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Commentary

JAK/STAT signal transduction: Regulators and implication in hematological malignancies

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Abbreviations:
BCR, break cluster region
EPO, erythropoietin
ETV6, ets translocation variant gene 6
GH, growth hormone
GM-CSF, granulocyte-macrophage colony-stimulating factor
IL, interleukin
JAK, Janus kinase
JH, JAK homology
PCM 1, pericentriolar material 1
PIAS, protein inhibitors of activated STAT
PRL, prolactin
PTP, protein tyrosine phosphatase
SHP, Src-homology 2 (SH2)-containing phosphatase
SOCS, suppressors of cytokine signaling
STAT, signal transducers and activators of transcription
TEL, translocated ETS leukaemia
TPO, thrombopoietin
TYK2, tyrosine kinase-2

ABSTRACT

Signal transducers and activators of transcription (STATs) comprise a family of several transcription factors that are activated by a variety of cytokines, hormones and growth factors. STATs are activated through tyrosine phosphorylation, mainly by JAK kinases, which lead to their dimerization, nuclear translocation and regulation of target genes expression. Stringent mechanisms of signal attenuation are essential for insuring appropriate, controlled cellular responses. Among them phosphotyrosine phosphatases (SHPs, CD45, PTP1B/TC-PTP), protein inhibitors of activated STATs (PIAS) and suppressors of cytokine signaling (SOCS) inhibit specific and distinct aspects of cytokine signal transduction. SOCS proteins bind through their SH2 domain to phosphotyrosine residues in either cytokine receptors or JAK and thus can suppress cytokine signaling. Many recent findings indicate that SOCS proteins act, in addition, as adaptors that regulate the turnover of certain substrates by interacting with and activating an E3 ubiquitin ligase. Thus, SOCS proteins act as negative regulators of JAK/STAT pathways and may represent tumour suppressor genes. The discovery of oncogenic partner in this signaling pathway, more especially in diverse hematologic malignancies support a prominent role of deregulated pathways in the pathogenesis of diseases. Fusion proteins implicating the JH1 domain of JAK2 (*TEL-JAK2*, *BCR-JAK2*), leading to deregulated activity of JAK2, have been described as the result of translocation. Somatic point mutation in JH2 domain of JAK2 ($JAK2^{V617F}$), leading also to constitutive tyrosine phosphorylation of JAK2 and its downstream effectors was reported in myeloproliferative disorders. Furthermore, silencing of *socs-1* and *shp-1* expression by gene methylation is observed in some cancer cells.

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1. Introduction

Cytokines are secreted glycoproteins, which regulate diverse biological process through their interaction with multi-subunit receptor complexes. Binding of cytokine to cell surface receptors results in receptor oligomerization and activation of the Janus Kinase (JAK) family of tyrosine kinases [JAK1, JAK2, JAK3 and tyrosine kinase-2 (TYK2)]. Activated JAKs phosphorylate the cytoplasmic domain of the receptor, thereby creating recruitment sites for signaling proteins, such as STATs (signal transducers and activators of transcription). STATs are phosphorylated by JAKs, dimerize and subsequently migrate to the nucleus where they regulate gene transcription. The pathways that convey signals from the cell surface to the nucleus are tightly controlled. 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), CD45 and PTP1B/TC-PTP, protein inhibitors of activated STATs (PIAS) and the suppressors of cytokine signaling (SOCS). SOCS gene expression is rapidly induced upon cytokine stimulation, and by a wide range of other stimuli including LPS, insulin and chemokines.

A number of diseases including cancer are linked to deregulation of tyrosine kinases. Constitutive activation of JAK2 and STATs are believed to mediate neoplastic transformation and promote abnormal cell proliferation in various malignancies.

In this review, we will discuss a number of deregulation of tyrosine kinase JAK2 associated with hematopoietic neoplasia and will focus on inducible negative regulators and their implication in oncogenesis.

1.1. *JAK/STAT signaling pathways*

JAK tyrosine kinases play critical roles in cytokine signaling in haematopoietic cells. Activation of JAKs occurs as a consequence of ligand-induced aggregation of receptor-associated JAKs and their subsequent autophosphorylation. JAKs are involved in the signal transduction of type I receptors (i.e. for IL2, IL3, IL4, IL5, IL6, IL7, IL13, GM-CSF, GH, PRL, EPO and TPO) as well as of type II cytokine receptors (for IFN-α, -β, -γ). Four human JAKs have been identified: JAK1, JAK2, JAK3 and TYK2. JAKs share common domains, termed JAK homology (JH) domains 1–7, numbered from the C- to the N-terminus (Fig. 1). The JH7 domain associates with the proline rich conserved region in cytokine receptors termed Box1, while the JH1

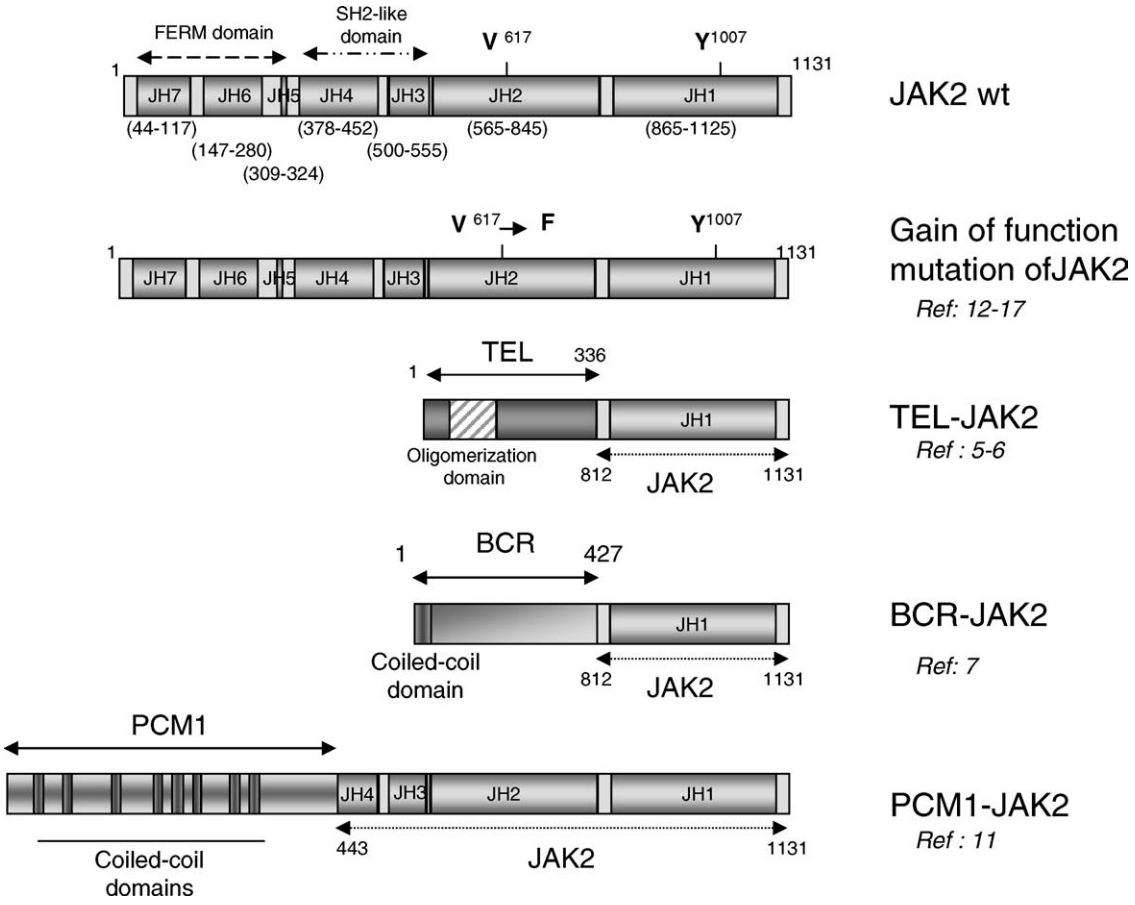

Fig. 1 – Schematic representation of JAK2, somatic point mutation of JAK2, TEL-JAK2, BCR-JAK2, and PCM1-JAK2 fusion proteins. Proteins are represented with their functional domains and motifs (grey boxes on PCM1 and BCR correspond to coiled-coils, shaded box on TEL to oligomerization domain). JH1–7 are the different domain of JAK2, with JH1 and JH2 corresponding to the kinase and the pseudokinase domain respectively.

Table 1 – Molecular abnormalities of regulators of JAK/STAT pathways frequently detected in myeloproliferative disorders

Molecular abnormality	Disease
PTPN11 mutations	Juvenile myelomonocytic leukaemia (JMML) [39] Myelodysplastic syndrome (MDS) Chilhood acute myeloid leukaemia (AML)
Loss of PIAS3 expression	Anaplastic lymphoma kinase-positive T/null-cell lymphoma (ALK^+ TCL) [52]
Loss of PIASy expression	Myelodisplastic syndrome (MDS) [53]
Mutations of *socs-1*	Primary mediastinal B-cell lymphoma [101]
Loss of SOCS-1 expression	Primary mediastinal B-cell lymphoma [102]

domain carries the catalytic phosphotyrosine kinase domain [1]. The function of JH2 domain, which is a pseudokinase domain, is not completely defined, but there is growing evidence that it regulates both the basal activity of the JAK kinase and receptor induced activation of the catalytic function [2]. The JH3–JH4 region shares some similarity with SH2 domains but lacks phosphotyrosine binding ability, and the JH4–JH7 region constitutes the band 4.1 domain [also known as FERM domain (four-point-one, Ezrin, Radixin, Moesin)] [3,4]. The FERM domain is involved in the interactions between JAKs and other kinases. The only JAK family member known so far to be involved in human leukaemia is JAK2 (Table 1).

Two fusion genes that involve the JH1 domain of JAK2 have been described, the first one being *ETV6/TEL-JAK2*, which arises as a consequence of a *t*(9;12) in patients with chronic myeloproliferative diseases or acute lymphoblastic leukaemias [5,6]. The second one *BCR-JAK2* was observed in a single individual with atypical chronic myelogenous leukaemia (CML) [7]. The amino-terminal region of TEL contains the pointed (PNT) domain, which mediates oligomerization of TEL fusion proteins [8]. Similarly, BCR has been shown to contain an oligomerization motif which is required to activate the BCR fusion proteins [9]. Another translocation fuses the *PCM1* gene to the *JAK2* gene in patients with atypical CML and related myeloproliferative disorders, but in this case the *PCM1-JAK2* fusion protein implicates the JH1 and JH2 domain of JAK2 [10,11] (Fig. 1).

JAK2 kinase has been also described to be activated as the result of somatic point mutation in JH2 domain ($Val^{617}Phe$) in myeloproliferative disorders (MPD) (such as polycethemia vera (PV), essential thrombocythemia (ET) or myelofibrosis with myeloid metaplasia (MMM), atypical MPD and myeloproliferative syndrome [12–17]) (Fig. 1). The pseudo kinase suppressed basal JAK2 activity by lowering the V_{max} of the kinase domain but does not affect the K_m value. Three inhibitory regions, namely IR1 (residues 619–670), IR2 (725–757) and IR3 (758–807), have been defined in the pseudokinase domain [2]. Val^{617} is just N-terminal to IR1 and is conserved in JAK2 ortholog of various animals from fish, frog, bird to mice [17]. One can predict that the replacement of Val by Phe in JAK2 should cause major conformation changes. This may disrupt the inhibitory function of the pseudokinase domain and thus cause deregulation of the kinase domain. Functional analysis demonstrates that this mutation confers cytokine-independent growth in vitro, deregulates signaling pathways downstream of JAK2 and causes autonomous proliferation in a murine model.

Fused to a ''dimerizing'' protein such as TEL, BCR or PCM1, JAK2 is probably more strongly activated than that which occurs as a consequence of $Val^{617}Phe$ mutation. This dimerization leads to diseases such as acute leukaemia or CML which are more aggressive than PV or ET. The chromosomal region containing *JAK2* gene has been also described to be amplified in Hodgkin's lymphomas [18].

In normal physiological conditions, activated JAKs phosphorylate additional targets, including both the receptor itself and the STATs. STATs are latent transcription factors that reside in the cytoplasm until they become activated by tyrosine phosphorylation. Activated STATs rapidly translocate into the nucleus, where they bind to specific sequences in the promoter regions of their target genes and stimulate their transcription [19]. STATs, like many signal-responsive transcription factors, are found to be regulated by coactivators belonging to the histone acetyl transferases involved in chromatin remodelling [20,21]. The terminal transactivation domain of some STATs contains a serine residue that can modulate the transcriptional activity of STAT [22] and seems to be important in coregulator recruitment, which interacts either directly or through other coactivator proteins [23,24].

The role of constituvely activated STATs, particularly STAT3 and STAT5, in cellular transformation has been established by in vitro and in vivo studies and is extensively reviewed elsewhere [25 and references herein]. The mechanisms by which activated STAT can promote tumourigenesis, appear to be involved at least in part, in deregulated cell growth and/or prevention of apoptosis [26]. Activated STAT3, observed in ovarian cancer cell, is localized not only to nuclei but also to focal adhesion in these cells and may therefore contribute to enhanced cell invasiveness [27]. The reports that STAT1-deficient mice develop spontaneous and chemically induced tumours more rapidly compared to wild-type mice and that STAT1-deficient cells are more resistant to agents that induce apoptosis strongly support the argument that STAT1 acts as a tumour suppressor [28].

2. Regulation of STAT activity

2.1. *Constitutive suppressors*

2.1.1. *Tyrosine phosphatases*

As STATs are activated by tyrosine phosphorylation, phosphotyrosine phosphatases are likely to play a role in STAT deactivation. Three families of tyrosine phosphatases are implicated in negative regulation of JAK/STAT signaling pathways.

The first to be described were the SH2-containing tyrosine phosphatases that include SHP1 (previously named PTP1C) and SHP2 (previously named PTP1D). These phosphatases are characterized by the presence of two SH2 domains N-terminal of the canonical 250 amino-acid-long tyrosine phosphatase domain. These enzymes are mainly cytoplasmic; their SH2 domains allow association with phospho-tyrosines present on

activated receptors or on signaling molecules as well as on activated JAKs. This association triggers activation of the phosphatase domain and the subsequent dephosphorylation of the substrate. In case of a receptor, STATs will no longer be recruited to it and thus will no longer be phosphorylated and activated. SHP1 is mainly expressed in hematopoietic cells but it is also present in epithelial or smooth muscle cells [29], as opposed to SHP2 which is ubiquitously expressed. SHP1and SHP2 shared 55% homology at the protein level. Mice deficient for SHP1 display important immunological and hematopoietic dysfunctions, and hyperphosphorylation of JAK1 and JAK2 following IFNα, GH or EPO treatment was observed. This gene is frequently altered in cancer cells. Hypermethylation of normally unmethylated CpG islands of tumour suppressor genes is associated with transcriptional silencing and thus is assumed to play an important role in cancer development and progression [30,31]. High-frequent silencing of haematopoietic cell-specific protein tyrosine phosphatase *shp-1* gene by promoter methylation was detected in various kinds of leukaemia and lymphomas [32,33], myeloma [34] and acute myeloid leukaemia [35].

Deletion of SHP2 gene is embryonic lethal for mice, thus revealing a major role for SHP2 in development. SHP2 can inhibit signaling from IL6 and gp130-containing receptors, but SHP2 can also function as a positive factor by stabilizing JAK2 protein or inducing src kinase activation [36]. Mutations of SHP2 (PTPN11), occur in ~50% of individuals with Nooman syndrome. Some of these patients develop myeloproliferative disease, which usually resolves but can develop into leukaemia (Table 1) [37–39]

The second type of tyrosine phosphatase that was reported to negatively affect JAK/STAT signaling pathways is the transmembrane tyrosine phosphatase CD45. CD45 is expressed in hematopoietic cells [40]. It exhibits two phosphatase domains in its intracellular part, but only one seems to be active. CD45 regulates T-cell and B-cell antigen receptor signals in T and B lymphocytes [41]. Mice deficient for CD45 show hyperactivation of JAK1 and JAK3, associated with a loss of antigen responses in T and B lymphocytes. However, CD45 has no major effect on cytokine signaling. Moreover, both the SHPs and CD45 do not directly associate with the JAK kinase domain, suggesting that other tyrosine phosphatases may deactivate the JAKs.

The PTP1B (Phospho-Tyrosine Phosphatase 1B) and TC-PTP (T Cell Protein Tyrosine Phosphatase) tyrosine phosphatases show great similarities in their catalytic domain. PTP1B is expressed in many tissues and is located on the cytosolic face of the endoplasmic reticulum due to a hydrophobic sequence at its C-terminal end [42]. TC-PTP is mainly hematopoietic and alternative splicing of its gene allows expression of two distinct proteins, a p45 nuclear form called TC45 or TC-PTPα, and a p48 cytoplasmic form called TC48 or TC-PTP1β. Both PTP1B and TC-PTP selectively recognize a motif centered on the characteristic double tyrosine residues present in the JAK activation loop, but each exhibits different specificity for surrounding residues: PTP1B only interacts with the D/E-pYpY-K/R sequence present in JAK2 and TYK2 while TC-PTP interacts with the D/E-pYpY-T/V sequence present in JAK1 and JAK3. This interaction leads to selective JAK dephosphorylation and subsequent deactivation. In addition, the nuclear TC45/TC-PTP1β is responsible for deactivation of nuclear STAT1 and STAT3, and probably also STAT5 [43]. In accordance with these observations, mice deficient for PTP1B showed hyperphosphorylation of JAK2, while the absence of functional TC-PTP in mice induces anemia and splenomegaly responsible for perinatal death of the animals.

2.1.2. *Protein inhibitors of activated STATs (PIAS)*

Mammalian protein inhibitors of activated STAT (PIAS) were initially identified as negative regulators of STAT signaling. The PIAS family consists of PIAS1, PIAS3, PIASx and PIASy (for review see [44]). Recent studies indicate that PIAS have a small ubiquitin-like modifier (SUMO)-E3-ligase activity (for review see [45]). Co-immunoprecipitation assays have shown that PIAS3 and PIASx interact with STAT3 and STAT4 respectively, while PIAS1 and PIASy are able to interact with STAT1 [45]. These PIAS-STAT interactions negatively regulate the activity of the specific STAT(s) in the complex. It seems that the SUMO E3 ligase activity of STAT is not implicated in the regulation of STAT. It has been proposed that PIAS1 and PIAS3 function by blocking the DNA binding activity of STAT1 and STAT3 respectively [46,47]. In contrast PIASx and PIASy repress the transcriptional activity of STAT1 and STAT4 by recruiting corepressor molecules such as histone deacetylases (HDACs) [48,49]. Gene-targeting studies in mice have been carried out to understand the physiological functions of PIAS proteins in cytokine signaling. *Pias1*$^{-/-}$ mouse embryonic fibroblasts showed an unexpected specificity in gene regulation. After stimulation by IFN, the removal of PIAS1 results in the increased expression of only some IFN-induced genes [50]. It has been shown that the differential effect of PIAS1 on the binding of STAT1 to the promoters of STAT1 target genes contributes to the observed PIAS1 specificity. PIAS1 has more profound effects on genes containing weak STAT1-binding sites than on genes containing a strong STAT1-binding site. In contrast no alteration of IFN responsive genes was observed in *piasy*$^{-/-}$ cells which can be the result of redundancy in PIAS proteins [51].

Cells derived from patients with anaplastic lymphoma kinase-positive T/null-cell lymphoma which express the nucleophosmin (NPM)/ALK chimeric kinase as the result of a translocation show continuous activation of STAT3 due to the loss of PIAS3 (Table 1) [52]. Furthermore DNA microarray analysis indicate that *piasy* is active in normal hematopoietic stem cells (HSC) or pluripotent stem cells in the indolent stage of myelodysplastic syndrome (MDS), but that the expression of the gene is suppressed in the cells on transition to the advanced stage of MDS (Table 1) [53]. This indicates that the loss of *piasy* may therefore contribute directly to the growth of MDS blasts and stage progression.

2.2. *Inducible suppressors: suppressors of cytokine signaling (SOCS) proteins*

In addition to the constitutive negative regulators of cytokine signaling, the inducible SH2 containing proteins belonging to the SOCS family have been described. Cytokine-inducible SH2 containing protein (CIS) was the first member of the family identified, as an immediate-early gene product induced by IL-2, IL-3 and EPO [54]. CIS then binds to

phosphorylated Y401 of the EPO receptor, the binding site for STAT5, thereby suppressing STAT5-mediated signaling [55]. Subsequently, three groups identified a protein similar to CIS, termed Suppressor of cytokine signaling-1 (SOCS-1), JAK-Binding protein (JAB), and STAT-induced STAT Inhibitor-1 (SSI-1) [56–58]. SOCS proteins are a family of at least eight members which bind through their SH2 domain to phosphotyrosine residues either in cytokine receptors (in the case of SOCS-2, SOCS-3, and CIS) [54,59,60] or JAKs (in the case of SOCS-1) [57]. They can suppress cytokine signaling either by inhibiting the activity of JAKs, or by competition with STATs for phosphorylated docking sites on the receptors, or by targeting bound signaling proteins to the ubiquitin proteasome pathway through the SOCS box, which is part of an E3 ubiquitin ligase [61]. Thus, the SOCS box may act as a bridge between SOCS-SH2 interacting proteins and E3 ubiquitin ligase, and regulate protein turnover by targeting proteins for polyubiquitination and proteasome-mediated degradation. SOCS-1 can ubiquitinate and regulate the half life of VAV [62], of JAK [63–65], the IRS1 and IRS2 adaptor proteins [66]. It has been observed that SOCS proteins also promote polyubiquitination and degradation of Focal Adhesion Kinase (FAK) in a SOCS box-dependent manner and inhibit FAK-dependent signaling events [67].

The level of SOCS-1 seems to be tightly controlled at different level. Transcription of *socs-1* mRNA is rapidly induced by many cytokines. The presence of GAS elements in the proximal region of CIS, SOCS-1 or SOCS-3 promoters has been described by several laboratories, the implication of the activated STAT in their regulation has been shown upon EPO, IFN and IL-4 stimulation [68–71].

It has been also reported that SOCS-1 protein stability is tightly regulated. Stabilization of SOCS proteins by inhibitors of the proteasome, suggests that cells may regulate SOCS-1 level through the proteasome pathway [72]. Association of Elongin BC and the SOCS box has been suggested to alter the stability of the SOCS-1 protein. A recent report indicates that SOCS-1 is found to colocalize and biochemically copurify with the microtubule organizing complex (MTOC) and its associated 20S proteasome. SOCS-1 may target JAK1, in a SH2 dependent manner, to a perinuclear location resembling the MTOC-associated 20S proteasome, this observation was only shown in the case of overexpression of the two proteins SOCS-1 with JAK1 [73]. Phosphorylation by PIM kinases prolongs the half-life of the SOCS-1 protein and potentiates the inhibitory effect of SOCS-1 on JAK-STAT activation [74].

SOCS-1 may function as a tumour suppressor gene and its down regulation may contribute to tumour progression [75]. Overexpression of SOCS-1 suppresses the growth of cells transformed either by an oncogenic form of the KIT receptor or by the TEL-JAK2 fusion protein, but implicates different function of SOCS-1 proteins, since the presence of SH2 domain of SOCS-1 is required for the inhibition of TEL-JAK2, whereas it is not required for the inhibition of KIT [76]. Direct transcriptional repression of the *socs-1* tumour suppressor gene by proto-oncoproteins has been described either for the transcriptional repressor GFI-1B [77] or the transcription factor ETS-1 [71]. More recently it has been shown that the hepatitis C virus (HCV) core protein down regulates the expression of *socs-1* gene, by interfering with the intracellular signaling pathway, and may thus contribute to the pathogenesis in HCV infection including hepatocarcinogenesis [78].

In the exploration of the mechanisms underlying the down-regulation of *socs-1* gene, it has been reported that the silencing of the *socs-1* gene by hypermethylation is associated with the development of hepatocellular carcinoma [79–81]. Methylation of CpG islands in the region of tumour suppressor genes induces a block to transcriptional initiation. The sites responsible for silencing tumour suppressor genes generally reside in the promoter (or 5′ UTR) region. However some exceptional observations have been reported, where these sites are located in exons or introns [82]. In the case of the *socs-1* gene, hypermethylation occurs also inside the translated exon2. Subsequent studies found hypermethylation of *socs-1* in different solid tumours [83–89] as well as in haematopoietic diseases [90–97]. In most cases, the restoration of SOCS-1 in cell lines suppressed cell growth. The inactivation of *socs-1* gene by aberrant methylation leads, at least partially, to an activation of effectors of JAK/STAT pathways [97]. The finding that *socs-1* is frequently silenced in malignancies, mainly as a result of hypermethylation, reinforced the idea that *socs-1* may function as a tumour suppressor gene. The expression of *socs-3*, which shows the highest homology to *socs-1*, has also been reported to be invalidated by methylation in hepatocellular carcinoma. The loss of *socs-3* expression confers cell advantage in growth and migration by enhancing JAK/STAT and FAK signaling [98]. *socs-3* methylation is also frequently observed in head and neck squamous cell carcinoma [99] and as for all the previous described examples it is associated with activation of the JAK/STAT pathway and of expression of downstream target genes.

Recently, amplification of the JAK2 gene and constitutive phosphorylation of JAK2 in primary mediastinal large B-cell lymphoma (MedB-1 cell line) has been described [100]. The constitutive activation is not associated with an overexpression at the protein level, but is due to delayed protein degradation. This is caused by a biallelic mutation of SOCS-1 which abrogates SOCS box function of the protein [101]. Thus the assumption is that mutated and loss of function of *socs-1* might be the cause for sustained activation and low turnover of JAK2. A large biallelic chromosomal deletion on 16p13.13 including the entire SOCS-1 gene was observed in Karpas 1106P, another primary mediastinal large B-cell lymphoma cell line [102]. In addition the simultaneous loss of function by gene methylation of *shp-1* and *socs-1* is observed in the pathogenesis of myeloma [34].

Constitutive activation of JAK/STAT pathways or inactivation of negative regulators of JAK/STAT pathways is observed in several myeloproliferative diseases. Although activation of JAK/STAT pathways leads to enhanced expression of *socs-1*, constitutive activation of JAK2 by either point mutation or by fusion proteins exhibit transforming properties often linked to STAT activation [5,75,103]. Escape from the SOCS-1 negative feedback could be due to the nature of the JAK2 fusion protein in which the JH1 conformation might be different from the wild type JAK2. The connection between aberrant activation of JAK/STAT pathways and silencing of *socs-1* is not demonstrated even though it is tempting to speculate that the events leading to most aggressive

pathologies might implicate at the same time deregulation of positive and negative regulators of JAK/STAT pathways.

3. Conclusion

Negative regulation of signal transduction is necessary for an appropriate cellular and physiological response to cytokine stimulation. Over the past few years, several different mechanisms by which cytokine signaling is attenuated have been identified. The discovery of oncogenic partner in this signaling pathway, more especially in diverse hematologic malignancies supports a prominent role of deregulated pathways in the pathogenesis of diseases. The functional relationship between JAK2 activation and SOCS-1 hypermethylation remains speculative, but they may collaborate in pathogenesis.

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