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Epigenetic Regulation of Signal Transducer and Activator of Transcription 3 in Acute Myeloid Leukemia

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Abstract

We have demonstrated that constitutive signal transducer and activator of transcription (STAT) 3 activity, observed in approximately 50% of acute myeloid leukemia (AML) cases, is associated with adverse treatment outcome. Constitutive STAT3 activation may result from the expression of oncogenic protein tyrosine kinases or from autocrine stimulation by hematopoietic growth factors. These causes are generally neither necessary nor sufficient for leukemogenesis; additional transforming events or growth stimulatory processes are needed. Here we review the literature addressing epigenetic regulation as a mechanism controlling STAT3 signaling in AML. A better understanding of mechanisms of dysregulation of STAT signaling pathways may serve as a basis for designing novel therapeutic strategies that target these pathways in leukemia cells.

Introduction

We have shown that samples from approximately 50% of acute myeloid leukemia (AML) patients demonstrate constitutive signal transducer and activator of transcription (STAT) 3 activity [1] and that the disease-free survival is significantly shorter in those patients whose leukemia cells exhibit constitutive STAT3 activity compared to those with cells without such constitutive activity [2]. Our work proposes that constitutive STAT3 activity is related, at least partially, to activation of protein tyrosine kinases, which, among others, phosphorylate and activate STATs [3]. This review focuses on the potential contribution of epigenetic regulation through DNA hypermethylation to constitutive STAT3 activity in AML.

There are several ways through which DNA methylation could contribute to the STAT phenotype of leukemic cells. The potential of methylation to alter STAT3 function is generally assessed by determining the effects of demethylating agents on target cells. Those studies have led to the following observations:

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- - Methylation could affect expression of STATs. There was no evidence from our own demethylation experiments in epithelial tumor cell lines *in vitro* [4].
- - Methylation affects expression of cytokine receptors (likely alters sensitivity to cytokines). This does not apply to constitutive activation of STATs [4].
- - Since methylation normally is associated with reduced or silenced gene expression, the most probable scenario is that the methylation process determines expression of factors that are involved in the activation STATs and/or supporting STAT function. Loss of an inhibitor function in such processes will lead to an increase of STAT activity. The latter outcome would explain the phenotype observed in leukemic cells. The focus of this review is whether dysregulation of STAT3 pathway involves altered DNA methylation and thus magnifies STAT3 activity.

STAT Signaling

The interaction of a cytokine with its ligand-binding receptor alpha subunit is the first step in the formation of a signaling-competent receptor complex. This process involves the oligomerization of the ligand-bound subunit with either another subunit or a separate, signal-transducing beta subunit. Alternatively, ligand binding occurs to a preformed, but inactive receptor complex [5]. Ligand-mediated receptor oligomerization or rearrangement initiates signal transduction by activation of the receptor-associated Janus family tyrosine kinases (JAK) through cross-phosphorylation (Fig. 1). Immediate substrates of the activated JAKs are the cytoplasmic portions of the receptors and receptor-associated proteins. The tyrosine phosphorylated sites become docking elements for Src homology 2 (SH2)- and phosphotyrosyl binding domain-containing proteins present in the membrane or the cytoplasmic compartment. Prominent among these are the STATs. Receptor-recruited STATs are phosphorylated on a single tyrosine residue in the carboxy terminal portion. The modified STATs are released from the cytoplasmic region of the receptor subunits to form homo- or heterodimers through reciprocal interaction between the phosphotyrosine of one STAT and the SH2 domain of another. Following dimerization, STATs rapidly translocate to the nucleus and interact with specific regulatory elements at target genes and often induce transcription.

Rigorous mechanisms exist to regulate STAT signaling to ensure appropriate controlled cellular response. Among the most studied ones are phosphotyrosine phosphatases (SHPs, CD45, PTPN1/PTPN2), suppressors of cytokine signaling (SOCS), protein inhibitors of activated STATs (PIAS), and a nuclear ubiquitin E3 ligase (STAT-interacting LIM protein, SLIM) [6–9] (Fig. 2). In addition, serine phosphorylation through various protein tyrosine kinases, in particular protein kinase C and mitogen activated protein kinases, has been noted to modify STATs and thus alter activity and tumor property [5]. In the following, we will briefly discuss the primary attenuating factors for STATs as we propose that hypermethylation of their genes leads to reduction in their expression and hence loss of STAT regulation. We will then review the literature for evidence of this phenomenon in different malignancies with emphasis on AML.

Phosphotyrosine phosphatases

STAT phosphorylation at a tyrosine residue is required for its activation. Therefore, phosphotyrosine phosphatases serve as rapid and reversible deactivators. Three different phosphotyrosine phosphatases were described: SH2-containing tyrosine phosphatases (SHP) 1 and 2, protein tyrosine phosphatase, receptor type C (CD45) and protein tyrosine phosphatase, non-receptor, type 1 (PTPN1) and type 2 (PTPN2). SHP1 and SHP2 contain two consecutive N-terminal SH2 domains and a C-terminal phosphatase domain. They reside in the cytoplasm and associate with tyrosine phosphorylated receptors via their SH2 domains. This binding results in activation of the phosphatase domain and subsequent dephosphorylation of the substrate (JAK or STAT; Fig. 1). The C-terminal domain also contains tyrosine residues that undergo phosphorylation upon activation allowing recruitment of adaptor proteins. SHP1 is mainly expressed in hematopoietic cells while SHP2 is ubiquitously expressed. CD45 is a transmembrane tyrosine phosphatase expressed in hematopoietic cells. It contains two phosphatase domains. PTPN1 is located at the cytosolic side of the endoplasmic reticulum while PTPN2 exists as two distinct proteins, one nuclear and the other cytoplasmic. PTPN1 is ubiquitously expressed while PTPN2 is expressed mainly in hematopoietic cells. Both target JAKs while the nuclear PTPN2 also dephosphorylates nuclear STATs.

Suppressors of cytokine signaling (SOCS)

SOCS represent a classical negative feedback loop in the JAK-STAT pathway, i.e., their expression is increased in response to signals from cytokines and growth factors. There are eight members in this family: the cytokine-inducible SH2 domain-containing protein (CIS) and SOCS1 to SOCS7. All have a central SH2 domain, to allow binding to phosphotyrosine residues, and a C-terminal SOCS box (Fig. 2). The SOCS box recruits the ubiquitin-transferase complex. SOCS can directly inhibit JAK activity, compete with STATs for phosphorylated docking sites on the receptors or target bound proteins to the ubiquitin proteasome leading to their degradation (Fig. 1).

Recently, SOCS1 was demonstrated to contribute to SHP1 function in negatively regulating cytokine-receptor signaling [10]. SHP-1 was shown to be recruited to the receptor-JAK complex, get phosphorylated on its C-terminal tyrosine residues and thereby recruit growth factor receptor-bound protein 2 (GRB-2). GRB-2 then associated SOCS1, which subsequently targeted JAK2, leading to inhibition of the cytokine-receptor signaling. Loss of SOCS1 expression led to attenuation of SHP-1 function.

Protein inhibitors of activated STATs (PIAS)

The family consists of PIAS1, PIAS3, PIASx and PIASy. PIAS1 and PIASy bind STAT1; PIAS3 and PIASx interact with STAT3 and STAT4 (Fig. 1). PIASy and PIASx repress the transcriptional activities of STAT1 and STAT4 by recruiting co-repressor molecules such as histone deacetylases. In addition, PIAS1, PIAS3 and PIASx exhibit small ubiquitin-related modifier (SUMO)-ligase activity (Fig. 2), though it seems that this activity may not be involved in the regulation of STATs.

STAT-interacting LIM protein (SLIM)

The SLIM protein contains a LIM domain and a PDZ domain (Fig. 2) that interact in the nucleus with phosphorylated STAT and inhibit gene transcription mediated by STAT1 and STAT4 [11]. The LIM domain, whose designation is derived from the first letter of the founders LIN-11, Isl1, and MEC-3, is one of the modular protein-binding domains found in numerous eukaryotic proteins, and consists of a conserved cysteine/histidine-rich sequence coordinating two zinc ions that organize a double zinc finger structure essential for LIM domain function. The PDZ domain is a modular protein interaction domain consisting of 80–90 amino acids. It was originally identified in postsynaptic density protein PSD95, *Drosophila* tumor suppressor disc large, and epithelial tight junction protein zona occludens-1. SLIM is a ubiquitin E3 ligase that enhances the ubiquitination and degradation of STAT proteins (Fig. 1). No data are available to date on their expression in human diseases.

Hypermethylation

Hypermethylation is characterized by the addition of methyl groups to cytosine residues in the DNA leading to reduction in gene expression. In addition to DNA, proteins are also subject to methylation at lysine and arginine side groups. DNA hypermethylation events in promoters of tumor suppressor genes result in transcriptional silencing. This suggests that hypermethylation may serve as one of the events in the multistep process of tumorigenesis, and probably follows a unique pattern in different tumor types. Since tumor suppressor genes are usually involved in the regulation of the cell proliferation, silencing them would lead to the activation of growth-promoting genes.

Hypermethylation occurs at the CpG islands and results from the attachment of a methyl ($-CH_3$) group to the 5′ carbon of the cytosine residue located prior to a guanosine in the CpG dinucleotide. Approximately 10% of the CpG dinucleotides are found in dense clusters called CpG islands that localize in the promoter regions of the genes and may extend into the first exon and sometimes into the subsequent intron [12]. These CpG islands may range from 0.5–4kb in length. Characteristically, they have a GC content of over 50% whereas the genome wide average is 40% [13–15]. The CpG islands are almost exclusively unmethylated with the exception of genes on the inactive X-chromosomes in female cells and some genes that are selectively silenced (imprinted) during embryonic development. CpG island methylation has been associated with changes in chromatin structure and repression of gene transcription [16].

DNA methylation patterns are established and maintained by interplay of several DNA methyl transferase (DNMT) enzymes that transfer a methyl group from the methyl donor, S-adenosine-L-methionine, to cytosine [17, 18]. The known DNMT proteins are DNMT1, DNMT2, DNMT3a and 3b. Of these, DNMT1, DNMT3a and 3b are DNA methyltransferases while DNMT2 has tRNA related function [19]. DNMT1 is known as a maintenance methylase, being mainly responsible for preserving DNA methylation patterns during replication. DNMT3a and 3b preferentially methylate unmethylated DNA and thus take part in *de novo* methylation. Their mechanism of action is only partially elucidated.

Transcriptional repression of tumor suppressor genes has been hypothesized to be caused by at least three possible mechanisms [18]. One of the mechanisms is direct recognition of DNA or chromatin by DNMTs' transcriptional repressor domains; the other involves recruitment of co-repressor proteins such as histone deacetylases, and the third might target the RNA-mediated interference system to DNA sequences. Although methylation controls gene activity, some promoters also depend on the chromatin structure to silence transcription [16]. Specifically, hypermethylated CpG islands can compact the DNA-histone structure to prevent access to regulatory proteins that promote transcription. Further, acetylation of histones is required to maintain transcription, while histone deacetylation results in gene silencing. Binding of histone deacetylases to hypermethylated DNA, as directed by DNMTs, results in prevention of access to the transcriptional machinery and hence transcriptional silencing.

We reviewed the literature on the methylation status of the three known families of STAT inhibitors (phosphatases, SOCS and PIAS); SLIM was not yet tested in human diseases. We describe only those members that were studied in human malignancies.

Hypermethylation of STAT inhibitors in solid tumors

Many solid tumors are characterized by constitutively activated STAT proteins. Among them are breast [20], ovarian [21, 22] and lung [23] cancers characterized by constitutively activated STAT3 and STAT5, and pancreatic [24] and hepatocellular [25] cancers characterized by constitutively activated JAK/STAT3. These data suggest that at least in some of these solid tumors, the STAT inhibitors may be hypermethylated.

SHP1 methylation status was not studied in solid tumor samples; conflicting data exist about its expression level in solid tumor cell line and tissues [26]. For example, membrane-associated SHP1 expression is required for receptor-mediated cytotoxic signaling and for intracellular acidification and subsequent apoptosis in breast cancer cell lines [27]. On the other hand, SHP1 mRNA and protein are overexpressed in ovarian cancer cell lines and tumor tissues [28]. More work is needed to determine the role of SHP1 expression and regulation of cytokine and growth factor receptor signals in solid tumors.

Table 1 summarizes our review of the literature on SOCS hypermethylation in solid tumors. CIS was found to be more frequently hypermethylated in hepatocellular carcinoma compared to non-neoplastic tumor tissues [29]. Similarly, SOCS1 was shown to be frequently hypermethylated in hepatocellular [25, 29–31] and pancreatic [32,33] carcinoma. Further, reduced expression of SOCS1 due to hypermethylation is common in gastric cancer and was shown to correlate with lymph node metastasis and advanced tumor stage [34]. On the other hand, SOCS1 hypermethylation was rarely detected in colorectal cancer [35, 36] though it may be associated with younger age at presentation [35]. Finally, SOCS3, which is prominently induced by cytokines in various cell types, was found to be frequently hypermethylated in lung [37, head and neck [38] and a subset of hepatocellular [29] cancer samples. Restoration of SOCS3 activity resulted in down-regulation of STAT3 activity and growth suppression in lung and head and neck models [37, 38].

Interestingly, forced expression of PIASy in human kidney 293T cells was shown to be accompanied by induction of apoptosis [39]. Further, PIAS3 overexpression in breast cancer cell lines was shown to significantly modulate STAT5-mediated gene expression and induce cellular apoptosis [40]. Finally, overexpression of PIAS3 suppresses cell growth and restores the drug sensitivity of human lung cancer cells [41]. To date, only cell line work supports the evidence for PIAS downregulation of the STAT pathway but no clear evidence exists for hypermethylation of the PIAS promoter in human solid tumors.

In summary, silencing SOCS by hypermethylation in solid tumors at least partially supports our hypothesis about the contribution of epigenetic events to constitutive STAT activity.

Hypermethylation of STAT inhibitors in non-leukemic hematologic malignancies

Dysregulation of the JAK/STAT pathway is frequently observed in lymphoma and multiple myeloma. For example, STAT5 is constitutively activated in T cell lymphoma [42] while STAT3 is constitutively activated in Burkitt's (B lineage) cells [43]. Finally, interleukin (IL) 6 signaling mediated by STAT3 transcriptional activity is the major pathway involved in growth and differentiation of malignant plasma cells [44, 45]. Therefore we searched the literature for evidence of hypermethylation of STAT inhibitors in non-leukemic hematologic malignancies.

SHP1 protein expression was shown to be either diminished or normal in a variety of T cell lymphoma cell lines [26]. In a subset of cell lines, lack of SHP1 expression was related to hypermethylation, and demethylation treatment was associated with dephosphorylation of JAK3 [46]. In another T cell lymphoma cell line, treatment with DNMT1 anti-sense oligonucleotides and STAT3 small-interfering RNA induced DNA demethylation and expression of the SHP1 gene. These data suggested that STAT3 activation may, in part, be induced in transformed cells by SHP1 silencing in cooperation with DNMT1 and histone deacetylase 1 [47]. In line with these findings, SHP1 protein expression was decreased in most human Burkitt's lymphoma cell lines [26] but no data are available about the methylation status of its gene in those lines.

Table 2 summarizes our review of the literature on SHP1 and SOCS1 hypermethylation in patient samples with non-leukemic hematologic malignancies. SHP1 was found to be frequently hypermethylated in multiple myeloma [48] patient samples but no studies described its methylation status in lymphoma patient samples. SOCS1 hypermethylation is rare in lymphoma patient samples [49]. The data on SOCS1 hypermethylation in multiple myeloma patient samples are contradictory. Two reports [49, 50] describe frequent methylation in the 3′ region while the third one [48] found no methylation in the 5′ promoter's region. The reason for the discrepancy is because the third group found the 3′ area to be unreliable since half of the normal volunteers' samples demonstrated methylation at that site. Interestingly, one of the reports [49] describing hypermethylation of SOCS1 did not find evidence for its methylation in the 3′ portion in samples from normal volunteers while the other one [50] did not report testing normal volunteer samples. In conclusion, based on our review of the literature, SHP1 is frequently hypermethylated in multiple

myeloma but it is unclear whether SOCS1 methylation plays a role in activating STAT in this disease. SOCS1 is rarely hypermethylated in lymphoma.

Hypermethylation of STAT inhibitors in myelodysplastic syndromes

Little information is available about constitutive STAT activation in myelodysplastic syndromes (MDS). The disease is challenging because the malignant cell tends to co-exist with its normal counterparts and does not lend itself well to isolation. We have found one report analyzing samples from seven early phase (refractory anemia) MDS and eight AML patients [51]. They showed that STAT5 was activated in the MDS patient samples only upon exposure to cytokines but was constitutively activated in all AML derived samples, suggesting that deregulated STAT5 activity may contribute to the phenotypic transformation from MDS to AML.

MDS are the first group of diseases to receive Food and Drug Administration approval for treatment with demethylating agents, 5-aza-cytidine (Vidaza®) [52] and decitabine (Dacogen®) [53] based on randomized clinical trials [54, 55]. In these trials, complete responses were seen in 7% and 9% of the patients, respectively. Later studies correlated responses with either global DNA or specific gene methylation [56, 57]. Therefore, we felt it prudent to review the literature for the possibility that STAT inhibitors are methylated in MDS patient samples (Table 3). No evidence of SHP1 methylation was detected in MDS patient samples [58]. However, SOCS1 hypermethylation was detected in MDS patient samples [58, 59]. Finally, PIASy mRNA was studied in blasts from 37 patients; it was present in stem cells from 13 MDS patients in the early phase (refractory anemia) of the disease but its expression was suppressed in samples from patients in the more advanced stages (nine with refractory anemia with excess of blasts and 13 MDS associated leukemia) of the disease [60] to suggest that methylation of STAT regulators may be involved in the transformation of MDS to AML.

In summary, it is still unclear whether the responses following therapy with 5-azacytidine and decitabine in MDS, are related to demethylating STAT inhibitors.

Hypermethylation of STAT inhibitors in acute leukemias

As already eluded in the MDS section, and described in several reviews [5, 61, 62], STAT3 and STAT5 were found to be constitutively expressed in *de novo* and secondary AML. Therefore, it seemed appropriate to examine the literature on the methylation status of the different STAT inhibitors in these malignancies.

SHP1 hypermethylation in AML patient samples was found frequently by one group [63] and infrequently by another [58] (Table 4). The majority of the patients studied by the second group, whose samples demonstrated hypermethylated SHP1, had leukemia characterized by t(8;21) or inv(16), also known as core factor binding leukemia (Table 4). SOCS1 hypermethylation was frequently detected in AML patient samples by two groups [64, 65] but not by two other groups [58, 63] (Table 4). Only the first group [64] reported correlation with karyotype (Table 4). Interestingly, samples from patients with t(15;17) (acute promyelocytic leukemia) had frequent SOCS1 hypermethylation. Since the product of

the t(15;17) is not known to activate STAT signaling, it may be that the FMS-like tyrosine kinase 3 (FLT3) mutation, reported commonly in this disease [66], is inducing STAT5 activation. Hypermethylation of SOCS1 may therefore further contribute to leukemogenesis. This group [64] also looked for correlation between hypermethylation and outcome (achievement of complete remission, disease-free and overall survival) and did not detect any difference between patients whose samples demonstrated SOCS1 hypermethylation and those without. The reason for the conflicting results about SOCS1 hypermethylation in AML samples is unclear. One explanation may be the area of the SOCS1 promoter studied, as a similar discrepancy was previously described above for multiple myeloma.

PIASy was shown to be hypermethylated in patients with MDS-derived AML (see above) [60]. We could not find any reports on PIAS hypermethylation in *de novo* AML. Interestingly, overexpression of PIASy resulted in growth suppression in a myeloid leukemia cell line model [60].

In summary, our review of the literature provides conflicting evidence for hypermethylation of SHP1 and SOCS1 in AML patient samples. One group reported PIASy hypermethylation in MDS-related AML. More work is warranted in this area, especially in view of the current increase in the number of clinical trials [67–69] studying the role of demethylating agents in AML. Some of these studies analyzed the effect of the hypomethylating agents on the methylation status of either global genomic DNA or specific genes. Correlation between hypomethylation and clinical response was reported by some [68, 69].

Studies on the role of STAT proteins in acute lymphoblastic leukemia (ALL) are much less extensive [5]. As compared to AML, where STAT3 activation seems prevalent, STAT5 is more commonly activated in ALL. Of note, the t(9;12)(p24;p13) in patients with T-cell ALL, pre-B-cell ALL, and atypical chronic myeloid leukemia (CML) generates the chimeric protein TEL-JAK2, with constitutive tyrosine kinase activity. Expression of SOCS1 has been demonstrated to inhibit TEL-JAK2-mediated transformation of Ba/F3 cells with impaired phosphorylation of STAT5 [70].

We found only one study analyzing the methylation status of SHP1 in ALL [71]. SHP1 was found to be hypermethylated in 17% of T-ALL and 11% of B-ALL samples. The cohort included both pediatric and adult patients but no further clinical data were provided to understand the characteristics of those patients with SHP1 hypermethylation. No published studies reported on SOCS or PIAS methylation status in ALL.

Hypermethylation of STAT inhibitors in chronic leukemias

The important role of STAT5 activation in Bcr-Abl-induced cell growth and transformation was confirmed using dominant negative STAT5 isoforms that inhibit Bcr-Abl-dependent STAT5 phosphorylation, with subsequent inhibition of gene transcription and cell growth [72, 73].

Members of the SOCS family were reported to be overexpressed in CML patient samples74–76 suggesting inability of the SOCS proteins to downregulate constitutive STAT activity induced by BCR-ABL. One report studied SOCS1 mRNA expression in CML

patient samples who were treated with interferon α [74]. In their work, constitutive SOCS1 expression was more frequently observed among high-risk patients and was independently associated with shorter median progression-free survival and poor cytogenetic response to interferon α therapy. Another report demonstrated overexpression of SOCS2 mRNA by reverse transcriptase (RT) PCR in samples from patients in blast crisis as compared to samples from patients in chronic phase or normal volunteers, though no significant differences were detected between these latter two groups [75]. Of interest is also the finding that therapy with imatinib (Gleevec®), the ABL kinase inhibitor, was associated with concomitant downregulation of SOCS2 mRNA. Finally, constitutive SOCS3 mRNA expression was found in samples from patients in blast crisis [76]. Based on cell line work, this group also suggested that constitutive SOCS3 expression was associated with interferon resistance. In summary, these data imply lack of negative feedback on STAT by the SOCS family in CML. No reports described hypermethylation of any of the STAT regulators in CML. However, in line with the promising results following treatment with decitabine in patients with imatinib-resistant disease [77], it may be justified to further study the genes to be hypomethylated.

Chronic myelomonocytic leukemia is a clonal myeloproliferative disorder occasionally associated with the chromosomal translocation t(5;12)(q33;p13), which results in TEL-PDGFβR tyrosine kinase fusion protein. This translocation is associated with constitutive STAT5 activity [78]. No data were found on hypermethylation of any of the STAT regulators in this disease.

Chronic lymphocytic leukemia (CLL) is characterized by STAT1 and STAT3 activity but as opposed to STAT phosphorylation on tyrosine in all other malignancies, here STAT proteins are serine phosphorylated [79]. Therefore it was not surprising that expression of SHP1, a tyrosine phosphatase, was maintained in 13 of 13 patient samples tested [80]. No data on hypermethylation of STAT inhibitors in CLL patient samples were found.

The myeloproliferative disorders, which include several pathologies (polycythemia vera, essential thromobocythemia and myelofibrosis with myeloid metaplasia) sharing the common feature of mutation in the JAK2 kinase, are associated with constitutive activation of JAK2 and subsequently STAT5 [81]. The main question is how a single mutation can explain such disease heterogeneity. It was tempting to speculate that, at least in some of these diseases, silencing of STAT5 inhibitors would contribute to the disease phenotype. Interestingly, SOCS1 mRNA expression was significantly higher in $JAK2^{V617F}$ samples compared to the control group [82]. Furthermore, SOCS3 enhanced the proliferation of cells expressing $JAK2^{V617F}$ [83]. This suggests that, as in CML, the expected compensatory feedback mechanism failed in $JAK2^{V617F}$ diseases. No reports on hypermethylation of STAT inhibitors in $JAK2^{V617F}$ diseases were found.

Conclusions and future directions

In the past several years, compelling evidence has accumulated emphasizing the role of STAT proteins in leukemogenesis. Constitutive activation of STATs has now been clearly demonstrated in acute and chronic leukemias. These raised the hypothesis that STAT

regulators will be silenced at least in some of these diseases. Our review of the literature supports this hypothesis. Of note, several studies [84–88] analyzed the effect of demethylating agents administered *in vivo* on target genes but demonstrated heterogeneous results. Some demonstrated changes in gene methylation but no clinical benefit, while others demonstrated clinical effect without changes in gene methylation. Alternatively, microRNAs (miRNAs) are recently discovered small non-coding transcripts with a broad spectrum of functions described mostly in invertebrates [89]. As post-transcriptional regulators of gene expression, miRNAs trigger target mRNA degradation or translational repression. It was recently shown that inhibition of miR-9 results in upregulation of STAT3 while overexpression of miR-9 and miR-124a results in reduced STAT3 activity [90]. While many questions remain open, we believe that the available data, described above, allow us to propose that future studies with demethylating agents will incorporate prospective analysis of candidate target and hypermethylated genes, including the STATs and their inhibitors.

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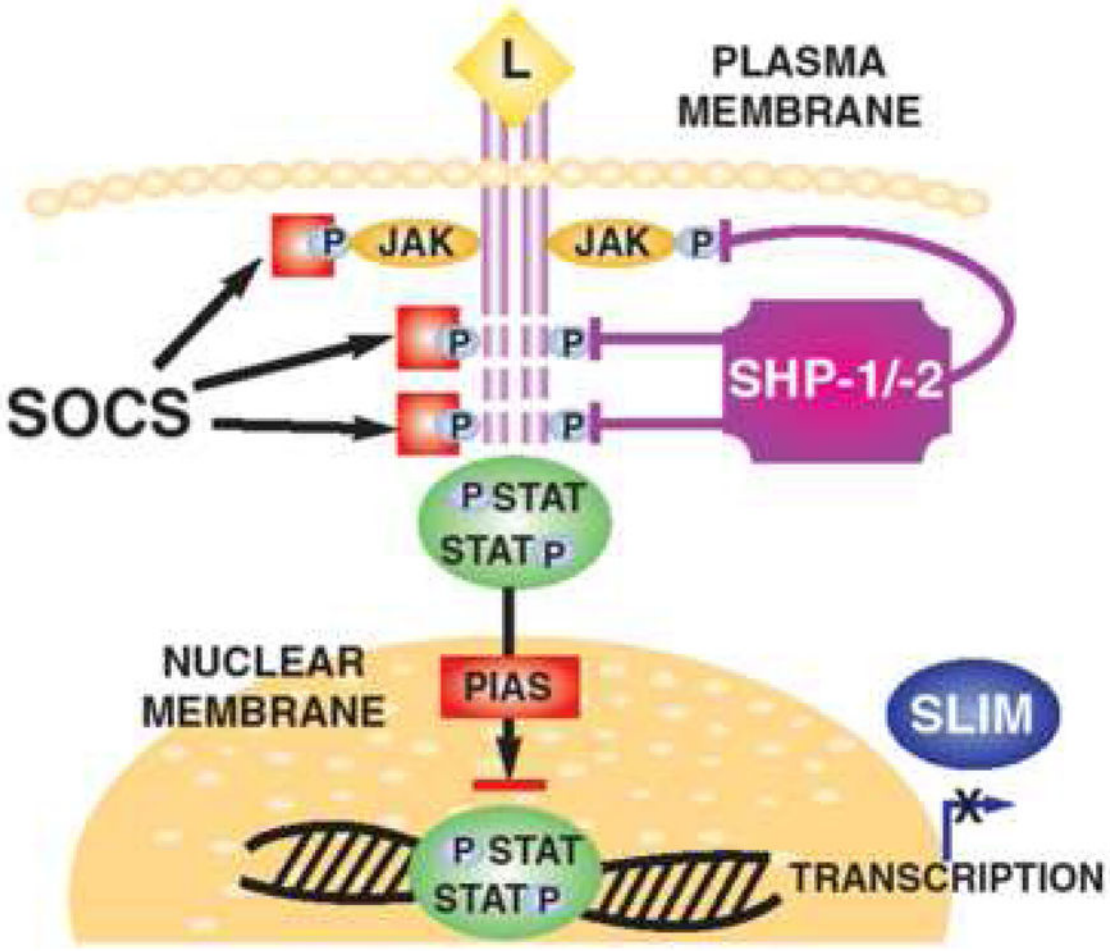

Figure 1.

Regulation of the JAK-STAT signaling pathway. Ligand-induced receptor oligomerization activates JAKs which subsequently phosphorylate tyrosine residues on the cytoplasmic portion of the receptor. The quiescent STAT monomers are then recruited to the activated receptor complex via the interaction of the SH2 domains with phosphotyrosine docking sites. STATs are phosphorylated by the JAKs on a conserved tyrosine residue in the c-terminal domain to form STAT homo- or heterodimers. STATs dissociate from the receptor after the dimerization and translocate into the nucleus. In the nucleus, STATs bind to specific response elements and induce gene transcription. Signaling is inhibited by four different mechanisms: SHP dephosphorylates activated JAKs and STATs, SOCS inhibits JAKs and STATs, and PIAS and SLIM inhibit transcriptional activities.

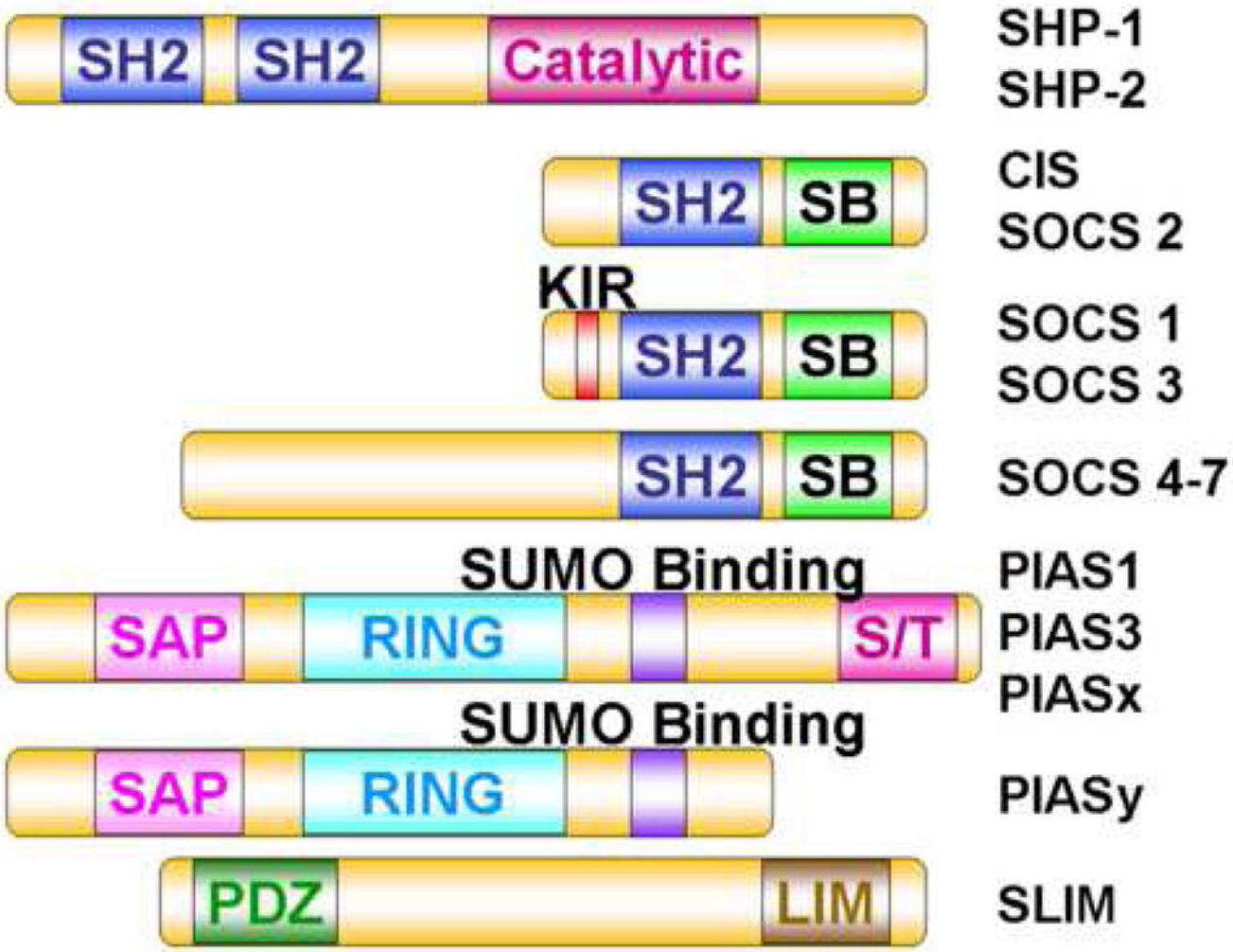

Figure 2.

Modular structure of the STAT inhibitors. SH2, Src homology 2; SB, SOCS Box; KIR, kinase inhibitory region; SUMO, small ubiquitin-related modifier; SAP, represents three proteins SAFA/B, ACINUS, PIAS; Ring, zinc binding domain; S/T, serine/threonine-rich region; PDZ, represents three proteins PSD95, *Drosophila* tumor suppressor disc large, and epithelial tight junction protein zona occludens-1; LIM, represents three proteins LIN-11, Isl1, and MEC-3.

Table 1

SOCS Hypermethylation in solid tumors patient samples

Gene	Cancer Type	Number of cases	Number of cases studied	Percent	Reference
CIS	Hepatocellular	N/A	N/A	88	29
SOCS1	Hepatocellular	6	18	33	25
		17	26	65	30
		7	15	47	31
		62	80	77	29
	Pancreatic	13	60	22	32
		8	14	57	33
	Gastric	33	75	44	34
	Colorectal	6	74	8	35
		7	185	4	36
SOCS3	Lung	14	18	78	37
	Head and Neck	85	94	90	38
	Hepatocellular	N/A	N/A	39	29

Abbreviations: N/A, not available.

Table 2

Hypermethylation of STAT inhibitors in non-leukemic hematologic malignancies patient samples

Gene	Cancrer Type	Number of cases	Number of cases studied	Percent	Reference
SHP1	Multiple myeloma	27	34	79	48
SOCS1	Lymphoma	2	62	3	49
	Multiple myeloma	23	35	63	49
		38	51	75	50
		0*	34	0	48

*5′ promoter methylation (please see text for details)

Table 3

Hypermethylation of STAT inhibitors in MDS patient samples

Gene	Number of cases	Number of cases studied	Percent	Reference
SHP1	0	107	0	58
SOCS1	8	74	11	58
	27	86	31	59

Table 4

Hypermethylation of STAT inhibitors in AML patient samples

Gene	Cytogenetic aberration	Number of cases	Number of cases studied	Percent	Reference
SHP1	Overall	26	50	52	63
	Normal karyotype	8	16	50	
	t(8;21)	1	3	33	
	Complex karyotype	2	7	29	
	Overall	13	121	11	58
	inv(16)	5*	38	13	
	t(8;21)	4†	20	20	
SOCS1	Overall	53	89	60	64
	inv(16)	2	3	67	
	t(8;21)	1	9	11	
	t(15;17)	13	17	76	
	t(7;11)	2	3	67	
	t(9;11)	1	2	50	
	Sole +8	4	5	80	
	Overall	64	88	72	65
	Overall	0	56	0	58
	Overall	0‡	50	0	63

*Four of the five patient samples possessed either c-KIT 419 (three cases) or FLT3 835 (one case) mutation

†Two of the four patient samples exhibited each either a FLT3-internal tandem duplication or N13 RAS mutation

‡5′ promoter methylation