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Protein tyrosine phosphatases in the JAK/STAT pathway

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1. ABSTRACT

The Janus kinase (JAK)/signal transducer and activator of transcription (STAT) pathway is crucial in controlling cellular activities in response to extracellular cytokines. Dysfunctions of the JAK/STAT pathway result in various hematopoietic and immune disorders. The central events in regulating this pathway are tyrosine phosphorylation and dephosphorylation of the signaling components, which are carried out by protein tyrosine kinases and protein tyrosine phosphatases (PTP), respectively. Here, we review recent advances in the regulatory roles of PTPs, in particular, SHP2 phosphatase, in the JAK/STAT signaling pathway.

2. INTRODUCTION

Intracellular signal transduction pathways are essential for connecting extracellular cytokine stimulations to appropriate cellular responses, such as proliferation, differentiation, and apoptosis. One of the most important pathways activated by cytokines is the Janus kinase (JAK)/signal transducer and activator of transcription (STAT) pathway, which was initially discovered in the studies on gene induction by interferon (IFN) (1). It is now clear that protein phosphorylation and dephosphorylation play a central role in controlling the activity of the JAK/STAT pathway. The majority of phosphorylation and dephosphorylation occur at tyrosine residues of the

component proteins, which are carried out by protein tyrosine kinases and protein tyrosine phosphatases (PTP), respectively (2-4). This short article summarizes latest insights into how PTPs, including SHP2, SHP1, CD45, PTP1B, T-cell PTP (TC-PTP), PTPRT, and PTPBL, regulate the JAK/STAT pathway.

In the current model of the JAK/STAT signaling pathway, the engagement between a cytokine and its cell surface receptor results in receptor oligomerization and subsequent activation of receptor-associated JAK tyrosine kinases. Activated JAKs phosphorylate specific tyrosine resides in the cytoplasmic domain of the receptor which in turn serves as the docking sites for cytoplasmic transcription factors known as STATs. STATs are therefore recruited to the phosphorylated receptor and subsequently phosphorylated by JAKs. The phosphorylated STATs then dimerize, leave the receptor, and translocate to the nucleus where they activate gene transcription (5, 6). In mammalian cells, four JAK members (JAK1, JAK2, JAK3, and TYK2) have been identified. Each JAK contains a conserved kinase domain and a catalytically-inactive domain at the carboxyl (C-) terminus which might regulate the activity of the kinase domain. There are seven members (STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b, and STAT6) in the mammalian STAT family. Each of them contains a DNA-binding domain, a transactivation domain located at the C-terminus (7, 8), and a SRC homology 2 (SH2) domain. The SH2 domain is required for STAT activation and dimerization (9, 10) while the amino (N-) terminal region of STAT is involved in the formation of STAT tetramers (11) and tyrosine dephosphorylation (12). Genetic knockout studies have revealed various but specific functions of JAKs and STATs. One who is interested in details about the structures and functions of JAKs and STATs could look at the reviews (2, 13-15). The following sections of this review will discuss how PTPs regulate JAK/STAT signaling with a focus on the SHP2 phosphatase, since the role of SHP2 in this context is more complicated than other PTPs.

3. REGULATION OF THE JAK/STAT PATHWAY BY PTPS

3.1. SHP2

3.1.1. Structure, regulation, and function

SHP2 is a ubiquitously expressed SH2 domain-containing PTP. It contains two tandem SH2 domains (N-SH2 and C-SH2), a classic tyrosine phosphatase domain at the C-terminus, and a C-terminal tail with two important tyrosine resides (Tyr542 and Tyr580) and some other functional motifs (16-19). The SH2 domains, in particular N-SH2, specifically recognize phosphorylated tyrosine residues on other molecules and mediate interactions between SHP2 and these molecules. Biochemical and structural studies indicate that, in the basal state, the N-SH2 domain is wedged into the PTP domain and forms a "backside loop" to prevent substrate accessing. Upon binding of an appropriate phosphotyrosyl (p-Tyr) peptide, N-SH2 alters its conformation and releases the PTP domain from the auto-inhibitory confirmation (20-22). In addition, mutagenesis and protease-resistance studies suggest that phosphorylation of Tyr542 and Tyr580 in the C-terminal tail also regulates SHP2 activity. Phosphorylated Tyr542 and Tyr580 could be engaged to the N-SH2 and C-SH2 domains, respectively, in an intramolecular manner to stimulate SHP2 activity (23).

SHP2 plays an essential role in hematopoiesis and lymphopoiesis. Homozygous deletion of *Exon* 3 of SHP2, including the N-SH2 domain-encoding region (amino acids 46-110) ($SHP2^{\Delta/\Delta}$), resulted in embryonic lethality at mid-gestation with a defect in mesodermal patterning (24, 25). *In vitro* differentiation of $SHP2^{\Delta/\Delta}$ embryonic stem (ES) cells revealed that loss of SHP2 function caused suppression of ES cell differentiation to erythroid and myeloid progenitors (26). These results suggest a positive role of SHP2 in hematopoietic development. Consistent with the *in vitro* differentiation data, chimeric mouse analyses showed that no $SHP2^{\Delta/\Delta}$ ES cell-derived progenitors for erythroid or myeloid lineages were detected in the fetal liver or bone marrow of the chimeric mice generated from $SHP2^{\Delta/\Delta}$ ES cells. In addition to erythroid and myeloid lineages, SHP2 is also required for lymphoid development. The RAG-2–deficient blastocyst complementation assay showed that no mature T and B cells or even Thy-1 positive or B220 positive precursor lymphocytes derived from the mutant ES cells were detected in the chimeric mice generated from $SHP2^{\Delta/\Delta}$ ES cells and Rag-2–deficient blastocysts (27). It appears that the blackage of the hematopoietic development of the SHP2 mutant ES cells occurred at a very early stage, since primitive hematopoiesis in the yolk sac of $SHP2^{\Delta/\Delta}$ embryos was also defective (28). Notably, reintroduction of WT SHP2 into $SHP2^{\Delta/\Delta}$ ES cells restored both primitive and definitive hematopoietic potential of the mutant ES cells (29), suggesting that the defective hematopoietic development is directly attributable to the loss of SHP2 function and that the defect is cell autonomous.

Consistent with the notion that SHP2 phosphatase plays a positive role in hematopoietic cell development, somatic mutations in SHP2-encoding gene *PTPN11* that cause hyperactivation of its catalytic activity have been identified in various childhood leukemias, such as juvenile myelomonocytic leukemia (JMML), B cell acute lymphoblastic leukemia, and acute myeloid leukemia (AML) (30-32). SHP2 represents the first identified proto-oncogene that encodes a tyrosine phosphatase (33, 34). The SHP2 disease mutations are located in the N-SH2 and PTP domains and cause changes in the amino acid residues at the interface formed by the two domains in the self-inhibited SHP2 conformation. It is thus thought that the mutations cause a decrease in the affinity of the binding between the N-SH2 and the phosphatase domains, leading to hyperactivation of SHP2 catalytic activity by allowing access to the active site on the enzyme. The SHP2 mutations appear to play a causal role in the development of related diseases, as SHP2 mutations and other well-known JMML-associated Ras or Nf1 mutations are mutually exclusive in the patients (30, 31, 35). Moreover, recent studies have shown that single SHP2 activating mutations are sufficient to induce cytokine hypersensitivity

in myeloid progenitor cells and JMML-like myeloproliferative disease in mice (36-40).

The molecular mechanisms underlying the positive role of SHP2 phosphatase in hematopoietic cell development and function are not completely understood. Since cytokines play an essential role in the hematopoietic process, SHP2 may promote hematopoiesis through the JAK/STAT pathway that is activated by cytokines. However, the SHP2 function in this pathway is rather complicated. Unlike the promoting role that SHP2 plays in the Ras/Erk and PI3/Akt signaling pathways (20), SHP2 both enhances and inhibits signaling in the JAK/STAT pathway, depending on the acting sites. In addition, different JAK/STAT pathways initiated by different extracellular signals may be differentially regulated by SHP2 phosphatase.

3.1.2. SHP2 negatively regulates JAK/STAT

SHP2 negatively regulates the IFN-induced JAK1/STAT1(2) pathway. INF-γ and INF-α are known to suppress cell viability through the JAK/STAT pathway. In $SHP2^{\Delta/\Delta}$ mouse fibroblasts, IFN-γ and IFN-α treatment resulted in elevated tyrosine phosphorylation levels of STAT1 and STAT2, and augmented suppression of cell viability. Reintroduction of WT SHP2 protein reversed these effects (41). Specifically, phosphorylation at the tyrosine residue Tyr701 of STAT1 induced by IFN-γ was enhanced and prolonged in $SHP2^{\Delta/\Delta}$ cells (42). Moreover, abolishment of protein kinase C-mediated inhibition of IFN-α signaling was observed in the SHP2 mutant cells (43). Consistent with these observations, purified GST-SHP2 dephosphorylated STAT1 at both tyrosine and serine residues when immunoprecipitated phospho-STAT1 or phosphor-peptides corresponding to the sequence surrounding Tyr701 or Ser727 of STAT1 were used as the substrates (42). These results thus indicate that SHP2 negatively regulates the INF-induced JAK/STAT pathway by dephosphorylating STAT1(2).

SHP2 also interacted with STAT5a in a tyrosine phosphorylation-dependent manner and purified SHP2 protein directly dephosphorylated STAT5 or tyrosine-phosphorylated peptides derived from STAT5 in the *in vitro* tyrosine phosphatase assay (44-46). In agreement with these results, tyrosine-phosphorylated STAT5 associated with a substrate-trapping mutant of SHP2 (SHP2 Cys459Ser) (44, 45). Moreover, overexpression of WT SHP2 in Ba/F3 cells and in primary bone marrow hematopoietic progenitor cells resulted in a decreased phosphorylation level of STAT5 in response to IL-3 stimulation (46). This was largely because STAT5 dephosphorylation was accelerated by overexpression of SHP2 (46), since STAT5 dephosphorylation was markedly delayed in $SHP2^{\Delta/\Delta}$ cells (44).

In addition, there is also evidence that SHP2 negatively regulates the activity of STAT3, a crucial signaling protein involved in maintaining the self-renewal feature of ES cells and in hematopoietic cell response to the cytokines that function through the gp130 receptor. $SHP2^{\Delta/\Delta}$ mutant mouse ES cells showed defective differentiation and more efficient self-renewal in the presence of leukemia inhibitory factor (LIF), which was at least in part due to an increased STAT3 activity in the absence of functional SHP2 (29, 47). Similar negative regulation of STAT3 by SHP2 was observed in SHP2-deficient neural cells generated from SHP2 conditional knockout mice (48, 49). In addition to the LIF-induced gp130 signaling pathway, the IL-6-activated gp130 pathway was also inhibited by SHP2. A mutation of the SHP2 binding site (Y759F) in gp130 in the knock-in mice resulted in lymphoadenopathy, splenomegaly, and an enhanced acute-phase immune response (50). Therefore, SHP2 appears to directly inhibit activation of STATs in various pathways. Intriguingly, although SHP2 normally dephosphorylates STAT5, IL3-induced phosphorylation of STAT5 was enhanced rather than decreased in hematopoietic cells harboring SHP2 E76K and D61G leukemia mutations that cause hyperactivation of the SHP2 catalytic activity (38, 40). Further investigations revealed that dephosphorylation of STAT5 by SHP2 E76K was delayed (40). Since overexpression (5-to-6 fold) of WT SHP2 in hematopoietic cells accelerated dephosphorylation of STAT5 and attenuated hematopoietic potential (46), the dampened dephosphorylation activity of SHP2 E76K indicates that the substrate specificity of the mutant SHP2 is altered by the E76K mutation, although the underlying mechanisms remain to be determined.

3.1.3. SHP2 positively regulates JAK/STAT signaling

A large body of data also support that SHP2 promotes JAK/STAT pathways. One of the most compelling evidence is that the activity of STAT5 was suppressed in the $SHP2^{-/-}$ mouse mammary gland cells in SHP2 conditional knockout mice (51). This result suggests a positive role of SHP2 in the prolactin-induced JAK2 activation pathway. JAK2 tends to associate with suppressor of cytokine signaling (Socs)1, which targets JAK2 to a ubiquitin-dependent degradation pathway and serves as a negative regulator for the JAK2/STAT5 pathway. The interaction between JAK2 and Socs1 is mediated by phosphorylation of Tyr1007 in JAK2. *In vitro* studies demonstrated that SHP2 was able to dephosphorylate this Tyr site and prevent the formation of the JAK2-Socs1 complex and subsequent degradation of Jak2 (52). Upon being released from the inhibitory effects of Socs1, JAK2 is recruited to the prolactin receptor (PrlR) and phosphorylates STAT5 (52). The physical interaction between SHP2 and the JAK2-PrlR complex is required for STAT5 activation and translocation into the nucleus to activate gene expression (52). In addition, it has been shown that SHP2 shares the same binding sites with the signaling suppressor Socs3, owing to similar binding preference of their SH2 domains (53, 54). Thus, it is also possible that SHP2 promotes cytokine signaling by limiting the negative feedback mediated by Socs3 and that this SHP2 function depends on its SH2 domains rather than the catalytic domain. Using catalytically-inactive SHP2 (SHP2 C459S) overexpressing Ba/F3 cells and immortalized $SHP2^{\Delta/\Delta}$ hematopoietic cells derived from SHP2 knockout embryos, our laboratory showed that SHP2 functioned at multiple sites in the IL-3-induced JAK2/STAT5 signaling in both catalytic-dependent and -independent fashion (55).

SHP2 acts immediately downstream of the receptor, facilitating IL3-induced activation of JAK2. In $SHP2^{\Delta/\Delta}$ cells in which the truncated SHP2 was barely detectable, JAK2 activation was abolished (55). Consequently, phosphorylation of STAT5, the substrate of JAK2 kinase, was impaired in the SHP2 mutant cells (55). It seems that SHP2 functions in JAK2 activation as an adaptor protein. However, further studies showed that the catalytic activity of SHP2 was required for optimal activation of JAK2. JAK2 activation in SHP2 C459S overexpressing cells was decreased. As a result of reduced JAK2 activation, phosphorylation of STAT5 was also decreased (55). Consistent with this notion, expressing catalytically-deficient mutant SHP2 in COS7 cells inhibited the induction of tyrosine phosphorylation and DNA-binding activity of STAT5 upon prolactin stimulation (56). Additionally, the positive role of SHP2 catalytic activity in JAK2 activation is also supported by the recent observation that JAK2 activation in hematopoietic cells harboring the SHP2 activating mutation E76K was enhanced (40).

Collectively, these studies suggest that SHP2 has dual functions in the same JAK2/STAT5 pathway. This may be true, since both a decrease in STAT5 phosphorylation and a delay in STAT5 dephosphorylation (sustained low-level STAT5 phosphorylation) were observed in catalytically-inactive mutant SHP2 overexpressing or $SHP2^{\Delta/\Delta}$ cells (44, 52, 55, 57). How SHP2 functions at multiple sites in the same pathway, however, remains to be further characterized. SHP2 may first act as a positive regulator for the activation of JAK2 and then inhibits the activated pathway by dephosphorylating STAT5. Alternatively, different fractions of the cytoplasmic SHP2 act at different sites, simultaneously functioning in both catalytic-dependent and –independent fashion.

3.2. Other PTPs

3.2.1 SHP1

SHP1 phosphatase shares a similar overall structure and a high homology with SHP2. However, unlike SHP2 which is ubiquitously expressed, SHP1 is restricted in hematopoietic cells (20). The functions of SHP1 in hematopoietic cells and lymphocytes have been revealed by numerous studies using *motheaten* mice, which are deficient in SHP1 expression. *Motheaten* mice display hyperproliferation and abnormal activation of granulocytes and macrophages, and an autoimmunity-like phenotype. These defects are at least in part attributable to the loss of SHP1 as a negative regulator for the JAK/STAT pathway (2, 58-61).

SHP1 down regulates erythropoietin (EPO)-induced proliferative signals by binding to the EPO receptor (EPOR) and dephosphorylating the JAK2 associated with EPOR. Cells expressing a mutant EPOR defective in SHP1 binding displayed hypersensitivity to EPO stimulation and prolonged EPO-induced autophosphorylation of JAK2 (62, 63). SHP1 is also involved in the dephosphorylation of JAK1. The IFN-α-induced tyrosine phosphorylation of JAK1 was enhanced in SHP1 deficient macrophages (64). In addition, expression of an inactive SHP1 (R459M) in Ba/F3 cell line increased the proliferative response to IL-3 and cell survival following IL-3 withdrawal (65). The overall level of IL-3-induced tyrosine phosphorylation of STAT5 was reduced upon expression of WT SHP1 and increased when R459M SHP1 was expressed (65).

Consistent with the negative role of SHP1 in the JAK/STAT pathway, silencing of SHP1 by promoter methylation is often associated with various kinds of leukemia and lymphomas, myeloma and acute myeloid leukemia, and the effect caused by SHP1 silencing is at least partially attributed to an increased activities in the JAK/STAT pathway (66). Eighty percent of myeloma samples showed SHP1 hypermethylation, concomitant with a constitutive STAT3 phosphorylation. Reintroduction of SHP1 resulted in barely detectable phosphorylated STAT3, suggesting that STAT3 may also be a substrate of SHP1 (67-69). Recently, defective SHP1 expression has also been detected in most cases of ALK positive anaplastic large-cell lymphoma (ALK(+)ALCL) (70). Transfection of SHP1 or induction of SHP1 with an inhibitor of DNA methyltransferase (5-AZA) in ALK(+)ALCL cell line, Karpas 299, caused an attenuated phosphorylation level of JAK3 and STAT3, subsequent down-regulation of STAT3 targets including cyclin D3, mcl-1 and bcl-2, and a significant G_1 cell cycle arrest. Co-immunoprecipitation studies showed that SHP1 was physically associated with JAK3. These results suggest that loss of SHP1 contributes to the pathogenesis of ALK(+)ALCL by leaving the phosphorylation and activation of JAK3/STAT3 unchecked (71, 72).

By contrast, SHP1 has been noted to have a positive role in promoting JAK/STAT signaling in some circumstances. For example, the epidermal growth factor (EGF)- and IFN-γ-induced STAT activation was suppressed by expressing a catalytically-inactive form of SHP1 in HeLa cells, while this pathway was essentially unaffected by the expression of WT SHP1 (73). The precise mechanism of how this molecule achieves opposing functions in different systems remains to be clarified.

3.2.2. CD45, PTP1B, TC-PTP, PTPRT, and PTP-BL

CD45 is a receptor tyrosine phosphatase highly expressed by hematopoietic cells. It plays an important role in controlling antigen-receptor signaling in T and B cells (74, 75). CD45 is shown to be able to dephosphorylate all JAKs in murine cells (76), and dephosphorylate JAK1 and JAK3 in human cells (77). CD45 deficient cells experienced prolonged JAK/STAT activation in response to IL-7 stimulation. The removal of CD45 also increased the erythroid colony formation and antiviral activity, which is consistent with the idea that CD45 negatively regulates EPO and IFN signaling by dephosphorylating JAKs (76, 78). However, the physiological significance of the role of CD45 in controlling the JAK/STAT pathways still needs to be further determined.

PTP1B and TC-PTP are two highly related PTPs, sharing great similarities in the catalytic domains. While PTP1B is expressed in many tissues, TC-PTP is mainly in

hematopoietic cells (79). PTP1B binds phosphorylated JAK1 upon leptin and IFN-γ treatment, and is implicated in the negative regulation of these signaling pathways. Increased phosphorylation of JAK2, STAT3, and STAT5 has been observed in PTP1B deficient embryonic fibroblasts (80-83). TC-PTP can also target JAK1, JAK3, STAT1, STAT3 and STAT5. Phosphorylation of JAK1 and STAT1 is enhanced in TC-PTP knockout cells (84-86).

More recently, it has been shown that PTPRT, a receptor-type tyrosine phosphatase, also dephosphorylates STAT3 at Tyr705, an essential tyrosine residue for the function of STAT3 (87). Accordingly, overexpression of PTPRT reduces the expression of STAT3 target genes. In addition, PTP-Basophil like (PTP-BL), a large non-transmembrane PTP, has been shown to dephosphorylate STAT proteins both *in vitro* and *in vivo*, and has been identified as a STAT PTP. In CD4 positive T cells, PTP-BL deficiency led to increased and prolonged activation of STAT4 and STAT6, and consequently enhanced Th1 and Th2 cell differentiation (88).

In summary, many PTPs participate in the regulation of the JAK/STAT signaling pathway and different PTPs recognize specific substrates. The role of PTPs in the JAK/STAT pathway has important implications in physiology and diseases. Nevertheless, many issues regarding the biochemical bases of the interactions of PTPs with the JAK/STAT pathway still remain to be resolved. Some prominent questions are: Why do some phosphatases, in particular, SHP2 promote local signaling? What is the direct biochemical significance of their phosphatase activities? Since several phosphatases dephosphorylate the same targets, how are functions of PTPs coordinated temporally and spatially? Addressing these questions will lead to a better understanding of how JAK/STAT signaling is modulated and why malfunction of this pathway results in hematopoietic and immune disorders. The information gathered may also lead to rational design of new therapeutics for treatment of the relevant diseases.

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Abbreviations: JAK: Janus kinase; STAT: signal transducer and activator of transcription; PTP: Protein tyrosine phosphatase; IFN: Interferon; SH2: Src homoglogy 2; ES cells: embryonic stem cells; Socs: Suppressor of cytokine signaling.

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