

Review Article

Suppressors of cytokine signaling: Potential immune checkpoint molecules for cancer immunotherapy

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Inhibition of immune checkpoint molecules, PD-1 and CTLA4, has been shown to be a promising cancer treatment. PD-1 and CTLA4 inhibit TCR and co-stimulatory signals. The third T cell activation signal represents the signals from the cytokine receptors. The cytokine interferon- γ (IFN γ) plays an important role in anti-tumor immunity by activating cytotoxic T cells (CTLs). Most cytokines use the Janus kinase (JAK)/signal transducer and activator of transcription (STAT) pathway, and the suppressors of cytokine signaling (SOCS) family of proteins are major negative regulators of the JAK/STAT pathway. Among SOCS proteins, CIS, SOCS1, and SOCS3 proteins can be considered the third immunecheckpoint molecules since they regulate cytokine signals that control the polarization of CD4⁺ T cells and the maturation of CD8⁺ T cells. This review summarizes recent progress on CIS, SOCS1, and SOCS3 in terms of their anti-tumor immunity and potential applications.

T cells and natural killer (NK) cells have been manipulated therapeutically to promote endogenous anti-tumor immunity. Cytotoxic T lymphocytes (CTLs), also known as CD8⁺ effector T cells, selectively recognize the antigen peptide presented on class I-MHC (or HLA) and kill antigen-expressing cells. While NK cells kill transformed cells that have lost the expression of class I-MHC. CD4⁺ helper T cells orchestrate diverse immune responses that activate CTLs and innate immune cells, including macrophages and dendritic cells, and promote antibody production from B cells. For antitumor immunity, Th1 cells that produce interleukin-2 (IL-2) and interferon- γ (IFN γ) play a positive role, while CD4⁺ regulatory T cells (Tregs) suppress anti-tumor immunity.

T cell activation is initiated through antigen recognition by the T cell receptor (TCR) and co-stimulatory signals such as CD28 (Fig. 1). On the other hand, the inhibitory signals for T cell activation (i.e., immune checkpoints) are crucial for the maintenance of self-tolerance and prevention of autoimmunity as well as excess immune responses. The two immune checkpoint receptors that have been most actively studied in the context of clinical cancer immunotherapy, cytotoxic T-lymphocyte-associated antigen 4 (CTLA4, also known as CD152) and programmed cell death protein 1 (PD1, also known as

CD279), are both inhibitory receptors. PD1 recruits the tyrosine phosphatase, which inhibits TCR signaling, while CTLA4 inhibits CD28-mediated co-stimulatory signals. Clinical efficacies of the antibodies that block either of these receptors proved that antitumor immunity can be enhanced by inhibiting immune checkpoints. The expression and activation of immune checkpoint proteins are important immune resistance mechanisms of tumors. Multiple additional immune checkpoints represent promising targets for therapeutic blockade based on preclinical experiments, and inhibitors for many of these are under active development.

In addition to TCR and co-stimulatory signals, T cell activation requires the third signal: signals from the cytokine receptors (Fig. 1). For example, IL-2 is necessary for the proliferation of T cells, and IL-12 and IFN γ are important for Th1 differentiation and CTL activation. Various roles of IFN γ in anti-tumor immunity have been reported.⁽¹⁾ IL-15 has been shown to be necessary for memory T cell survival. Thus, negative regulators of the cytokine signaling must be important immune checkpoint molecules that regulate anti-tumor immunity. In this review, we will focus on the suppressors of cytokine signaling (SOCS) family proteins and their relationship to anti-tumor immunity. The future direction of the application of

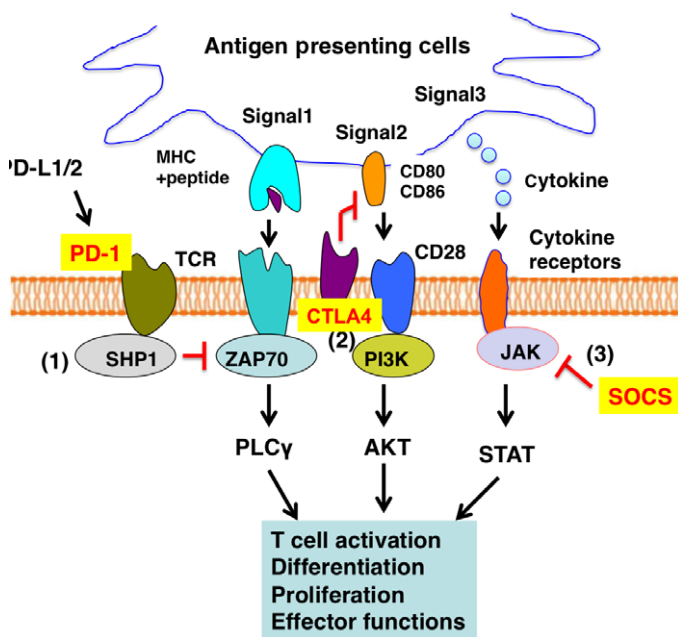


Fig. 1. T cell immune checkpoints for anti-tumor immunity. There are three major T cell activation signals, signal 1, signal 2 and signal 3. Signal 1 is MHC-antigen-peptide complex-TCR signal that activate tyrosine kinases including ZAP70. Signal 2 is the co-stimulatory signals from CD28, in which mostly PI3 kinase is involved. Signal 3 is the cytokine receptor signals that activate the JAK/STAT pathway. T cell activation by TCR, co-simulators, and cytokines is blocked by (1) PD-1, (2) CTLA4, and (3) SOCS.

SOCS inhibitors for anti-tumor immunity enhancement will also be discussed.

The JAK/STAT Pathway

Cytokines play several essential roles in the development, differentiation, and function of myeloid and lymphoid cells. Most cytokines, including interleukins, IFNs, and hematopoietic growth factors, activate the janus kinase (JAK)/signal transducer and activator of transcription (STAT) pathway.⁽²⁾ In this pathway, cytokine binding results in cognate receptors oligomerization, which initiates the activation of JAK kinases (JAK1, JAK2, JAK3, and Tyk2). JAK3 is associated with the IL-2 receptor γ (common γ chain), and is activated by IL-2-related cytokines, such as IL-4, IL-7, IL-9 and IL-15. The activated JAKs in turn phosphorylate the receptor cytoplasmic domains, which creates docking sites for SH2-containing signaling proteins. The STAT proteins are the major substrates that are recruited to the receptors and activated by JAKs. For example, IFN γ receptors activate JAK1 and JAK2, and then phosphorylate and activate STAT1, while IL-6 binds to the complex of IL-6 receptor- α (IL6R α) chain and gp130, which mainly activate JAK1 and STAT3.⁽³⁾ An anti-inflammatory cytokine IL-10 also activates STAT3.⁽⁴⁾ In case of T cells, STAT5 is mainly activated by IL-2, which promotes cell proliferation and survival. Differentiation of the helper T cell subsets, Th1, Th2, and Th17 requires STAT4 (activated by IL-12), STAT6 (by IL-4), and STAT3 (by IL-6 and IL-23), respectively. IL-4 in combination with TGF- β has been shown to induce Th9 *in vitro*.⁽⁵⁾ IL-2/STAT5 is also essential for regulatory

T cell (Treg) development, and IL-21/STAT3 is essential for follicular helper T (Tfh) cell differentiation,⁽⁶⁾ and regulates CD8⁺T cells.⁽⁷⁾

The CIS/SOCS Family: Molecular Mechanisms

Suppressors of cytokine signaling proteins and cytokine inducible SH2-containing (CIS, also known as CISH) protein compose a family of intracellular proteins^(8–12) (Fig. 2). There are eight CIS/SOCS family proteins: CIS, SOCS1, SOCS2, SOCS3, SOCS4, SOCS5, SOCS6, and SOCS7, each of which has a central SH2 domain, an amino-terminal domain of variable length and sequence, and a carboxy-terminal 40-amino-acid module known as the SOCS box.^(13–15) The SOCS box interacts with elongin B/C, Cullins, and the RING-finger-domain-only protein RBX2, which binds E2 ubiquitin-transferase.⁽¹⁴⁾ Thus, CIS/SOCS family proteins, as well as other SOCS-box-containing molecules, function as E3 ubiquitin ligases and mediate the degradation of proteins that are associated with these family members through their N-terminal and SH2 regions.

The central SH2 domain determines the target of each SOCS and CIS protein. The SH2 domain of SOCS1 directly binds to the activation loop of JAKs.⁽¹¹⁾ The SH2 domains of CIS, SOCS2, and SOCS3 bind to phosphorylated tyrosine residues of the activated cytokine receptors (Fig. 2). SOCS3 binds to gp130-related cytokine receptors. In addition to the general SOCS-box function in this family, both SOCS1 and SOCS3 have a unique N-terminal motif that can inhibit JAK tyrosine kinase activity directly through their “kinase inhibitory region” (KIR). KIR has been proposed to function as a pseudosubstrate of JAKs, and it is essential for the suppression of cytokine signals (Fig. 2).^(16,17) The recent study of the ternary co-crystal structure among SOCS3, the JAK2 kinase domain, and a fragment of gp130 has supported this hypothesis (Fig. 3). The KIR of SOCS3 occludes the substrate-binding groove on JAK2, and biochemical studies show that it blocks substrate association. SOCS3, and probably SOCS1, inhibit the catalytic activity of JAK1, JAK2, and TYK2, but not JAK3, because the KIR and a part of the SH2 domain interact with an evolutionarily conserved “GQM” sequence that is present in all vertebrate forms of JAK1, JAK2, and TYK2, but not JAK3, where it lines one edge of the substrate-binding groove⁽¹⁸⁾ (Fig. 3). The SH2 domain of SOCS3 binds to the phosphorylated gp130, while that of SOCS1 binds to the activation-loop of JAKs.⁽¹⁷⁾ Consistently, suppression of type I IFN signaling by SOCS1 was shown not to require any of the phosphorylation sites in the IFNAR1 receptor.⁽¹⁹⁾ SOCS1 and SOCS3 have been shown to be concentrated in exosomes and microparticles, respectively, for uptake by alveolar epithelial cells and subsequent inhibition of STAT activation.⁽²⁰⁾ Secretion and transcellular delivery of vesicular SOCS proteins were diminished by cigarette smoking, suggesting a novel mechanism of dysregulated inflammation by smoking.

CIS and Anti-Tumor Immunity

CIS was discovered as an inducible gene in response to various cytokines including EPO, IL-2, IL-3, and IL-5, which mostly activate STAT5.^(10,11) CIS does not possess the KIR and cannot inhibit JAK tyrosine kinase activity directly. However, CIS binds to phosphorylated cytokines receptors, such as the EPO receptor, IL-2 receptor, IL-3 receptor β chain, prolactin receptor, and the growth hormone (GH) receptor, which

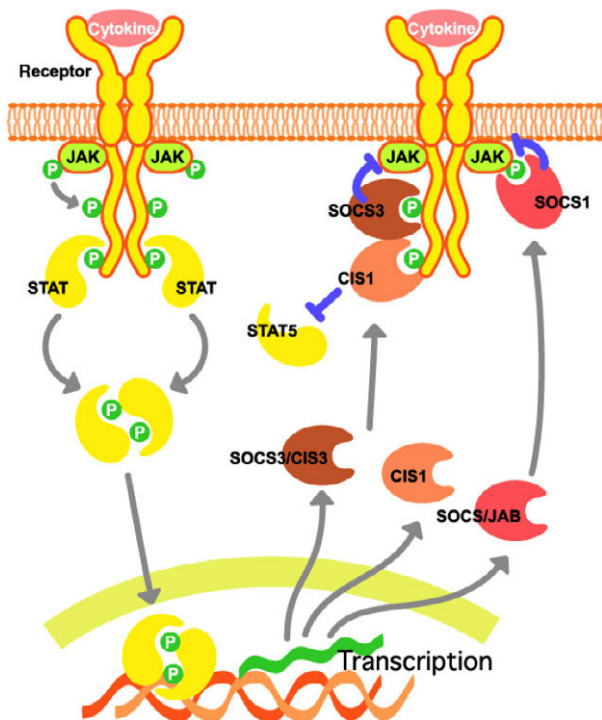


Fig. 2. General mechanism of the action of CIS, SOCS1, and SOCS3. Cytokine stimulation activates the JAK-STAT pathway, leading to the induction of CIS, SOCS1 and/or SOCS3. SOCS1 binds to the JAKs and inhibits catalytic activity, SOCS3 binds to JAK-proximal sites on cytokine receptors and inhibits JAK activity, and CIS blocks the binding of STATs to cytokine receptors and induces receptor degradation.

mostly activate STAT5.^(10,11,21–23) Thus, CIS is believed to suppress STAT5 by masking STAT5-binding phosphotyrosine motifs on the receptors, and also by inducing ubiquitin/proteasome-dependent degradation of the activated receptors^(24,25) (Fig. 3). However, recent studies using *Cish* (encodes CIS protein)-deficient mice have suggested that CIS is a negative feedback regulator of IL-4 rather than IL-2, although the precise biochemical mechanism remains to be clarified.⁽²⁶⁾

CIS has been shown to be an important immune checkpoint molecule for adoptive cancer immunotherapy. Genetic deletion of *Cish* in CD8⁺ T cells enhances their expansion and function, resulting in pronounced and durable regression of established tumors.⁽²⁷⁾ Another recent paper suggest that CIS is a critical negative regulator of IL-15 signaling in NK cells and that deletion of *Cish* enhances anti-tumor immunity.⁽²⁸⁾ CIS was rapidly induced in response to IL-15, and deletion of *Cish* rendered NK cells hypersensitive to IL-15, as evidenced by enhanced proliferation, survival, IFN- γ production, and cytotoxicity toward tumors. In this study, CIS has been shown to selectively interact with the tyrosine kinase JAK1, inhibiting its enzymatic activity and targeting JAK1 for proteasomal degradation. *Cish*-deficient mice were resistant to melanoma, prostate, and breast cancer metastasis *in vivo*, and this was intrinsic to NK cell activity. These papers suggest possibilities for new cancer immunotherapies directed at blocking CIS function.

SOCS1 and T Cells

SOCS1 can inhibit nearly all cytokines using JAKs because it binds to and directly inhibits JAKs except for JAK3. Thus,

SOCS1 specificity is regulated by its inducible expression. SOCS1 plays an essential negative regulatory role in IFN- γ , IL-2, IL-4, IL-7, and IL-15 functions in T cell.⁽²⁹⁾ SOCS1-deficient mice showed an aberrant CD4/CD8 ratio, implicating the role of SOCS1 in thymic T cell development.^(30,31) SOCS1 is involved in maturation and proliferation of CD8⁺ T (CTL) cells. In SOCS1-knockout mice, the number of CTLs has increased and showed CD44^{high}CD62L^{high} central memory type characteristics partly due to higher responses to IL-17 and IL-15.^(32,33) IL-21 induces SOCS1 expression in CD8⁺ T cells,⁽⁷⁾ and in the absence of SOCS1, IL-21 dramatically potentiates IL-7- and IL-15-induced proliferation in CD8⁺ T cells.

SOCS1 plays critical roles in the helper T cell (Th) subset differentiation. SOCS1^{-/-} CD4 naive T cells differentiated into Th1, even under non-skewing conditions, while Th17 differentiation was strongly suppressed.⁽³⁴⁾ This Th17 suppression by SOCS1 deficiency is probably a result of hyper-production and enhanced signal transduction of IFN- γ . STAT3 activation was reduced in SOCS1-deficient T cells, mostly because of upregulation of SOCS3 gene expression, which can account for reduced IL-6 responses and Th17 differentiation.⁽³⁴⁾ In addition, SOCS1^{-/-} T cells are less responsive to TGF- β , although the mechanism has not been clarified.⁽³⁴⁾

SOCS1 also plays an important role in the regulation of regulatory T cells (Tregs), which has been shown to regulate anti-tumor immunity.⁽³⁵⁾ SOCS1 has been reported to play an important role in Treg cell integrity and function by protecting Tregs from a harmful effect of excessive inflammatory cytokines at inflammatory conditions.⁽³⁶⁾ SOCS1-deficient Tregs easily lose Foxp3 expression and are converted into Th1- or Th17-like effector cells, probably because of STAT1 and STAT3 hyperactivation. We have shown that SOCS1-deficiency in Tregs resulted in strong enhancement of anti-tumor immunity (Takahashi *et al.*, unpublished data). In humans, a negative correlation between SOCS3 and Foxp3 levels has been reported.⁽³⁷⁾

SOCS1 is a target of miRNA-155 and miR-146a in Tregs.^(38–40) Lu *et al.* showed that during thymic differentiation of Tregs, upregulation of Foxp3 is associated with high miR155 expression, which in turn promotes the competitive fitness and proliferative potential of Treg cells by inducing SOCS1 downregulation. In this case, IL-2 signal is enhanced by SOCS1 downregulation. miR-155 deficiency also attenuates liver ischemia-reperfusion injury through upregulation of SOCS1, which was associated with promotion of M2 macrophage polarization and suppression of Th17 differentiation.⁽⁴¹⁾

SOCS1 and Anti-Tumor Immunity

SOCS1 is now considered to be an immune checkpoint molecule for anti-tumor immunity, because SOCS1 negatively regulates signaling of IFN- γ and IL-12, essential cytokines for anti-tumor immunity. Previously, we and others showed that SOCS1-silenced DCs induce stronger anti-tumor immunity.^(42–44) Myeloid cell-specific SOCS1-deficient mice were resistant to tumor growth in an IFN- γ dependent manner⁽⁴⁵⁾ (Fig. 4). In CD8⁺ T cells, even though SOCS1-deficiency caused defective expansion following *in vivo* antigen stimulation,⁽⁴⁶⁾ SOCS1-silenced CD8⁺ T cells showed stronger anti-tumor activity.⁽⁴⁷⁾ Because SOCS1 is an important target of miRNA-155, miRNA-155 over-expression reduced SOCS1 expression levels, thereby enhancing antitumor responses. Indeed, enforced SOCS1 expression in CD8⁺ T cells phenocopied with the miRNA-155 deficiency,

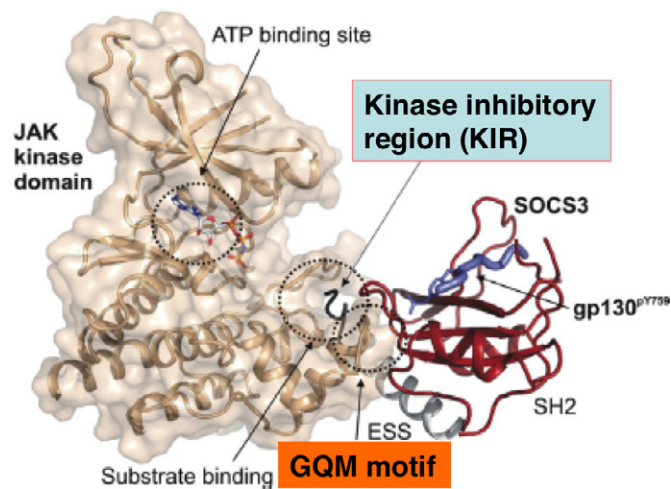


Fig. 3. Structure of the complex of JAK2 and SOCS3, and gp130 phosphopeptide. SOCS3 binds the kinase domains of JAK1, JAK2, and TYK2 and inhibits its catalytic activity by blocking the substrate-binding site with its kinase inhibitory region. SOCS3 remains bound to gp130 while in complex with JAK (beige). Regions for GQM motif and KIR are highlighted. The figure is from Babon JJ, Varghese LN, and Nicola NA. Inhibition of IL-6 family cytokines by SOCS3. *Semin Immunol.* 2014; 26: 13–19.⁽⁶¹⁾ Permission was obtained from Dr. Babon.

whereas SOCS1 silencing augmented tumor eradication.⁽⁴⁷⁾ In addition, higher levels of miR155 facilitates tumor growth modulating myeloid-derived suppressive cells (MDSC) through SOCS1 repression.⁽⁴⁴⁾ These observations indicate that SOCS1 is a key regulator of anti-tumor immunity in both DCs and CD8⁺ T cells.

SOCS3; Essential Regulator for STAT3-Related Cytokines

SOCS3 is highly specific for several key cytokines that are related to the gp130 family, because the SOCS3-SH2 domain has a high affinity for phosphorylated gp130. Tissue-specific conditional tissue deletion of SOCS3 demonstrated a non-redundant ability to inhibit signaling from IL-6 and also from LIF, leptin, and G-CSF.⁽⁸⁾ In SOCS3-deficient macrophages, IL-6 functions like IL-10, which is a potent inhibitory regulator of macrophages and DCs.⁽⁴⁸⁾ This is probably due to sustained activation of STAT3 in the absence of SOCS3 because the IL-10 receptor does not have SOCS3-binding sites. Macrophages expressing mutant gp130 that are unable to bind SOCS3 displayed sustained STAT3 activation and anti-inflammatory effects in response to IL-6. However, mice lacking SOCS3 in the skin or mice carrying a gp130 mutant develop exacerbated inflammation, chronic disease, and cancer.⁽⁴⁹⁾ Thus, the biological functions of the IL-6/STAT3 pathway are strictly dependent on cell types.

SOCS3 and Cancer

SOCS3 is believed to be an anti-oncogene. Reduced SOCS3 expression has been observed in various human cancers and is associated with constitutive STAT3 activation.⁽⁴⁹⁾ Recently, we reported that stomach-specific deletion of SOCS3 resulted in the development of gastric tumors, and this was dependent on leptin.⁽⁵⁰⁾ A *SOCS3* SNP was reported to be associated with human gastric cancer.⁽⁵¹⁾ Similarly, gp130 mutant mice carrying the Y757F mutation, which loses its binding ability to SOCS3, developed gastric tumors.⁽⁵²⁾ In this case, IL-11 and TGFβ have

been shown to play important roles.⁽⁵³⁾ Loss of SOCS3 also promoted pancreatic cancer driven by the oncogenic Ras mutation.⁽⁵⁴⁾ SOCS3 mutation (or variant) in the SH2 domain was discovered in a patient with polycythaemia vera.⁽⁵⁵⁾ In addition, many previous reports demonstrated that STAT3 activation in tumor-associated immune cells might promote immunosuppressive environment by mediating the generation of immune suppressor cells, including myeloid-derived suppressors (MDSCs) and Treg cells and/or by inducing production of immune suppressive factors, such as VEGF, IL-10, and IL-6.^(56–58)

However, to our surprise, deletion of SOCS3 in myeloid cells using *LysMCre-SOCS3-flox* (cKO) mice showed reduced melanoma metastasis.⁽⁵⁹⁾ In a subcutaneous transplantation model of B16F10 melanoma cells, tumor sizes were not significantly different, and SOCS3-cKO mice survived longer than wild-type (WT) mice did. SOCS3-deficient macrophages stimulated with tumor lysates *in vitro* exhibited prolonged STAT3 phosphorylation and produced a smaller amount of TNFα and IL-6, and a larger amount of monocyte attractive chemokine, MCP2/CCL8 than WT macrophages did. MCP2/CCL8 was induced via STAT3 and suppressed tumor metastasis in WT mice. We also observed a significant reduction of tumor size of subcutaneously transplanted MC38 colon adenocarcinoma cells in T cell-specific SOCS3-cKO mice (Mise-Omata *et al.*, unpublished data). Thus, the targeted inhibition of SOCS3 activity in macrophages as well as in T cells may be therapeutic for the suppression of tumor growth and/or metastasis. Nevertheless, as discussed above, there are some contradictory reports for the role of SOCS3 in anti-tumor immunity. Thus further study is necessary to determine the usefulness of SOCS3 suppression for cancer treatment.

Design of SOCS Inhibitors and Application for Promoting Anti-Tumor Immunity

Since deletion or suppression of SOCS1 and SOCS3 in either T cells or myeloid cells enhanced anti-tumor immunity, SOCS inhibitors could be ideal drugs that target an immune checkpoint controlled by cytokines. Since SOCS is an intracellular molecule, inhibitors should be membrane-permeable small compounds. As mentioned, the N-terminal region containing KIR of SOCS1 and SOCS3 is essential for the tight binding to JAK's GQM region and for the inhibition of the tyrosine kinase activity.⁽¹⁸⁾ We have shown that a KIR-mutant SOCS1 functions as a dominant negative form not only for SOCS1 but also for SOCS3⁽⁶⁰⁾ (Fig. 5). Thus, to suppress SOCS-JAK interaction, inhibition of the SH2 domain-phosphotyrosine interaction is not always necessary. Compounds capable of blocking the interaction between KIR and GQM KIR could be drugs that enhance JAK activity by preventing the function of SOCS1 and SOCS3 (Fig. 6). Such compounds may protect dephosphorylation and inactivation of JAKs by limiting SOCS1 binding to the kinase activation loop. Such compounds will be developed by *in silico* drug design because the interactions between KIR and GQM have already been resolved at atomic levels.

Concluding Remarks

Over the past two decades, following the discovery of the SOCS family proteins, we have extended our understanding of the structure and function of these proteins. Therapeutic anti-tumor effects of SOCS1 inhibition have been demonstrated by using SOCS anti-sense oligonucleotide and shRNA. We now understand that the SOCS-JAK interaction is a potentially

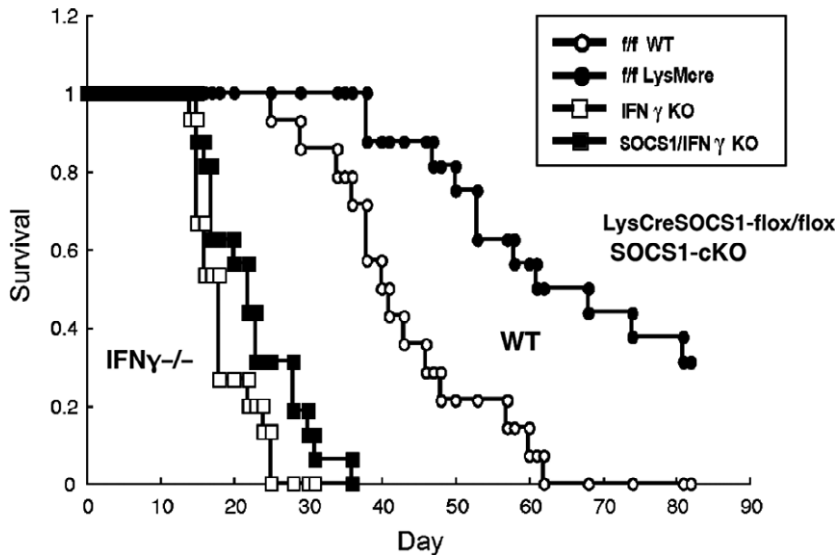


Fig. 4. Anti-tumor activity of myeloid cell-specific SOCS1 conditional knockout (cKO) mice. WT, SOCS1-cKO, IFN γ ^{-/-} and SOCS1^{-/-} IFN γ ^{-/-} mice were subcutaneously challenged with B16 melanoma cells. Kaplan-Meier survival curves are depicted as time after tumor challenge. Data are modified from Hashimoto *et al.* Silencing of SOCS1 in macrophages suppresses tumor development by enhancing antitumor inflammation. *Cancer Science*. 2009; 100: 730–736.⁽⁴⁵⁾ Copyright (c) (2009) AY.

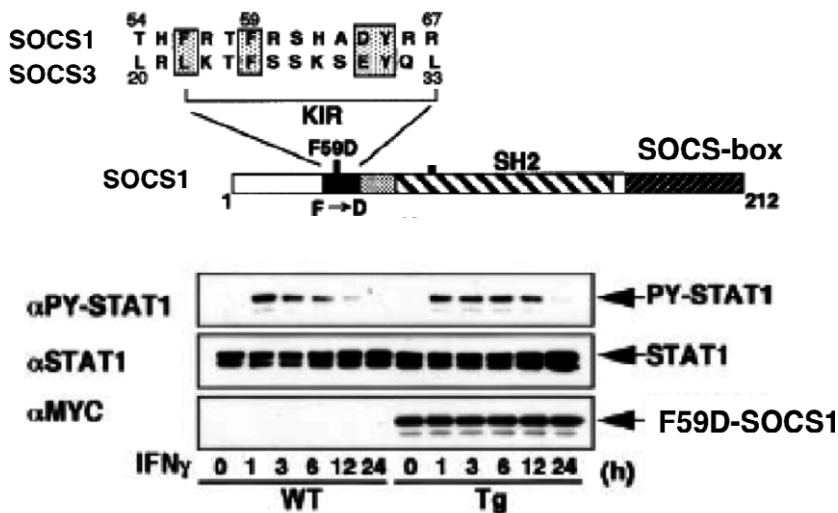


Fig. 5. Effects of KIR mutations on IFN γ -STAT1 activation. Transgenic thymocytes loading a point mutation in KIR domain which does not interact with JAK GQM motif exhibited prolonged STAT1 activation induced by IFN γ stimulation. This research was originally published in the *Journal of Biological Chemistry* [A mutant form of JAB/SOCS1 augments the cytokine-induced JAK/STAT pathway by accelerating degradation of wild-type JAB/CIS family proteins through the SOCS-box. *J Biol Chem*. 2001; 276: 40746–54].⁽⁶⁰⁾

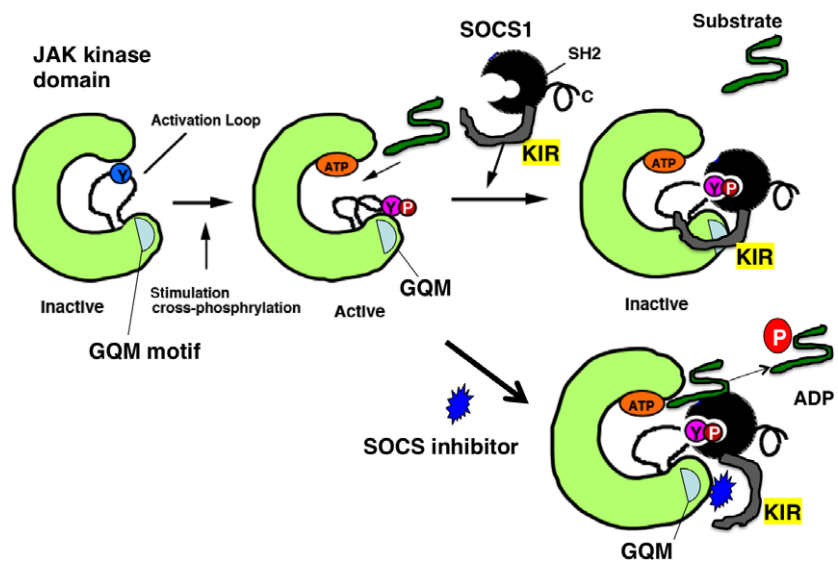


Fig. 6. Model of the effect of a SOCS inhibitor for JAK tyrosine kinase activity. A hypothetical SOCS inhibitor which blocks interaction between KIR region of SOCS1 and GQM motif of JAK will inhibit the action of KIR, therefore the substrate is accessible to the catalytic pocket of JAK. Furthermore, binding of SOCS1-SH2 domain may protect dephosphorylation of JAK's "kinase activation loop"; therefore, the kinase activation will be prolonged.

druggable target as an immune checkpoint modulator. Development of SOCS inhibitors, based on a structural analysis of the JAK/SOCS complex is highly desirable for cost-effective tumor therapy.

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References

- Ikeda H, Old LJ, Schreiber RD. The roles of IFN gamma in protection against tumor development and cancer immunoeediting. *Cytokine Growth Factor Rev* 2002; **13**: 95–109.
- O'Shea JJ, Gadina M, Schreiber RD. Cytokine signaling in 2002: new surprises in the Jak/Stat pathway. *Cell* 2002; **109**(Suppl): S121–31.
- Guschin D, Rogers N, Briscoe J et al. A major role for the protein tyrosine kinase JAK1 in the JAK/STAT signal transduction pathway in response to interleukin-6. *EMBO J* 1995; **14**: 1421–9.
- Weber-Nordt RM, Riley JK, Greenlund AC, Moore KW, Darnell JE, Schreiber RD. Stat3 recruitment by two distinct ligand-induced, tyrosine-phosphorylated docking sites in the interleukin-10 receptor intracellular domain. *J Biol Chem* 1996; **271**: 27954–61.
- Tamiya T, Ichiyama K, Kotani H et al. Smad2/3 and IRF4 play a cooperative role in IL-9-producing T cell induction. *J Immunol* 2013; **191**: 2360–71.
- Vogelzang A, McGuire HM, Yu D, Sprent J, Mackay CR, King C. A fundamental role for interleukin-21 in the generation of T follicular helper cells. *Immunity* 2008; **29**: 127–37.
- Gagnon J, Ramanathan S, Leblanc C, Ilangumaran S. Regulation of IL-21 signaling by suppressor of cytokine signaling-1 (SOCS1) in CD8(+) T lymphocytes. *Cell Signal* 2007; **19**: 806–16.
- Yoshimura A, Naka T, Kubo M. SOCS proteins, cytokine signalling and immune regulation. *Nat Rev Immunol* 2007; **7**: 454–65.
- Tamiya T, Kashiwagi I, Takahashi R, Yasukawa H, Yoshimura A. Suppressors of cytokine signaling (SOCS) proteins and JAK/STAT pathways: regulation of T-cell inflammation by SOCS1 and SOCS3. *Arterioscler Thromb Vasc Biol* 2011; **31**: 980–5.
- Matsumoto A, Masuhara M, Mitsui K et al. CIS, a cytokine inducible SH2 protein, is a target of the JAK-STAT5 pathway and modulates STAT5 activation. *Blood* 1997; **89**: 3148–54.
- Yoshimura A, Ohkubo T, Kiguchi T et al. A novel cytokine-inducible gene CIS encodes an SH2-containing protein that binds to tyrosine-phosphorylated interleukin 3 and erythropoietin receptors. *EMBO J* 1995; **14**: 2816–26.
- Endo TA, Masuhara M, Yokouchi M et al. A new protein containing an SH2 domain that inhibits JAK kinases. *Nature* 1997; **387**: 921–4.
- Kamizono S, Hanada T, Yasukawa H et al. The SOCS box of SOCS-1 accelerates ubiquitin-dependent proteolysis of TEL-JAK2. *J Biol Chem* 2001; **276**: 12530–8.
- Kamura T, Maenaka K, Kotoshiba S et al. VHL-box and SOCS-box domains determine binding specificity for Cul2-Rbx1 and Cul5-Rbx2 modules of ubiquitin ligases. *Genes Dev* 2004; **18**: 3055–65.
- Hilton DJ, Richardson RT, Alexander WS et al. Twenty proteins containing a C-terminal SOCS box form five structural classes. *Proc Natl Acad Sci U S A* 1998; **95**: 114–9.
- Kershaw NJ, Murphy JM, Liao NP et al. SOCS3 binds specific receptor-JAK complexes to control cytokine signaling by direct kinase inhibition. *Nat Struct Mol Biol* 2013; **20**: 469–76.
- Yasukawa H, Misawa H, Sakamoto H et al. The JAK-binding protein JAB inhibits janus tyrosine kinase activity through binding in the activation loop. *EMBO J* 1999; **18**: 1309–20.
- Babon JJ, Kershaw NJ, Murphy JM et al. Suppression of cytokine signaling by SOCS3: characterization of the mode of inhibition and the basis of its specificity. *Immunity* 2012; **36**: 239–50.
- Piganis RA, De Weerd NA, Gould JA et al. Suppressor of cytokine signaling (SOCS) 1 inhibits type I interferon (IFN) signaling via the interferon alpha receptor (IFNAR1)-associated tyrosine kinase Tyk2. *J Biol Chem* 2011; **286**: 33811–8.
- Bourdonnay E, Zaslona Z, Penke LRK et al. Transcellular delivery of vesicular SOCS proteins from macrophages to epithelial cells blunts inflammatory signaling. *J Exp Med* 2015; **212**: 729–42.
- Aman MJ, Migone TS, Sasaki A et al. CIS associates with the interleukin-2 receptor beta chain and inhibits interleukin-2-dependent signaling. *J Biol Chem* 1999; **274**: 30266–72.

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Disclosure Statement

We have no conflict of interest.

- Ram PA, Waxman DJ. Role of the cytokine-inducible SH2 protein CIS in desensitization of STAT5b signaling by continuous growth hormone. *J Biol Chem* 2000; **275**: 39487–96.
- Endo T, Sasaki A, Minoguchi M, Joo A, Yoshimura A. CIS1 interacts with the Y532 of the prolactin receptor and suppresses prolactin-dependent STAT5 activation. *J Biochem* 2003; **133**: 109–13.
- Okabe S, Tauchi T, Morita H, Ohashi H, Yoshimura A, Ohyashiki K. Thrombopoietin induces an SH2-containing protein, CIS1, which binds to Mpl: involvement of the ubiquitin proteasome pathway. *Exp Hematol* 1999; **27**: 1542–7.
- Lewis RS, Noor SM, Fraser FW, Sertori R, Liongue C, Ward AC. Regulation of embryonic hematopoiesis by a cytokine-inducible SH2 domain homologue in zebrafish. *J Immunol* 2014; **192**: 5739–48.
- Yang XXO, Zhang HY, Kim BS et al. The signaling suppressor CIS controls proallergic T cell development and allergic airway inflammation. *Nat Immunol* 2013; **14**: 732–40.
- Palmer DC, Guittard GC, Franco Z et al. Cish actively silences TCR signaling in CD8+ T cells to maintain tumor tolerance. *J Exp Med* 2015; **212**: 2095–113.
- Delconte RB, Kolesnik TB, Dagley LF et al. CIS is a potent checkpoint in NK cell-mediated tumor immunity. *Nat Immunol* 2016; **17**: 816–24.
- Yoshimura A, Suzuki M, Sakaguchi R, Hanada T, Yasukawa H. SOCS, inflammation, and autoimmunity. *Front Immunol* 2012; **3**: 20.
- Catlett IM, Hedrick SM. Suppressor of cytokine signaling 1 is required for the differentiation of CD4(+) T cells. *Nat Immunol* 2005; **6**: 715–21.
- Luckey MA, Kimura MY, Waickman AT, Feigenbaum L, Singer A, Park JH. The transcription factor ThPOK suppresses Runx3 and imposes CD4(+) lineage fate by inducing the SOCS suppressors of cytokine signaling. *Nat Immunol* 2014; **15**: 638–45.
- Chong MMW, Cornish AL, Darwiche R et al. Suppressor of cytokine signaling-1 is a critical regulator of interleukin-7-dependent CD8(+) T cell differentiation. *Immunity* 2003; **18**: 475–87.
- Davey GM, Starr R, Cornish AL et al. SOCS-1 regulates IL-15-driven homeostatic proliferation of antigen-naive CD8 T cells, limiting their autoimmune potential. *J Exp Med* 2005; **202**: 1099–108.
- Tanaka K, Ichiyama K, Hashimoto M et al. Loss of suppressor of cytokine signaling 1 in helper T cells leads to defective Th17 differentiation by enhancing antagonistic effects of IFN-gamma on STAT3 and smads. *J Immunol* 2008; **180**: 3746–56.
- Nishikawa H, Sakaguchi S. Regulatory T cells in cancer immunotherapy. *Curr Opin Immunol* 2014; **27**: 1–7.
- Takahashi R, Nishimoto S, Muto G et al. SOCS1 is essential for regulatory T cell functions by preventing loss of Foxp3 expression as well as IFN-gamma and IL-17A production. *J Exp Med* 2011; **208**: 2055–67.
- Lan F, Zhang N, Zhang J et al. Forkhead box protein 3 in human nasal polyp regulatory T cells is regulated by the protein suppressor of cytokine signaling 3. *J Allergy Clin Immunol* 2013; **132**: 1314–21.
- Lu LF, Thai TH, Calado DP et al. Foxp3-dependent MicroRNA155 confers competitive fitness to regulatory T cells by targeting SOCS1 protein. *Immunity* 2009; **30**: 80–91.
- Lu LF, Boldin MP, Chaudhry A et al. Function of miR-146a in controlling Treg Cell-mediated regulation of Th1 responses. *Cell* 2010; **142**: 914–29.
- Lu LF, Gasteiger G, Yu IS et al. A single miRNA-mRNA interaction affects the immune response in a context- and cell-type-specific manner. *Immunity* 2015; **43**: 52–64.
- Tang B, Wang ZR, Qi GY et al. MicroRNA-155 deficiency attenuates ischemia-reperfusion injury after liver transplantation in mice. *Transpl Int* 2015; **28**: 751–60.
- Hanada T, Tanaka K, Matsumura Y et al. Induction of hyper Th1 cell-type immune responses by dendritic cells lacking the suppressor of cytokine signaling-1 gene. *J Immunol* 2005; **174**: 4325–32.
- Shen L, Evel-Kabler K, Strube R, Chen SY. Silencing of SOCS1 enhances antigen presentation by dendritic cells and antigen-specific anti-tumor immunity. *Nat Biotechnol* 2004; **22**: 1546–53.

- 44 Chen SQ, Wang L, Fan J *et al*. Host miR155 promotes tumor growth through a myeloid-derived Suppressor cell-dependent mechanism. *Cancer Res* 2015; **75**: 519–31.
- 45 Hashimoto M, Ayada T, Kinjyo I *et al*. Silencing of SOCS1 in macrophages suppresses tumor development by enhancing antitumor inflammation. *Cancer Sci* 2009; **100**: 730–6.
- 46 Ramanathan S, Dubois S, Gagnon J *et al*. Regulation of cytokine-driven functional differentiation of CD8 T cells by suppressor of cytokine signaling 1 controls autoimmunity and preserves their proliferative capacity toward foreign antigens. *J Immunol* 2010; **185**: 357–66.
- 47 Dudda JC, Salaun B, Ji Y *et al*. MicroRNA-155 is required for effector CD8 (+) T Cell responses to virus infection and cancer. *Immunity* 2013; **38**: 742–53.
- 48 Yasukawa H, Ohishi M, Mori H *et al*. IL-6 induces an anti-inflammatory response in the absence of SOCS3 in macrophages. *Nat Immunol* 2003; **4**: 551–6.
- 49 Inagaki-Ohara K, Kondo T, Ito M, Yoshimura A. SOCS, inflammation, and cancer. *JAKSTAT* 2013; **2**: e24053.
- 50 Inagaki-Ohara K, Mayuzumi H, Kato S *et al*. Enhancement of leptin receptor signaling by SOCS3 deficiency induces development of gastric tumors in mice. *Oncogene* 2014; **33**: 74–84.
- 51 Wang X, Li T, Li M, Cao N, Han J. The functional SOCS3 RS115785973 variant regulated by MiR-4308 promotes gastric cancer development in Chinese population. *Cell Physiol Biochem* 2016; **38**: 1796–802.
- 52 Jenkins BJ, Grail D, Nheu T *et al*. Hyperactivation of Stat3 in gp130 mutant mice promotes gastric hyperproliferation and desensitizes TGF-beta signaling. *Nat Med* 2005; **11**: 845–52.
- 53 Judd LM, Bredin K, Kalantzis A, Jenkins BJ, Ernst M, Giraud AS. STAT3 activation regulates growth, inflammation, and vascularization in a mouse model of gastric tumorigenesis. *Gastroenterology* 2006; **131**: 1073–85.
- 54 Lesina M, Kurkowski MU, Ludes K *et al*. Stat3/Socs3 activation by IL-6 transsignaling promotes progression of pancreatic intraepithelial neoplasia and development of pancreatic cancer. *Cancer Cell* 2011; **19**: 456–69.
- 55 Suessmuth Y, Elliott J, Percy MJ *et al*. A new polycythaemia vera-associated SOCS3 SH2 mutant (SOCS3(F136L)) cannot regulate erythropoietin responses. *Br J Haematol* 2009; **147**: 450–8.
- 56 Yu H, Liu YD, McFarland BC *et al*. SOCS3 deficiency in myeloid cells promotes tumor development: involvement of STAT3 activation and myeloid-derived suppressor cells. *Cancer Immunol Res* 2015; **3**: 727–40.
- 57 Sun Y, Ju M, Lin Z *et al*. SOCS3 in retinal neurons and glial cells suppresses VEGF signaling to prevent pathological neovascular growth. *Sci Signal* 2015; **8**: ra94.
- 58 Zheng S, Hedl M, Abraham C. TAM receptor-dependent regulation of SOCS3 and MAPKs contributes to proinflammatory cytokine downregulation following chronic NOD2 stimulation of human macrophages. *J Immunol* 2015; **194**: 1928–37.
- 59 Hiwatashi K, Tamiya T, Hasegawa E *et al*. Suppression of SOCS3 in macrophages prevents cancer metastasis by modifying macrophage phase and MCP2/CCL8 induction. *Cancer Lett* 2011; **308**: 172–80.
- 60 Hanada T, Yoshida T, Kinjyo I *et al*. A mutant form of JAB/SOCS1 augments the cytokine-induced JAK/STAT pathway by accelerating degradation of wild-type JAB/CIS family proteins through the SOCS-box. *J Biol Chem* 2001; **276**: 40746–54.
- 61 Babon JJ, Varghese LN, Nicola NA. Inhibition of IL-6 family cytokines by SOCS3. *Semin Immunol* 2014; **26**: 13–9.