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Controlling cytokine signaling by constitutive inhibitors

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Abstract

Cytokines are secreted proteins that regulate diverse biological functions by binding to receptors at the cell surface to activate complex signal transduction pathways including the Janus kinase-signal transducer and activator of transcription (JAK-STAT) pathway. Stringent mechanisms of signal attenuation are essential for ensuring an appropriate, controlled cellular response. Three families of proteins, the SH2-containing phosphatases (SHP), the protein inhibitors of activated STATs (PIAS), and the suppressors of cytokine signaling (SOCS), inhibit specific and distinct aspects of cytokine signal transduction. The analysis of mice lacking genes for members of the SHP has shed much light on the roles of these proteins in vivo. In recent in vitro studies, the protein modifiers ubiquitin and small ubiquitin-like modifier (SUMO) have emerged as key players in the strategies employed by SOCS and PIAS to repress signaling. This review throws light on the mechanisms of action of these regulators as being evolved by the latest researches.

Keywords: Cytokines; JAK/STAT signalling; SOCS; SHP; PIAS; Inhibitors of signaling

1. Introduction

Cytokines are secreted glycoproteins which play a pivotal role in the development and pathology of human disease, including diseases of the immune system. Since their discovery and cloning, it has become abundantly clear that cytokines play critical roles in regulating immune and inflammatory cells. For instance, the development of lymphoid and myeloid cells is now known to be controlled to a major degree by cytokines such as interleukin IL-7, IL-3, granulocyte-monocyte colony-stimulating factor (GM-CSF), and granulocyte colony-stimulating factor, among others. Similarly, numerous studies have documented the role of IL-6 in promoting inflammatory responses. Other cytokines can be classified as immunoregulatory cytokines. For example, IL-2 controls lymphoid homeostasis both positively and negatively; in addition, the differentiation of $CD4^+$ T-helper (Th) cells into Th1 and Th2 subsets has been documented to be controlled in large measure by cytokines. For instance, IL-12 promotes the differentiation of naïve Th cells to those that produce interferon (IFN)-γ and lymphotoxin (Th1 cells), whereas IL-4 drives the differentiation of T cells to those that secrete IL-4, IL-5, and IL-10 (Th2 cells). Signaling receptors are members of two structurally related families, termed type I and type II cytokine receptors. Type I cytokine receptors include those for cytokines such as erythropoietin, prolactin, growth hormone (GH), thrombopoietin, granulocyte colony-stimulating factor, and GM-CSF. In addition, many, but not all of the receptors for different interleukins are part of this family: IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-11, IL-12, IL-13, and IL-15. The type II cytokine receptors include those for the IFNs (IFN-α, IFN-β, and IFN-γ) and IL-10. Of note, the receptors for IL-1, IL-18, IL-8, transforming growth factor-β, and tumor necrosis factor are not part of this family. Type I and II cytokine receptors lack intrinsic kinase activity and instead rely on Janus kinase (Jak) proteins to initiate signaling. Once a cytokine binds to its corresponding receptor, it leads to conformational changes in the receptor initiating activation of JAK. JAKs, a family of four non-receptor tyrosine kinases, selectively phosphorylate STATs, leading to their activation, dissociation from JAK, dimerization and translocation to the nucleus (Fig. 1). This pathway is crucial to many responses like hematopoiesis, immune regulation and oncogenesis.

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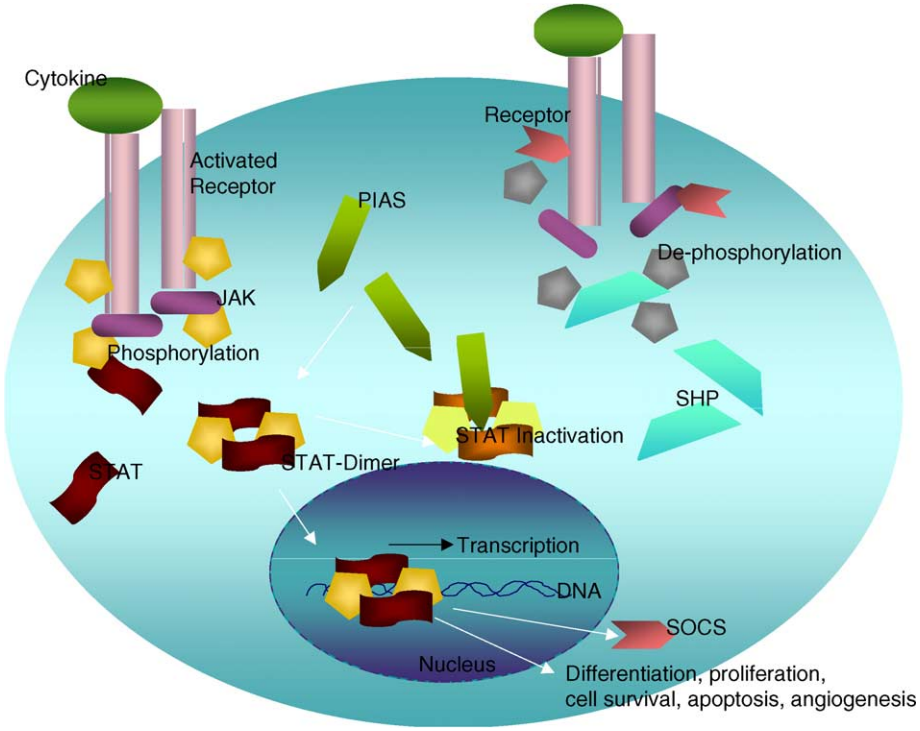

Fig. 1. Upon binding of a cytokine to its corresponding receptor, it leads to conformational changes in the receptor initiating activation of JAK. JAKs, a family of four non-receptor tyrosine kinases, selectively phosphorylate STATs, leading to their activation, dissociation from JAK, dimerization and translocation to the nucleus. This pathway is crucial to many responses like hematopoiesis, immune regulation and oncogenesis. Signal transduction is repressed by distinct mechanisms. At least three different classes of negative regulators exist to limit the strength and duration of cytokine responsiveness. These include constitutively present protein tyrosine phosphatases, such as Src-homology 2 (SH2)-containing phosphatase (SHP) and protein inhibitors of activated STATS (PIAS) and cytokine inducible suppressors of cytokine signaling (SOCS). SHP dephosphorylate activated receptors and Jak. PIAS sumoylates STAT dimers in the nucleus. SOCS act on JAK and receptors for deactivation and proteolysis.

Cytokine binding to these receptors can activate a variety of pathways within cells including mitogen-activated protein kinases (MAPKs) and phosphoinositide 3′ kinase (PI3K). However, tyrosine-phosphorylated transcription factors, the signal transducer and activator of transcription (STAT) provide great insight into the action of cytokines. Recently, research has focused on molecules that attenuate cytokine signaling. Excess positive signaling or insufficient negative signaling may lead to autoimmunity. Excess negative signaling or insufficient positive signaling may lead to immunodeficiency. Regulation of the initiation, duration, and magnitude of cytokine signaling occurs at multiple levels, including limiting the availability of cytokine to initiate a response, regulating the expression and half-life of cell surface receptor components, and controlling the duration of activation and half-life of intracellular signal transduction machinery. Control via a negative feedback loop is conceptually one of the simplest types of control. A number of hematological malignancies are characterized by constitutive activation of the JAK-STAT pathway [1]. Recent studies show STAT signals being down-regulated at several intermediate levels of the signaling cascade. Signal transduction is repressed by distinct mechanisms. At least three different classes of negative regulators exist to limit the strength and duration of cytokine responsiveness. These include protein tyrosine phosphatases, such as Src-homology 2 (SH2)-containing phosphatase-1 (SHP-1) and CD45, protein inhibitors of activated STATS (PIAS) and the suppressors of cytokine signaling (SOCS) (Fig. 1). SOCS proteins are induced in response to cytokine signaling and can inhibit JAK activity or target signaling components for ubiquitination and subsequent proteolysis. It is hypothesized that SOCS gene expression is rapidly induced on cytokine stimulation, and the resulting protein products then hinder further signaling by blocking the JAK-STAT pathway. Recently, it has emerged that SOCS gene expression can also be induced by a range of other stimuli, including lipopolysaccharide (LPS) and chemokines. The SOCS protein family consists of eight members: cytokine-inducible SH2 domain-containing protein (CIS) and SOCS1–7. Common familial features include a central SH2 domain and a conserved C-terminal SOCS box. It has been suggested that SOCS attenuate cytokine signal transduction by binding to phosphorylated tyrosine residues on signaling intermediates, such as receptor chains and JAKs, through their SH2 domains, whereas CIS is thought to act by blocking STAT recruitment to the receptor. The SOCS box motif has been implicated in E3 ligase activity through its association with elongin-B and elongin-C. In doing so, SOCS are thought to target any associated proteins for degradation through the ubiquitin pathway. SOCSs are also indispensable for reg-

ulating many biochemical processes like responses to pathogens, growth rate, leukocyte homeostasis and glucose turnover [2]. There is considerable amount of literature on the nature and biological functions of SOCS, whereas SHP and PIAS are relatively new players. This review will focus on each SHP and PIAS family member and discuss what is currently known about the function, regulation and disease implications of these negative regulators [3].

2. SH2-containing phosphatases (SHP)

A feature shared by many of the cytokine signal transduction pathways is the coupling of receptor activation to the tyrosine phosphorylation of signaling intermediates. Tyrosine phosphorylation is a rapid and reversible process and is therefore likely to be a target of regulatory molecules. Protein tyrosine phosphatases are obvious candidates for proteins that modulate these signals. Early observations indicated that phosphatase inhibitors could to some extent mimic the action of cytokines in mitogenic responses. Virtually all cellular processes, including proliferation, survival, migration, differentiation, and maintenance of metabolic homeostasis, are regulated by tyrosine phosphorylation of proteins within signal transduction networks. Tyrosine phosphorylation levels are determined by the antagonistic actions of protein tyrosine kinases and protein tyrosine phosphatases and are frequently deregulated in cancer cells. SHP-1, SHP-2, CD45, PTP1B and T cell PTP are some phophatases reported so far involved in regulating JAK/STAT signaling [4]. SH2-containing phosphatases, SHP are present constitutively and control signaling rapidly by dephosphorylating JAK and its receptors.

There are two members in this family, SHP-1 and SHP-2, consisting of two consecutive N-terminal SH2 domains and a C-terminal protein-tyrosine phosphatase domain. They bind with their SH2 domains to phosphotyrosine residues of a number of cytokine receptors. SHP-1 is expressed primarily in hematopoietic cells and SHP-2 is widely expressed in both embryonic and adult tissues [5].

The tyrosine phosphatase SHP-1, primarily expressed in hematopoietic cells has been hypothesized as one regulator that can interact with cytokine receptors and down-regulate their function. Mice having a mutation in the gene that encodes SHP-1 exhibit many characteristics of systemic autoimmunity. SHP-1 is thought to function by binding directly to cytokine receptors to dephosphorylate signaling components (Fig. 1). Whether SHP-1 or another nuclear tyrosine phosphatase is responsible for STAT dephosphorylation is not clear.

2.1. Role in cancer

Dysfunction in SHP-1 regulation can cause abnormal cell growth and induce different kinds of cancers. Extensive studies on SHP-1 protein and SHP-1 mRNA revealed that its expression was diminished or abolished in most cancer cell lines and tissues examined [6]. Growth of cancer cells was suppressed after introducing the SHP-1 gene into the corresponding cell lines [7]. It binds directly to JAK2 via an SH2-independent mechanism.

2.2. Role in brain and neural tissue

SHP-1, the intracellular regulator of many cytokine signaling pathways, has been implicated in mediating the activation of glial cells that regulate the central nervous system. Zhang et al. [8] and Zhao and Lurie [9] report that loss of SHP-1 leads to altered cytokine expression. Their findings indicate that decreases in anti-inflammatory cytokines, in combination with increased expression of the pro-inflammatory cytokine IL-1β, may initiate a robust inflammatory reaction within brain of uninjured mice contributing to the neuronal degeneration in the deafferented auditory brainstem. SHP-1 may therefore play a role in limiting CNS inflammation following injury and disease [8]. CNS myelination was significantly reduced in SHP-1-deficient mice relative to their normal littermates. Myelin basic protein and mRNA levels were reduced in SHP-1-deficient mice suggesting that SHP-1 is a critical regulator of developmental signals leading to terminal differentiation and myelin sheath formation by oligodendrocytes [9].

2.3. Role in vascular homeostasis

Angiotensin II has been shown to play an important role in the regulation of vascular homeostasis, with various implications for both cardiovascular diseases and tumor angiogenesis. It exerts its various actions to the cardiovascular and renal systems via interactions with its two receptor molecules, angiotensin II type 1 receptor (AT1) and angiotensin II type 2 receptor (AT_2). It has been documented that AT_2 receptors activate SHP-1 in PC12 cells. Moreover, the onset of SHP-1 activation clearly precedes the onset of JAK2 inhibition and apoptosis, thus suggesting that SHP-1 is an upstream, proximal effector in AT_2 signaling [10].

2.4. Involvement with SOCS

A novel mechanism has been demonstrated by which SHP-1 down-regulates the Janus kinase-2 (Jak2)/signal transducer and activator of transcription-5 (STAT-5) pathway downstream of the prolactin receptor (PRLR) and the erythropoietin receptor (EPOR) in a catalytic activity-independent manner [10]. Structural/functional analysis of SHP-1 defined the C-terminal tyrosine residues (Y278, Y303, Y538, Y566) within growth factor receptor-bound protein 2 (Grb-2) binding motif to be responsible for delivering the inhibitory effects. Their results further indicate that these tyrosine residues, via recruitment of the adaptor protein

Grb-2, are required for targeting the inhibitory protein suppressor of cytokine signalling-1 (SOCS-1) to Jak2 kinase. They demonstrate for the first time that SOCS-1 contributes to SHP-1 function in negative regulation of cytokine-receptor signaling, as the loss of SOCS-1 significantly compromises the ability of SHP-1 to down-regulate STAT5 activation. On the basis of these results, they propose that following ligand binding, SHP-1 is recruited to the receptor/Jak complex. SHP-1 recruitment leads to its phosphorylation on the C-terminal tyrosine residues, resulting in the recruitment of Grb2/SOCS-1 complex and targeting SOCS-1 to Jak2 kinase, consequently inhibiting cytokine-receptor signaling [11] (Fig. 2).

2.5. Role in growth and development

SHP-2 plays an important role in intracellular signaling elicited by growth factors, hormones, and cytokines, and it is required during development and hematopoiesis [12]. It is rapidly tyrosine phosphorylated upon stimulation with IL-2, IL-3, IL-5, IL-6, SCF, granulocyte-macrophage colony-stimulating factor (GM-CSF), macrophage colony-stimulating factor, and erythropoietin. SHP-2, a widely expressed cytoplasmic tyrosine phosphatase with two SH2 domains, has been implicated in a variety of signal transduction pathways elicited by growth factors, cytokines, hormones, antigens, and extracellular matrices. Recently, genetic evidence has indicated that SHP-2 is coupled to EGFR signaling in regulation of mouse growth and development [13,14]. Gain of functional mutations in protein tyrosine phosphatase, non-receptor type 11, *PTPN11*, the gene-encoding SHP-2, is observed in Noonan syndrome, a

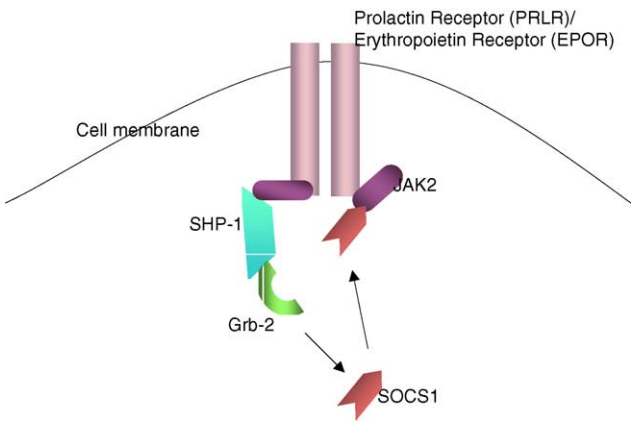

Fig. 2. A novel mechanism has been demonstrated by which SHP-1 down-regulates the Janus kinase-2 (Jak2)/signal transducer and activator of transcription-5 (STAT) pathway downstream of the prolactin receptor (PRLR) and the erythropoietin receptor (EPOR) in a catalytic activity-independent manner. SHP-1 is recruited to the receptor/Jak complex. SHP-1 recruitment leads to its phosphorylation on the C-terminal tyrosine residues, resulting in the recruitment of Grb2/SOCS-1 complex and targeting SOCS-1 to Jak2 kinase, consequently inhibiting cytokine-receptor signaling. SOCS-1 contributes to SHP-1 function in negative regulation of cytokine-receptor signaling, as the loss of SOCS-1 significantly compromises SHP-1's ability to down-regulate STAT5 activation.

developmental disorder characterized by cardiac and skeletal defects and related development disorders, as well as in myeloid malignancies. Somatic PTPN11 mutations are common in patients with juvenile myelomonocytic leukemia (JMML) and have been reported in some other hematologic malignancies. Leukemia-associated *PTPN11* mutations result in hyperactive RAS. Hyperactive RAS therefore appears to be a pivotal molecular lesion that drives the aberrant growth of malignant myeloid cells [15]. PTPN11 mutations are largely mutually exclusive in JMML, which suggests that mutant SHP-2 proteins deregulate myeloid growth through Ras. SHP-2 is an important cellular phosphatase that is mutated in myeloid malignancies [16]. Tartaglia et al. [17] reported that PTPN11 lesions occurred in childhood acute lymphoblastic leukemia (ALL). Leukemia-associated PTPN11 mutations were mis-sense and were predicted to result in SHP-2 gain-of-function [17]. SHP-2 also has been shown to inhibit the Jak-STAT signaling pathway initiated by IFN-α and IFN-γ stimulation [18]. Recently, genetic evidence has indicated that SHP-2 is coupled to EGFR signaling in regulation of mouse growth and development. Various studies indicate that activation of mitogen-activated protein kinase by growth factors such as EGF and cytokines is positively regulated by SHP-2. A major non-receptor partner of SHP-2 in cytokine signaling is Grb2-associated binder, GAB2, a recently identified pleckstrin homology domain-containing docking protein. GAB2/SHP-2 interaction promotes MAPK activation. This interaction might contribute to chronic myelogenous leukemia, perhaps through effects on Ras/MAPK signaling [19]. GAB1 interacts with multiple signaling molecules, including the p85 subunit of PI3-kinase, the SHP-2 tyrosine phosphatase, phospholipase C-γ, Shc, Nck, and Crk. Several studies indicate that Gab1 acts via SHP-2 to control Erk activation. Mutants of Gab1 or receptor-Gab1 chimeras lacking SHP-2 binding sites are unable to activate Erk or to induce morphogenesis in MDCK cells, suggesting that SHP-2 is one of the important binders of GAB1 [20,21]. GAB1 becomes rapidly phosphorylated at tyrosine residues by a variety of ligands and serves as a docking protein for a variety of signal relay molecules, such as the p85 subunit of PI3-kinase, SHP-2 etc. Various studies indicate that activation of mitogen-activated protein kinase by growth factors such as EGF and cytokines is positively regulated by SHP-2. Kapoor et al. [22] show that over-expression of SHP-2(WT) significantly upregulates EGFR-mediated NF-κB activity. An additive effect on NF-κB-mediated gene expression was observed when SHP-2 and GAB1 were coexpressed. SHP-2 has been reported to be a major binding partner of GAB1 in a variety of cell types. GAB1/SHP-2 complex formation is critically important in cell signaling. GAB1 can function to activate SHP-2, which in turn activates MAPK signaling. GAB1/SHP-2 complex is critical for the efficient relay of EGFR oncogenic signals in glioblastoma cells and thus may represent a therapeutic target in EGFR-transformed

cancer cells [22]. This association is a critical step in the formation of the signalosome linking EGFR to NF-κB activation. They also show that EGFR-induced NF-κB activation is mediated by the PI3-kinase/Akt activation loop [22]. This supports the observations that the Gab1/SHP-2 complex is critical for efficient EGFR signaling and suggests a positive regulatory role for SHP-2.

2.6. Role in apoptosis

Programmed death-1, PD-1, originally described as a transcript preferentially expressed in apoptotic cells, is thought to play a prominent role during the maintenance of peripheral tolerance. PD-1 engagement in ligation studies shows blocking of T cell activation. Chemnitz et al. [23] have reported that the ability of PD-1 to block T cell activation correlated with recruitment of SHP-1 and SHP-2. Cytoplasmic tail of PD-1 recruits both SHP-1 and SHP-2 in primary human T cells. Delineation of the role(s) that SHP-1 and SHP-2 play in PD-1-mediated signal transduction will provide further insight into the mechanism by which this receptor modulates T cell activation. This type of association has not been reported in B cell line so far [23].

3. Protein inhibitors of activated STATs (PIAS)

Protein inhibitors of activated STATs are also expressed constitutively. PIAS proteins contain a zinc ring finger domain, N-terminal LXXLL co-regulator motif, C-terminal acidic domain involved in binding TIF2 and recently found PINIT motif involved in nuclear retention [24]. PIAS proteins exhibit E3-SUMO (small ubiquitin-related modifier)-ligase activity stimulating SUMO attachment to target proteins acting in different pathways. Ubiquitin is known for its catabolic role in directing protein degradation when added to proteins in polyubiquitin chains, causing the targeted proteins to bind to and be degraded by the 26S proteasome. Monoubiquitin and its Ubl cousins appear to function as posttranslational modifiers of protein function and likely reflect a more ancestral role for the ubiquitin structural fold in regulating protein activity.

SUMO is highly conserved and shares 18% homology with ubiquitin. A C-terminal proteolytic cleavage exposes two C-terminal glycine residues essential for the isopeptide bond between SUMO and ε-amino acid group of a lysine residue of the target protein. A detailed account of origin, structure, function and interaction with different factors is given in the review by Schmidt and Muller [25]. SUMO modification proceeds by a three-step enzyme shuttle analogous to ubiquitin addition [26]. For ubiquitin, an ATP-dependent activation step couples ubiquitin by a thiolester bond to the E1, ubiquitin-activating enzyme. In turn, ubiquitin is transferred to the reactive cysteine of one of several E2 ubiquitin-conjugating enzymes. Typically, an E3 ubiquitin ligase combines with the charged E2

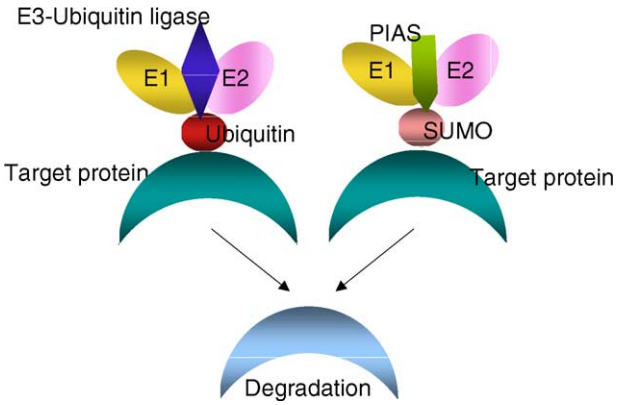

Fig. 3. Ubiquitin is coupled to E-1 ubiquitin-activating enzyme and in turn transferred to E-2 ubiquitin-conjugating enzyme. E3 ubiquitin ligase combines with the charged E2 and forms an isopeptide bond between ubiquitin and the target protein. PIAS proteins act as E3 ligases for SUMO. SUMO shares 18% homology with ubiquitin.

to facilitate formation of an isopeptide bond between ubiquitin and the target protein [27]. E3s typically use protein–protein interaction domains to bind to and select specific targets and either a zinc-binding RING finger domain or a HECT domain to stimulate polyubiquitin chain formation. How ubiquitin is then assembled into a polyubiquitin chain remains unclear, but the chain is sufficient for binding to the 26S proteasome and degradation, although other factors that assist in this process are becoming known [28] (Fig. 3). PIAS proteins can augment the covalent modification of proteins with the small ubiquitin-related modifier (SUMO). Multiple lines of evidence suggest that PIAS proteins act as E3 ligases for SUMO. First, PIAS proteins can cooperate with E1 and E2 enzymes in reconstituted systems to augment SUMO modification of target proteins. Second, PIAS proteins contain a cysteine-rich RING domain, which is a hallmark for ubiquitin ligases and is functionally important for the sumoylation activity of PIAS proteins. Finally, PIAS proteins display some specificity of protein substrates, although the specificity is not as pronounced as for ubiquitin ligases [29] (Fig. 3). In mammals, the PIAS proteins were first discovered as transcriptional co-regulators of the JAK-STAT pathway. The binding of cytokines to cell surface receptors activates the Janus, or JAK, family of tyrosine kinases, which phosphorylate a family of at least seven cytoplasmic transcription factors termed STATs. STATs mediate specific transcriptional responses. Protein inhibitor of activated STAT1 (PIAS1) was identified as a specific inhibitor of STAT1 signaling, but conversely can enhance the transcriptional activity of nuclear hormone receptors [30]. Briefly, five families are known so far: PIAS1, PIAS3, α and β PIASx and PIASy. PIAS1 and PIAS3 bind STAT1 and STAT3 and prevent DNA association. PIASx interacts with STAT4. PIASx and PIASy function in some other ways that are yet not clear. Apart from inhibition of STAT transcription factors, PIAS proteins play role in sumoylating transcription factors such as

p53, c-Jun, androgen receptor (AR), c-Myb, and lymphoid enhancer factor 1 (LEF-1) [31]. PIAS proteins may act like a buffer for maintaining the concentration of active STAT dimers within the cell. Studies have shown that PIAS1 and PIASxβ possess inherited transcription activity, whereas PIASxα and PIAS3 lack such activity [32].

PIAS1 binds only to activated STAT1 dimers and inhibits their DNA-binding activity. Monomeric forms of STAT1 are not bound [32,33]. PIAS1 is a physiological negative regulator of STAT1 and preferentially associates with unmethylated STAT1. Methylation prevents PIAS1 from binding to activated STAT dimers. Rogers et al. [34] demonstrated that STAT1 is a substrate for SUMO modification and that PIASx-α, but not PIAS1, functions as an E3 ligase to promote STAT1 modification. They showed that inhibition of STAT1 by PIAS proteins does not require SUMO modification of STAT1 itself. Lysine 703 to arginine mutant STAT1 showed modest but consistently stronger IFN-γ-induced gene activation relative to wild-type STAT1 [34]. PIAS1, PIAS3 and PIAS x sumoylate STAT1 at Lys-703- close to Tyr-701 where JAK is phosphorylated. Over-expression of PIASx-α, like PIAS1, was able to inhibit STAT1-mediated gene activation in cultured cells. Direct interactions between PIAS1 and STAT1 may interfere with the STAT1 ability to bind DNA [35] (Fig. 4). PIAS1 interferes with its recruitment to the promoters of endogenous genes. It is important in interferon-mediated innate immunity to pathogenic infection [36].

PIAS3 is a specific inhibitor of STAT3. N-terminal region of PIAS3 is necessary for transcriptional suppression activity. PIAS3 interferes with p65 binding to the CBP co-activator, thereby resulting in a decreased NF-κB-dependent transcription [37]. Over-expression of PIAS3 induces apoptosis in prostrate cancer cell line. PIAS3 functions as a SUMO-1 ligase for interferon regulatory factor-1, IRF-1, and represses its transcriptional activity [38]. Smad proteins that transduce TGF-β signal are phosphorylated by TGF-β receptor and accumulate in the nucleus. PIAS3 interacts with Smad proteins and activates TGF-β/Smad transcriptional responses. PIASy however inhibits Smad transcriptional activity and other transcriptional responses [39].

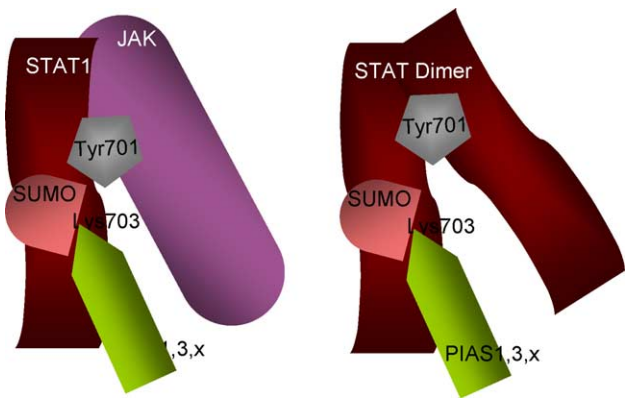

Fig. 4. PIAS1, PIAS3 and PIAS x sumoylate STAT1 at Lys-703- close to Tyr-701 where JAK is phosphorylated. STAT1 can be modified by SUMO at lysine residue 703. Direct interactions between PIAS1 and STAT1 may interfere with the STAT1 ability to bind DNA.

PIASx-alpha is a modulator of androgen receptor. It acts as a co-suppressor of STAT4 following IL-12 stimulation. PIASx does not inhibit the DNA binding activity of STAT4. Instead it is present in the STAT4-DNA binding complex [40]. PIASx gene encodes two SUMO E3 ligases that are highly expressed in the testis [41]. PIAS-NY may play an important role in testis development and/or spermatogenesis [42].

PIASy represses the transcriptional activity of the androgen receptor. AR binds to the RING-finger like domain of PIASy. PIASy contains two transcriptional repression domains, RD1 and RD2. Gross et al. [43] suggest that PIASy may repress AR by recruiting histone deacetylases, independent of its SUMO ligase activity. Mutant PIASy, defective in promoting sumoylation, retains the ability to repress AR transcription [43]. PIASy has been shown to inhibit the activation of gene expression by the IFN-responsive transcription factor STAT1 and the Wnt-responsive transcription factor lymphoid enhancer binding factor (LEF1). Roth et al. [44] recently analyzed mice carrying a targeted mutation of the *PIASy* gene. The analysis revealed a modest reduction of the transcriptional response to IFN-γ, a reduced viability of the homozygous mutant mice and that the activation potential of a Wnt-responsive reporter by LEF1 and β-catenin is decreased [44]. PIASy also mediates SUMO-2/3 modification of lymphoid enhancer factor 1, LEF-1, sequestering it into nuclear bodies, and SUMO-1 ligation to c-Myb, modulating its transcriptional activation properties [45].

4. Suppressors of cytokine signaling

In contrast to these constitutive inhibitors are the much talked about SOCS proteins that are produced in response to signals from a diverse range of cytokines and growth factors and which act to attenuate cytokine signal transduction. Members of the SOCS family form a classical negative feedback loop with key actions involving inhibition of the JAK-STAT signaling cascade. There are eight members of the SOCS protein family: the cytokine-inducible SH2 domain-containing protein and SOCS1 through SOCS7. All eight members contain an N-terminal region of varying length and sequence, a central SH2 domain, and a C-terminal SOCS box. Studies reflect the fact that SOCS1, SOCS2, SOCS3, and CIS mRNA and protein are generally present at low levels in un-stimulated cells, perhaps because of active repression. Their levels are induced rapidly in response to cytokines, with the STATs playing an important part in regulating SOCS gene transcription [46–48]. In addition, pathogens and LPS are also known to induce SOCS expressions [49–51]. Of the family members,

SOCS1 and SOCS3 are the most potent inhibitors of cytokine-induced signals. Forced expression of SOCS1 or SOCS3 down-regulates a variety of cytokine signal pathways including IFN-α.

SOCS1 can directly bind to the catalytic domain (phosphorylated Y1007) of JAK2 through the SH2 domain and inhibits kinase activity through the kinase-inhibitory region (KIR) in the N-terminal domain, which is proposed to function as a pseudosubstrate. The SOCS box functions to recruit the ubiquitin-transferase complex. Therefore, SOCS1 combines specific kinase inhibition and a generic mechanism of targeting interacting proteins into proteasomal degradation. SOCS1 also binds to the p65 subunit of NF-κB and promotes its degradation, which is a mechanism of suppression of the NF-κB pathway by SOCS1 [52]. KO mice. These mice die within three weeks of birth with a syndrome characterized by severe inflammation, activation of peripheral T cells and macrophage infiltration of major organs. The neonatal defects exhibited by SOCS1$^{-/-}$ mice appear to occur primarily as a result of hyperT-cell activation because mice do not die when the *SOCS1* gene is restored in T cells in a SOCS1$^{-/-}$ background [53]. SOCS1 gene silencing by small interfering RNA (siRNA) technology also leads to hyperactivation of dendritic cells [54].

SOCS3 initially was found to be induced by erythropoietin and granulocyte-macrophage colony stimulating factor in certain hematopoietic cells. It is involved in erythropoiesis regulation. Transient expression of SOCS3 inhibits leukemia inhibitory factor (LIF)-induced STAT3 reporter gene activation. It is induced in response to IL-10 in liver. Over-expression of SOCS3 inhibits IFN-α-induced reporter activity in hepatic cells. [55]. In presence of the receptor, SOCS3 has a greater ability to interact with JAKs and to inhibit kinase activity in growth hormone and IL-2-induced signaling pathway. SOCS3 has the highest affinity for gp130 subunit of LIF/IL-6 receptors. Tumor response to oncostatin M treatment is adversely affected by SOCS3. Recently it was shown that SOCS-3 does not inhibit Stat3 activation, growth, and survival in CTCL. In contrast, SOCS3 protects tumor cells against growth inhibition by IFN-α. Unlike SOCS-1, SOCS-3 is therefore not a tumor suppressor but rather a protector of tumor cells [56]. The mechanisms of action of the other four SOCS proteins, SOCS4–7, have not yet been established. A detailed account on SOCS mechanisms is given in several review articles focusing only on SOCS.

5. Conclusion

Given the important role of cytokines in mediating many biological functions, it is not surprising that cytokine signal transduction pathways are tightly regulated. It is becoming increasingly apparent that a variety of mechanisms are in place to modulate a cellular response to cytokine, and the rate at which the signal is turned off will be due to the net effect of all of these regulatory pathways. In contrast to SOCS, which are induced in response to cytokines, SHP-1 and PIAS are constitutively present in the cell and may therefore function as more acute, early response regulators. The timing and specificity of each of these mechanisms, as well as how the inhibitors interact and cooperate with each other, remain to be determined. Finally, dephosphorylation of three signaling intermediates (receptor, JAKs, and STATs) is required to switch off the signal. Not only do these processes contribute to normal host defense, but also to the pathogenesis of immune and inflammatory diseases. Much research has focused on the roles that such inhibitors play in diseases such as cancer, rheumatoid arthritis, systemic lupus erythematosus, and even such disparate illnesses as scleroderma and osteoarthritis. It is clear that both the pathogenesis and the clinical manifestations of these debilitating diseases are at least in part due to aberrant immune and inflammatory responses, both of which are critically dependent on proteins-activated downstream of cytokine activation. Sorting out the roles of negative regulators of cytokine signaling in the all the existing pathways that are activated in response to cytokine will certainly prove a challenge for the future. Once a full understanding of how these mediators interplay is gained, this knowledge could be used for therapeutic purposes in immune diseases by up- or down-regulating these key inhibitors.

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