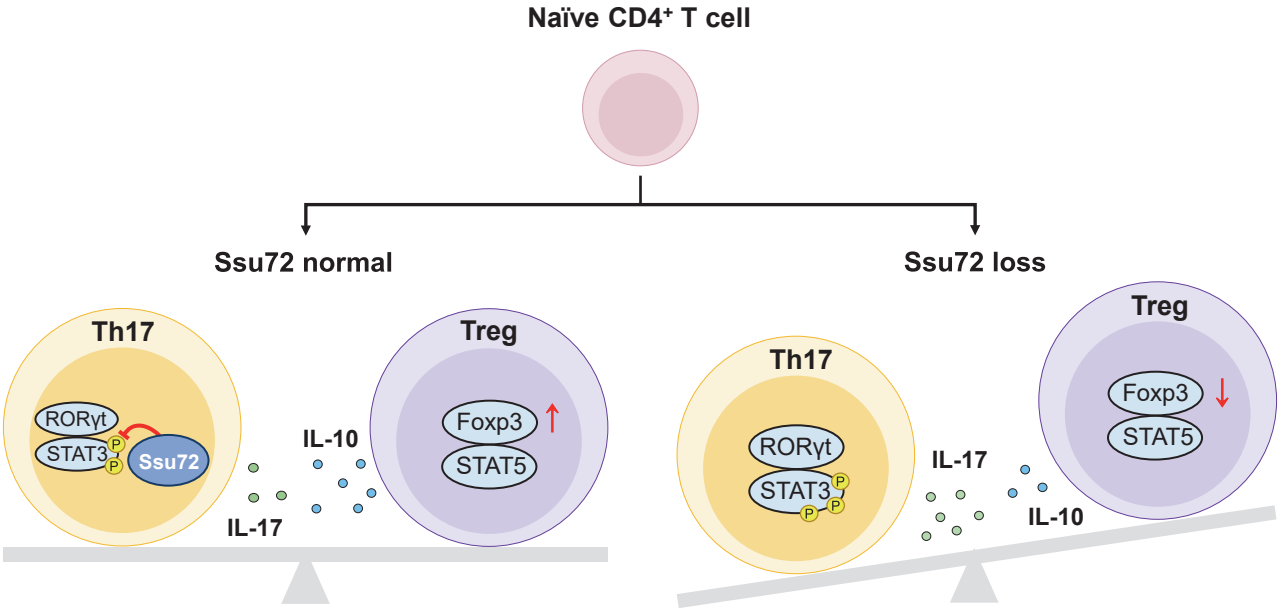

Figure 3. Ssu72 orchestrates the homeostatic balance between Th17 and Tregs. Ssu72 deficiency in CD4⁺ T cells promotes the polarization of CD4⁺ T cells toward pro-inflammatory Th17 programs by disrupting Foxp3 induction. Therefore, the molecular mechanism underlying differentiation into CD4⁺ T cell lineages by Ssu72 phosphatase is implicated in controlling TCR and cytokine receptor-mediating signaling cascades, including TGF, IL-2, and IL-6.

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On the other hand, treatment with purified Ssu72 in splenocytes isolated from collagen-induced arthritis (CIA) mice stimulated with IL-6 can decrease the differentiation into Th17 cells by dephosphorylating the level of phosphorylated STAT3 (13). Increased proportions of pathogenic IFNγ$^+$IL-17$^+$ Th17 cells in dextran sulfate sodium (DSS)-induced colitis mouse model and human inflammatory bowel disease (IBD) tissues support the possibility that Ssu72 can control IL-6 and/or IL-23 receptor-mediated signaling pathway (11). These comprehensive results indicate that Ssu72 can act as a critical homeostatic regulator of the Th17/Treg axis by affecting the cytokine microenvironment. A detailed description of the therapeutic effect of Ssu72 in these mouse disease models, including DSS-induced colitis and CIA mouse models, will be discussed below.

ASSOCIATION BETWEEN Ssu72 PHOSPHATASE AND PATHOGENESIS OF IMMUNE-MEDIATED DISEASES

Alterations of homeostatic balance between CD4$^+$ cell lineages can induce chronic T cell activation and increase the proportion of pro-inflammatory T cells, resulting in the development of autoimmune disorders (70,78). An appropriate phosphorylation status of intracellular molecules involved in receptor-mediated signaling is required for maintaining homeostatic proportions between CD4$^+$ cell lineages (70,79). Recent studies have suggested that the loss of phosphatase activity of Ssu72 can lead to failed signal transduction of multiple immune receptor signaling pathways, including TCR downstream signaling and cytokine receptor signaling pathways, promoting the differentiation of naïve CD4$^+$ T cells into effector CD4$^+$ T cells (10). Dysregulation of these signaling pathways, which form a complex network for immune responses, is associated with increased inflammatory responses and the development of autoimmune disorders, including IBD, multiple sclerosis, and rheumatoid arthritis (RA). Therefore, targeting the phosphatase activity of Ssu72 highlights a therapeutic approach that can effectively ameliorate the pathogenesis of autoimmune disorders.

IBD

Intestinal inflammatory diseases result from a chronic inflammatory response to intestinal microbes and hyperactivation of the innate and adaptive immune systems (80). There are two main types of IBD: Crohn's disease and ulcerative colitis (UC). UC and Crohn's disease are polygenic disorders characterized by chronic recurrent inflammation that causes intestinal pain, diarrhea, and intestinal bleeding (80,81). UC is restricted to the large intestine, appearing as a uniform continuous pattern of inflammation, whereas Crohn's disease can occur throughout the gastrointestinal tract as a patch. Additionally, the thickness of the inflammation is different between the 2 diseases. UC is localized to the mucous membrane, whereas Crohn's disease is present in both the mucosa and the underlying muscle tissue (14). It has been demonstrated that the CD4$^+$ T-cell subset contributes to chronic intestinal inflammation, which accumulates in the mucosa in both UC and Crohn's disease patients (82). It was originally thought that Crohn's disease was driven by Th1 cells, whereas UC was driven by Th2 cells (83). However, more recent studies have revealed that identified Th17 and Treg lineages are involved in the development of IBD (84). Furthermore, the effect of CD4$^+$ T cells on the pathogenesis of IBD has been further complicated by the recent discovery of CD4$^+$ T cell plasticity in the intestinal mucosa of both Crohn's disease and UC patients (85,86).

Ssu72 has been correlated with the maintenance of peripheral tolerance in mucosal sites such as lungs and intestine by controlling pTreg homeostasis (11). Under physiological

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conditions, Ssu72 deficiency in T cells can increase the population of active $CD4^+$ T cells in the intestinal lamina propria and lungs (11). In a mouse model of DSS-induced acute colitis, a well-established mouse model for analyzing T-cell responses to mucosal injury, features of acute colitis including body weight loss and bloody stools were observed in Ssu72-deficient mice (11,87). Ssu72-deficient mice also showed more severe mucosal hyperemia and colonic ulceration compared with wild-type mice (11). Consistent with this, the level of Ssu72 expression in both immune cells and epithelial cells was downregulated in patients with active UC (aUC) and aCD, whereas increased expression of Ssu72 was observed in healthy controls and inactive UC patients. Considering that a defect of Ssu72 in T cells inhibits the polarization of naïve $CD4^+$ T cells toward Tregs in the periphery and interferes with the maintenance of mucosal tolerance, the correlation between Ssu72 expression in Tregs and the severity of IBD provides a therapeutic potential for IBD. Although both $ROR\gamma t^+$ cells and $Foxp3^+$ cells in the lamina propria were increased in patients with aUC and aCD, the level of Ssu72 expression in infiltrating $Foxp3^+$ cells was reduced in tissues from IBD patients (11). These comprehensive findings suggest that Ssu72 depletion in T cells is associated with the pathogenesis of IBD by causing an imbalance between effector and regulatory $CD4^+$ T cells. Thus, Ssu72 is required for pTreg homeostasis and the maintenance of mucosal tolerance, indicating the potential of Ssu72 as a therapeutic target and diagnostic marker for IBD.

RA

Several studies have demonstrated that targeting the Th17/Treg axis has therapeutic potential for autoimmune diseases, including RA (60,73,88). RA is a chronic autoimmune disorder with persistent inflammation of multiple synovial joints, leading to progressive tissue destruction of bone resorption and articular cartilage (89). Failure to maintain Th17/Treg balance promotes Th17 cell-mediated pro-inflammatory responses and contributes to the pathogenesis of RA (71,73,90). Increased proportion of Th17 cells can augment pro-inflammatory programs by secreting pro-inflammatory cytokines (such as IL-17, IL-23, and IL-6) and increasing the expression of transcription factors associated with pathogenic inflammation, including RORγt and STAT3. These data suggest that restoring the balance between Th17 and Tregs has potential for an anti-rheumatic therapy. In connection with this, dysregulation of STAT3 activation, which results from the binding of IL-6 and its receptors, has been linked to immune-mediated diseases and cancer (44,50,91). One example of targeting IL-6-JAK-STAT3 signaling is tofacitinib. Tofacitinib, a JAK inhibitor, can potently inhibit the proportion of pathogenic Th17 cells and suppress IL-6-induced phosphorylation of STAT1 and STAT3 in synovial tissues from RA patients (92,93).
Thus, targeting protein tyrosine phosphatases that negatively regulate IL-6-JAK-STAT3 signaling has a determinant potential to attenuate the pathogenesis of various disorders, including RA (50,94). Interestingly, a recent study has shown that Ssu72 can intervene RA pathogenesis by controlling the plasticity between Th17 and Tregs (13). In the CIA mouse model in which RA was induced 1 week after collagen type II immunization, the Ssu72 overexpression group showed inhibited osteoclastogenesis and attenuated joint destruction and cartilage damage compared to the control group, contributing to improvement in the severity of CIA (13,95). The therapeutic contribution of Ssu72 to CIA pathogenesis is closely associated with homeostatic maintenance between Th17 and Tregs. Ssu72 can downregulate the differentiation into Th17 cells and the level of IL-17 and TNF-α by inhibiting the dephosphorylation of STAT3 (Tyr795 and Ser727) (13). Thus, Ssu72 phosphatase can maintain the homeostatic balance between Th17 and Treg differentiation by modulating the IL-6-JAK-STAT3 signaling pathway axis. These comprehensive findings indicate that targeting the phosphatase activity of Ssu72 could provide therapeutic insight into the pathogenesis of RA.

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Other diseases

To summarize, we have focused on Ssu72 as a mediator in immune receptor signaling that leads to the regulation of T cell homeostasis and its impact on disease pathogenesis. Although the role of Ssu72 in T cell-mediated immune responses against Ags has not been elucidated yet, dysregulation of Ssu72 in controlling TCR signaling such as chronic stimulation and excessive signal strength is likely to drive the development of viral infections and/or cancer in response to Ags. Since $CD8^+$ T cells, which bind to MHC class I molecules, focus on cytotoxic immune responses, $CD8^+$ T cells have important functions, especially in viral infections and/or cancer (96). Ssu72-deficient mice with reduced proportion of $CD8^+$ T cells are susceptible to activation-induced cell death following TCR engagement (10,11). The impairment of TCR signaling and TCR-mediated proliferation in Ssu72-deficient mice caused failure of cytotoxic functions in response to persistent Ags. In cancer and chronic viral infections, chronic TCR signaling can lead to T cell dysfunction such as T cell exhaustion and anergy (97). Thus, an immunological role of Ssu72 in $CD8^+$ T cells can be expected, although further studies on Ssu72 in $CD8^+$ T cells are required. In addition, our recent unpublished observation suggested that Ssu72 deficiency in T cells could impair Ag-specific $CD4^+$ and $CD8^+$ T cell responses in a lymphocytic choriomeningitis virus (LCMV) Armstrong infection, a frequently used mice model for studies on relationships between viral infections and immune responses (96). Nevertheless, whether Ssu72 in T cells is directly involved in adaptive immune responses (including immunological memory and Ab responses that coordinate with other immune cells such as Ag-presenting macrophages and Ab-secreting B cells) remains poorly understood. Therefore, further studies are needed to elucidate the function of Ssu72 in T cells of the adaptive immune system.

CONCLUSIONS

Ssu72 phosphatase is a predominant driver that orchestrates $CD4^+$ T cell differentiation, activation, and functions by modulating the phosphorylation status of intracellular molecules involved in immune receptor-mediated signaling, including ZAP70 and PLCγ1 (10,11,13). Sophisticated control of signaling pathways is indispensable for T cell differentiation and function. Ssu72 regulates T cell activation and the homeostatic balance between $CD4^+$ T cell lineages by closely downregulating TCR signaling. As such a homeostatic regulatory mechanism, it has been suggested that Ssu72 phosphatase can control the phosphorylation activity of ZAP70, which is influential in initial TCR signaling (10). In addition, Ssu72 can directly interact with PLCγ1, which affects $Ca2^+$-calcineurin signaling, and dephosphorylate its active sites (**Fig. 2**), thereby driving Treg differentiation in the periphery (11). This result provides evidence that Ssu72 can participate in signal transduction of $Ca2^+$-calcineurin, NF-κB, and MAPK signaling pathways, which are major TCR downstream signaling pathways (**Fig. 2**) (10,11). In addition to TCR signaling, Ssu72 is involved in T cell activation, differentiation into $CD4^+$ T cell lineages, and functions by mediating cytokine receptor signaling cascades, including IL-2, IFN-γ, IL-6, and TGF-β signaling (**Fig. 2**). Notably, Ssu72 can downregulate the phosphorylation status of STAT3 in response to IL-6. The expression of Ssu72 is altered after stimulation of TGF-β and IL-2. Therefore, it has been indicated that Ssu72 might act as a negative regulator of cytokine receptor signaling cascades, including IL-6-JAK-STAT3 and TGF-β and/or IL-2 signaling pathways (13).

Immunomodulatory roles of Ssu72 in maintaining $CD4^+$ T cell homeostasis by mediating multiple signaling pathways provide mechanistic insights to prevent disease progression and

suggest therapeutic potential. Protein tyrosine phosphorylation, where Ssu72 phosphatase modulates receptor-mediated signaling pathways, is closely correlated with the homeostatic balance between $CD4^+$ T cell lineages and their physiological functions. The phosphatase activity of Ssu72 raises the possibility that it can act as a potential target for several other downstream signaling cascades in addition to TCR and cytokine receptor signaling cascades. Since Ssu72 regulates the induction of Foxp3 strongly and the hypophosphorylation of STAT3 protein, it is likely to be involved in STAT5 and other signaling related to Foxp3 expression regulation (**Fig. 2**). In particular, it was confirmed that Ssu72 was involved in the regulation of the PI3K-Akt-mTOR cascade in our unpublished data. We have covered different aspects of $CD4^+$ T cells, ranging from their homeostasis that forms this complex signaling network to their pathophysiological aspects. Although previous studies have focused on $CD4^+$ T cell lineages in the periphery, the functional relevance between Ssu72 in T cells and multiple immune receptor-mediated signaling pathways indicates the potential immunological role of Ssu72 in other T cell lineages such as $CD8^+$ T cells, NKT cells, and γδ T cells (10,11). However, it is still unclear how the Ssu72 protein in the cytoplasm moves to the cell membrane in response to various T cell receptor signaling. Further studies are needed to elucidate additional immune functions and regulatory mechanisms of Ssu72 in immune-mediated pathophysiology. Understanding the association between functional alterations of Ssu72 under various physiological conditions and the pathology of immune-mediated diseases will provide insight into the treatment and prevention of autoimmune diseases and other diseases.

In addition to the immunological roles of Ssu72, recent studies have shown that targeting Ssu72 phosphatase could be a potential therapeutic strategy in a tissue-specific manner. Particularly, our unpublished observation indicates that Ssu72 phosphatase in brown adipose tissue (BAT), which is essential in maintaining body temperature and energy homeostasis, can be the promising therapeutic application for multiple metabolic disorders. Ssu72 deficiency in BAT results in metabolic dysfunction, including the impaired mitochondria function and thermogenesis. Given that targeting and enhancing BAT activity could be a promising therapeutic tool to treat metabolic diseases in humans, enhancing the expression and activity of Ssu72 can improves the treatment of metabolic diseases, including type 2 diabetes, obesity and NAFLD (98). Another study has implicated evidence for the therapeutic links between Ssu72 and steatohepatitis-associated HCC development (9). Ssu72 phosphatase mediates the dephosphorylation of hepatocyte nuclear factor 4α (HNF4α), a master transcriptional modulator of hepatocyte differentiation and functional liver maintenance. Loss of Ssu72 in hepatocytes leads to the downregulation of HNFα target genes, contributing to the progression of steatohepatitis-associated HCC (9). Now that identifying the mechanistic insights to clearly understand the conversion of nonalcoholic steatohepatitis (NASH)-to-HCC remains elusive, targeting Ssu72 phosphatase could be an attractive therapeutic approach (99). In summary, we have highlighted the emerging role of Ssu72 phosphatase which has important potential in the treatment and diagnosis of diseases, ranging from cancer, metabolic disease and immune disease.

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