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SOCS proteins in regulation of receptor tyrosine kinase signaling

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Abstract

The receptor tyrosine kinases (RTKs) are a family of cell surface receptors that play critical roles in signal transduction from extracellular stimuli. Many of this family of kinases are overexpressed or mutated in human malignancies and thus became attractive drug target for cancer treatment. The signaling mediated by RTKs must be tightly regulated by interacting proteins including protein-tyrosine phosphatases and ubiquitin ligases. The suppressor of cytokine signaling (SOCS) family proteins are well known negative regulators of cytokine receptors signaling consisting of eight structurally similar proteins, SOCS1-7 and CIS. A key feature of this family of proteins is the presence of an SH2 domain and a SOCS box. Recent studies suggest that SOCS proteins also play a role in RTK signaling. Activation of RTK results in transcriptional activation of SOCS encoding genes. These proteins associate with RTKs through their SH2 domains and subsequently recruit the E3 ubiquitin machinery through the SOCS box, and thereby limit receptor stability by inducing ubiquitination. In a similar fashion SOCS proteins negatively regulate mitogenic signaling by RTKs. It is also evident that RTKs sometimes can bypass SOCS regulation and SOCS proteins can even potentiate RTKs-mediated mitogenic signaling. Thus apart from negative regulation of receptor signaling, SOCS proteins may also influence signaling in other ways.

Keywords: SOCS1, SOCS2, SOCS3, SOCS6, FLT3, KIT, INSR, IGF1R, CSF1R, PDGFR.

1. Introduction

The receptor tyrosine kinase (RTK) family of proteins are cell surface receptors involved in the regulation of critical cellular processes including cell survival, proliferation, differentiation, migration, cell cycle control and metabolism. Within the more than 500 mammalian protein kinases described about 90 are classified as tyrosine kinases, of which about 60 belong to the RTK family [1, 2]. All RTKs possess a similar molecular architecture with three key features: an extracellular ligand binding domain, a transmembrane domain and a cytoplasmic kinase domain. In the human genome there are 58 known RTKs which are subdivided in 20 families according to structure of their extracellular domain (For review see [3]). RTKs function to transduce signals from extracellular stimuli by inducing changes in tyrosine phosphorylation that promote the formation of signaling complexes leading to changes in biological processes. RTK are involved in many human diseases [4-7]. Overexpression or mutation of RTKs and their aberrant activation of downstream signaling pathways have been linked to the oncogenic transformation, angiogenesis, atherosclerosis, bone disorder, inflammation and diabetes. For example, the epidermal growth factor (EGF) receptor (EGFR) is overexpressed in lung cancer and colon cancer, ERBB2 is overexpressed in certain types of breast cancer, FLT3 is mutated in acute myeloid leukemia and KIT is mutated in mastocytosis [8, 9]. The association of RTKs with human diseases has driven the development of novel class of drugs targeting these proteins. Many of RTK inhibitors show promising results in clinical trials and some have been approved for clinical use. The RTK signaling must be tightly regulated. The regulation occurs primarily through the action of ubiquitin ligases and protein-tyrosine phosphatases. Ubiquitin ligases attach ubiquitin moieties to the RTKs leading to degradation, while protein-tyrosine phosphatases terminate signaling by de-phosphorylating RTKs. Oncogenic mutations in RTKs help to bypass these regulations mainly due to the loss of binding site in receptor due to mutation. The E3 ubiquitin ligase CBL is the most studied ubiquitin ligase in respect to RTK regulation. Another class of proteins include suppressor of cytokine signaling (SOCS) which play important roles in regulation of various receptors signaling.

In 1995 Yoshimura and colleagues observed an immediate-early cytokine-responsive gene that was up-regulated in response to interleukin 3 (IL-3) treatment of hematopoietic cells [10]. The gene product was named cytokine-inducible SH2-containing protein (CIS), as the protein contains an SH2 domain. Later identification of seven more structurally similar proteins led to the discovery of a family of eight proteins named suppressor of cytokine signaling (SOCS) [11-14]. This family of proteins include SOCS1-7 and CIS [15] which are characterized by the presence of similar structural domains. Although the N-terminal part of the SOCS family of proteins varies from 50 to 400 amino acids in length, the C-terminus is well conserved, containing an SH2 domain followed by a SOCS box (Fig. 1). Among the SOCS members, SOCS1 and SOCS3 possess a unique KIR motif. All individual domains hold a distinct functional role. The SH2 domain functions as phosphotyrosine binding domain, the KIR domain acts as an inhibitor of kinase activity while the SOCS box facilitates association with Cullin 5/2 for the formation of an E3 ubiquitin ligase complex. Thus, the current consensus is that SOCS proteins act as substrate recognition subunits of Cullin-Ring E3 ligases thereby targeting tyrosine phosphorylated proteins for ubiquitination. Other modes of action have also been proposed. The KIR motif can inhibit kinase activity by impairing access to substrates, while the SH2 domain can competitively inhibit the binding of other proteins to phosphotyrosine residues. The interactions between RTKs and SOCS have been shown to be selective, occurring only with the ligand-stimulated wild-type receptors or with constitutively active mutant receptors [16-18].

2. SOCS proteins in RTK regulation

SOCS proteins have been extensively studied in regulation of cytokine and growth hormone receptor signaling. Recent studies disclosed a potential role in regulation of RTK signaling. Similar to cytokine receptors, activation of a number of RTKs induces SOCS mRNA expression (Table 1). Furthermore, expression of SOCS proteins can counteract RTK signaling. For example, SOCS1 deficient mice are viable with significantly lowered levels of blood sugar [19] and display a comparatively high sensitivity towards insulin [20]. Moreover, SOCS1 blocks insulin-induced insulin phosphorylation of insulin receptor substrate 1 (IRS1) [19]. These observations suggest that SOCS1 negatively regulates insulin receptor (INSR) signaling. SOCS proteins display a degree of specificity for the different receptors. It has been shown that KIT associates with SOCS6 and weakly interacts with SOCS4 and SOCS5 but not with SOCS2 and SOCS3 [18]. Thus, SOCS family proteins appear to be important components that regulate RTK signaling (Table 2).

The mechanisms by which RTK activation induces SOCS expression mostly overlaps with the cytokine induction pathway. Many RTKs are capable of promoting tyrosine phosphorylation of STAT proteins. Tyrosine phosphorylation of STAT proteins lead to dimerization and nuclear translocation of these proteins which in turn activates transcription of various genes including SOCS [16]. It is increasingly clear that additional mechanisms also contribute to the regulation of SOCS genes expression, some of which are known to be directly or indirectly influenced by RTK activation. For example, it was recently demonstrated that SOCS3 gene promoter contains AP-1 and SP1/SP3 transcription factors binding sites that mediate ERK-dependent activation of SOCS3 [21]. Given the ability of SOCS proteins to modulate RTK signaling, transcriptional regulation of SOCS genes can provide a means of cross-talk between RTKs and other signaling pathways. It was recently demonstrated that SOCS2 expression is induced by androgens in prostate cancer cells which in turns down-regulates Src tyrosine kinase, a key mediator of RTK signaling [22]. Epigenetic silencing by DNA methylation is another layer of regulatory control of SOCS gene expression that may be of relevance for their biological actions. Indeed, SOCS1 and SOCS3 genes have been shown to be methylated in several tumor types [23, 24]; although the consequences of these events for growth factor signaling in these tumors are not well understood. Clearly, a better understanding of the transcriptional and posttranslational mechanisms regulating SOCS expression in normal and disease tissue is needed in order to better understand the interplay with RTKs.

3. SOCS1

SOCS1 was initially identified as a JAK-binding protein in a yeast two-hybrid assay and was referred to as JAB [12]. Later it was found to associate with multiple RTKs including stem cell factor (SCF) receptor (KIT), FMS-like tyrosine kinase 3 (FLT3), platelet-derived growth factor (PDGF) receptor (PDGFR) and colony stimulating factor 1 (CSF1) receptor (CSF1R) in yeast two-hybrid assays [25]. The interaction in between SOCS1 and RTKs is mediated through SH2 domain of SOCS1 and is dependent on the presence of phosphorylated key tyrosine residues on the receptor. Thus, the interaction is dependent on activation of the tyrosine kinase activity of the receptor.

One hour of SCF stimulation induces SOCS1 expression in KIT expressing Ba/F3 cells and in bone marrow derived mast cells [25], suggesting that SOCS1 is an early response gene and that KIT activation leads to transcriptional activation of the SOCS1 gene. SOCS1 mRNA and protein levels were down-regulated in an acute myeloid leukemia (AML) cell lines expressing oncogenic FLT3 upon treatment with sorafenib, a multi-kinase inhibitor that inhibits the oncogenic mutant FLT3-ITD [26]. Thus it is suggested that, like in the case of KIT, FLT3 activation also lead to transcriptional activation of SOCS1. The resulting expression of the SOCS1 protein then negatively regulates KIT and FLT3-induced

mitogenic signaling in hematopoietic cells [25]. Therefore, it is apparent that activation of RTKs leads to transcriptional activation of SOCS1 which then counteracts RTK signaling. Since SOCS1 associates with RTKs through its SH2 domain, the SOCS box is still available for recruiting ubiquitin machinery and then transfer ubiquitin moieties to the receptor (Fig. 2). Thus, it is suggested that ubiquitination of receptor is promoted by SOCS1 which in turn negatively regulate mitogenic signaling of receptors.

Although it is likely that SOCS1 regulates receptor signaling by promoting degradation of the receptor, it has become increasingly clear that alternative mechanisms exist. SOCS1 was found to be directly associated with activated KIT but its inhibitory role in KIT signaling was independent of this interaction in Ba/F3 cells [27] indicating that an alternative mechanism is involved in regulation of receptor signaling by SOCS1. One possibility is that SOCS1 limits the availability of downstream signaling proteins either through ubiquitin-mediated degradation or by competing for the same binding site in the receptor. For example, SOCS1 regulates insulin signaling by ubiquitin-mediated degradation of the INSR substrates IRS1 and IRS2 [28], while it can associate with INSR in response to insulin [29]. Furthermore, SOCS1 binding is not dependent on INSR-pY960, a site required for both IRS1 and IRS2 interaction but overlaps with the alternative IRS2 binding site in the catalytic loop [29].

Interaction of SOCS1 with CSF1R is dependent on CSF1 stimulation. This interaction abrogates CSF1-induced cell proliferation of hematopoietic cells lines FDCP1 and EML [30]. The interaction sites of the SOCS1 SH2 in the CSF1R have been mapped to phosphorylated Y697 (mouse) and pY721 (mouse) residues in CSF1R [30]. Mutation on these two sites had no effect on the kinase activity of the CSF1R or CSF1-induced proliferation and differentiation of macrophages [31] suggesting that SOCS1 regulation of CSF1R maybe cell -specific. SOCS1 is also known to interacts with activated MET [32] and AXL [33] and to block hepatocyte growth factor-induced MET, GAB1 and ERK1/2 phosphorylation [32]. SOCS1 has been shown in a yeast two-hybrid assay to associate with insulin-like growth factor 1 (IGF1) receptor (IGF1R) [34] but its role in IGF1 signaling has not yet been defined.

In contrast to the previously explained findings, SOCS1 constitutively associated with the FGF receptor FGFR3. Constitutive association of SOCS1 has also been reported for the EGFR, where association is mediated through the cytoplasmic domain of receptor [35]. However, the precise mechanisms behind these interactions remain unknown. Interestingly, SOCS1 can block both fibroblast growth factor (FGF)- and EGF-induced STAT phosphorylation. Thus, it is suggested that SOCS1 association with FGFR3 and EGFR has similar regulatory functions as other RTKs. On the other hand, SOCS1 cooperated with FGFR3 to promote FGF induced MAPK phosphorylation through a still undefined mechanism [36]. A possible explanation is that SOCS proteins may not only act as ubiquitin ligases but also as adapters. Indeed, SOCS1 can recruits signaling proteins such as Grb2 and NCK to the KIT through its N-terminal proline-rich region [25].

Although SOCS1 overexpression negatively regulates wild-type KIT and FLT3 signaling, ectopic expression of SOCS1 in FLT3-ITD-expressing 32D cells did neither affect FLT3-ITD mediated phosphorylation of AKT, ERK1/2, STAT5 nor cell proliferation [26, 37]. SOCS1 blocked IL3-induced cell proliferation in 32D cells lacking Flt3-ITD expression. Furthermore, expression of SOCS1 along with FLT3-ITD confers resistance to IFN-α and IFN-γ induced cell death in primary murine bone marrow cells. While SOCS1 plays a role in control of multiple RTKs including KIT, FLT3 and MET [25, 38], it is unclear how oncogenic FLT3-ITD sustains SOCS1 regulation. Recently it was shown that tyrosine phosphorylation of the adaptor protein SLAP by an oncogenic mutant of KIT, D816V, impairs its ability to recruit the ubiquitin E3 ligase CBL and thereby reduces ubiquitination. This leads to sustained oncogenic signaling from KIT [39]. Thus it is possible that FLT3-ITD in a similar fashion phosphorylates SOCS1 on tyrosine residues and thereby inactivates it. However, this remains to be shown.

Thus, SOCS1 is involved in complex regulation of signaling downstream of the receptors. While receptor activation in general leads to transcriptional activation of SOCS1, expression of SOCS1 in many cases negatively regulates receptor signaling, suggesting a mechanism of feedback inhibition. Thus, it is in a way contradictory that oncogenic mutants of RTKs up-regulate SOCS proteins, since that would counteract their oncogenic potential. It might be that the negative feedback does not shut off the signal from the RTKs but rather modulates the signal to a reasonable level. It is well known that over-activity of RTKs under certain condition can lead to induction of apoptosis or senescence, rather than a proliferative signal [40, 41].

4. SOCS2

SOCS2 plays important roles in growth control. SOCS2 deficient mice display about 50% increased body size primarily due to the loss of negative regulation of growth hormone (GH) receptor function [42]. SOCS2 negatively regulates the GH receptor through ubiquitination-dependent degradation [43]. SOCS2 was also found to interact with IGF1R and INSR, and this interaction occurred only when the receptor was activated [34, 44] suggesting that similar to SOCS1 the interaction between receptor and SOCS2 is mediated through receptor phosphotyrosine residues and the SOCS2 SH2 domain. Despite this, there is no evidence that SOCS2 can regulate the activity of the insulin or the IGF1 receptor.

SOCS2 has been reported to associate with both wild-type and oncogenic FLT3 but not with KIT [16, 18]. The interaction between FLT3 and SOCS2 is mediated through phospho-tyrosine residues Y589 and Y919 in FLT3 and the SOCS2 SH2 domain. Unlike the IGF1R, FLT3 mediates tyrosine phosphorylation of SOCS2 [16, 34]. Thus, the SOCS2 SH2 domain displays a higher degree of selectivity in its interaction and is phosphorylated only by selected receptor. It is also likely that SOCS2 remains constitutively serine phosphorylated and that FLT3 slightly increases SOCS2 serine phosphorylation [44]. Since FLT3 has the ability to indirectly activate serine/threonine kinases [45] it is possible that FLT3 activation leads activation of a signaling cascade that in turn regulate SOCS2 serine phosphorylation.

Although mono-ubiquitination of RTKs lead to degradation in lysosomes [46], association of SOCS2 with FLT3 led to increased ubiquitination of FLT3, which was followed by degradation in the proteasomes. As SOCS2 directs FLT3 for proteasomal degradation it is expected that it will negatively regulate receptor signaling. However, it has been shown that SOCS2 expression negatively regulates wild-type FLT3 induced ERK1/2 phosphorylation but not AKT phosphorylation in Ba/F3 cells [16] suggesting a different mechanism of suppression of FLT3 signaling. Besides ERK1/2 phosphorylation, it partially inhibited oncogenic FLT3-ITD-induced STAT5 phosphorylation, cell proliferation as well as colony formation [16]. FLT3-ITD induces transcriptional activation of SOCS2 in transfected hematopoietic cells and AML cell lines expressing FLT3-ITD display elevated SOCS2 expression [16, 26] suggesting that FLT3 activation induces SOCS2 expression and then SOCS2 act as a negative regulator.

SOCS2 expression has been found to be inversely correlated with that of EGFR in breast cancer [47] probably due to the fact that elevated endogenous SOCS2 expression leads to accelerated EGFR degradation, while loss of SOCS2 expression stabilizes the EGFR. Furthermore, SOCS2 associates with tyrosine phosphorylated EGFR and probably competes with SRC for binding to the same binding site, pY845, in the EGFR and inhibits EGF-induced STAT5 phosphorylation [48]. Thus SOCS2 might regulate EGFR signaling by two parallel mechanisms, both by destabilizing EGFR and by competing with SRC for binding to the receptor.

To summarize, SOCS2 associates with FLT3, IGF1R and EGFR receptors upon ligand stimulation. SOCS2 expression is up-regulated by receptor activation and association of SOCS2 with the

receptor directs the receptor for degradation. Furthermore SOCS2 negatively regulates selective pathways downstream to RTKs.

5. SOCS3

The role of SOCS3 in cytokine and growth hormone signaling has been extensively investigated. Although SOCS3 knockout mice die in utero due to placental defects, conditional SOCS3 depletion induces inflammatory and metabolic disorder [49-51]. Thus, SOCS3 plays a role in cytokine as well as insulin signaling. SOCS3 negatively regulates insulin signaling by associating through pY960 in the INSR [52]. This effect is extended by suppression of insulin-induced IRS1 tyrosine phosphorylation and interaction with p85 [53]. It has been shown that SOCS3 directly interacts with the cytoplasmic domains of the activated IGF1R and INSR and that both insulin and IGF1-stimulation induces SOCS3 tyrosine phosphorylation [54-56]. Thus, unlike SOCS2, SOCS3 is a substrate of the IGF1R and the INSR. Insulin induces tyrosine phosphorylation of SOCS3 on Y204 but the role of this event is not yet known [56]. Since the pY960 is the major binding site for IRS1 and IRS2 in the INSR [29], it is possible that SOCS3 competes with INSR substrates resulting in reduced phosphorylation of IRS1 or IRS2 [53]. SOCS3 also binds to IRS1 and IRS2, and abrogates insulin signaling by ubiquitin-mediated degradation of these two substrate proteins [28]. Therefore SOCS3 inhibits INSR signaling by two different mechanisms: by destabilization of IRS1 and IRS2 and by abrogation binding of IRS proteins to the receptor.

Apart from its regulatory role in insulin signaling, transcriptional activation of SOCS3 was also elevated in response to multiple growth factors including EGF, FGF and PDGF [36, 57, 58]. Unlike what was the case with the INSR, SOCS3 constitutively binds to the EGFR cytoplasmic domain [35]. This binding results in negative regulation of EGF-induced STAT1 and STAT3 phosphorylation in 293 cells. Similar to INSR, EGFR induces tyrosine phosphorylation of SOCS3 [58]. Another RTK, FGFR3 was found to be constitutively associated with SOCS3 independent of FGF stimulation or the receptor's kinase activity [36, 57]. Thus it is most likely that SOCS3 associates with the RTKs and regulates downstream signaling by ubiquitination-dependent degradation of receptor. However, the role of SOCS3 in RTK ubiquitination and degradation has so far not been described. Another growth factor, PDGF induces SOCS3 tyrosine phosphorylation on residuesY202 and Y221 in NIH3T3 and recruits p120 RasGAP, thereby contributing to the activation of ERK kinases [58]. Overexpression of a SOSC3 mutant that lacks the two tyrosine residues inhibits PDGF-induced cell proliferation, while wild-type SOCS3 did not show any effect, suggesting that tyrosine phosphorylation by RTKs promotes an adaptor function for SOCS3 that may be of biological relevance.

6. SOCS4

There is limited evidence about regulation of RTKs by SOCS4. SOCS4 has mostly been studied in respect to the EGFR signaling. While EGF-treatment increases SOCS4 expression, the expression of SOCS4 significantly reduced EGFR expression in transfected CHO cells [59]. EGF induces transcriptional activation of SOCS4, probably through activation of STAT family proteins and then it markedly reduced EGF-induced STAT3 activation [59] suggesting that a negative feedback signaling is involved in receptor signaling. SOCS4 associates with the EGFR through pY1092 and regulates EGFR degradation but EGF stimulation did not induce SOCS4 tyrosine phosphorylation [59, 60]. Like other SOCS proteins, SOCS4 also act as an E3 ubiquitin ligase [60], and therefore it has been suggested that SOCS4 associates with EGFR through its SH2 domain and then induces ubiquitination of receptor followed by degradation. However so far we do not have any direct evidence to support this hypothesis. Although SOCS4 associates with EGFR through a specific phosphotyrosine residue, SOCS4-mediated EGFR degradation was independent of EGF stimulation, probably due to the constitutive activity of the

EGFR in the overexpression system used in the study. Besides regulation of EGFR signaling, SOCS4 weakly associates with KIT but its role in KIT signaling remains to be defined [18].

7. SOCS5

In Drosophila the SOCS5 ortholog SOCS36E negatively regulates the EGFR ortholog suggesting that SOCS5 plays a role in RTK signaling. EGF-stimulation increases SOCS5 expression and induces tyrosine phosphorylation of SOCS5 [59]. Expression of SOCS5 partially blocks EGF-induced STAT3 activation and a functional SH2 domain and SOCS-box are required for this regulation [59]. Additionally SOCS5 accelerates EGFR degradation [59, 61]. SOCS5-mediated EGFR degradation is dependent on both the SOCS box and the SH2 domain of SOCS5, but degradation is independent of EGF stimulation and occurs in a CBL-independent manner. These findings suggest that SOCS5 remains constitutively associated with EGFR and act as E3-ubiquitin ligase. In addition to regulating degradation of the EGFR, SOCS5 also accelerates degradation of ErbB2 and ErbB4 [59]. Furthermore, SOCS5 associates following growth factor stimulation with Shc1 at pY317 through its SH2 domain [62] suggesting that SOCS5 not only limits receptor stability but also induces degradation of downstream signaling proteins. SOCS5 also weakly associates with KIT but its role in KIT regulation has not been studied [18].

8. SOCS6

SOCS6 acts as a tumor suppressor in several malignancies. SOCS6 is frequently down-regulated in gastric cancer due to the allelic loss and promoter hyper-methylation. In line with this ectopic expression of SOCS6 reduces cell growth in AGS and AZ-521 cells [63]. Loss of SOCS6 expression is associated with poor prognosis in human lung cancer [64]. Although, mice lacking SOCS6 gene display mild growth retardation, no other abnormalities have been described [65]. SOCS6 is capable of associating with IRS2, IRS4 and p85 suggesting a role in insulin signaling like the other SOCS family proteins [65]. Insulin-stimulation induces SOCS6 expression both at the mRNA and protein levels, and thereby it associates with p85 without affecting the stability of p85 [66]. INSR has also been shown to associate with SOCS6 and this association blocks phosphorylation of AKT and ERK1/2 [67].

The pattern of SOCS6 expression overlaps with the expression of KIT and FLT3. Cells expressing these receptor induce SOCS6 mRNA expression in response to their respective ligands [17, 18] suggesting that KIT and FLT3 regulate SOCS6 expression. It has been shown that PMA, a potent activator of PKC, can stabilize SOCS6 proteins through activation of ERK1/2 [68]. Probably PKC activation leads to transcriptional up-regulation of SOCS6 mRNA through activation of the MAPK pathway. FLT3 and KIT activation can activate PKC through activation of PLD that releases phosphatidic acid, which in turn can be dephosphorylated to diacylglycerol, an activator of PKC [45]. Thus SOCS6 mRNA expression induced by KIT and FLT3 might occur through activation of PKC isoforms. Like other SOCS proteins, SOCS6 associates with KIT through phospho-tyrosine residue Y568 and an intact SOCS6 SH2 domain is required for this interaction. Binding of SOCS6 to the receptor increases receptor ubiquitination which, in turn, is followed by degradation of the receptor [69]. Thus, SOCS6 acts as an ubiquitin ligase, since mutation in SOCS box blocks receptor degradation, while it remains fully capable to associate with KIT.

SOCS6 has been shown to associate with KIT in a manner dependent on SCF-stimulation, which subsequently leads to ubiquitination and degradation of the receptor [18]. SOCS6 has also been found to associate with ligand-stimulated FLT3 as well as with oncogenic FLT3. This interaction increases FLT3 ubiquitination and degradation [17]. Association of SOCS6 with KIT and FLT3 apparently plays a role in control of receptor downstream signaling. SOCS6 selectively blocks ERK1/2 and p38 phosphorylation but not phosphorylation of AKT or STAT5β [17, 18]. Thus SOCS6 does not solely regulate KIT or FLT3

signaling by destabilizing receptor. It is possible that SOCS6 competes with other proteins for association with KIT or FLT3. The SOCS6 binding sites in KIT (pY568) and FLT3 (pY589 and pY591) are a well-known binding sites for SRC family proteins. CBL also associates with KIT or FLT3 through this residues. Thus, regulation of p38 and ERK1/2 phosphorylation by SOCS6 might be due to the interference with receptor signaling as well as interference with downstream signal transduction molecules.

SOCS6 has also been shown to be tyrosine phosphorylated in response to the SCF and FL-stimulation [17, 18]. However, so far no report has identified the phospho-tyrosine residues in SOCS6. According to mass spectroscopy data available at the website Phosphosite Plus, five tyrosine residues are likely to be phosphorylated in human SOCS6. Two of those sites, Y223 and Y443, are more likely to be the binding sites for the adaptor proteins Nck and CrkL which have the consensus binding sequence of pYXXP. It has been shown that tyrosine phosphorylation of SOCS3 on YXXP motif is required for interaction of Nck and CrkL [70]. Thus, regulation of KIT signaling by SOCS6 might not require physical interaction of receptor with SOCS6.

9. SOCS7

SOCS7 (also known as NAP4) was initially identified as a NCK interacting protein in a yeast two-hybrid assay [71]. Unlike other SOCS proteins SOCS7 contains four unique short proline-rich motifs in the uncharacterized N-terminal region and through these SOCS7 associate with SH3 domain-containing proteins such as Nck, Grb2, PLCγ and Vinexin [71, 72]. In the same study it was also shown that SOCS7 associates with tyrosine phosphorylated EGFR through its SH2 domain. Thus, SOCS7 acts as an adaptor between receptor and the signaling molecules. Furthermore, the SOCS7 SH2 domain binds to IRS2, IRS4 and p85 [65]. Insulin induces SOCS7 expression both on the mRNA as well as on the protein level which then associates with INSR and IRS1 [73]. SOCS7-deficient mice displayed altered glucose homeostasis, faster glucose metabolism and improved glucose tolerance [73] suggesting a function of SOCS7 in regulating insulin signaling. SOCS7 also negatively regulates IGF-I signaling [74].

10. CIS or CISH

FGF induces expression of CIS and this plays an inhibitory role in FGFR signaling [75]. Although expression of SOCS5 induces EGFR degradation and blocks STAT3 activation, CIS expression did not influence EGFR degradation, but it slightly potentiated EGF-induced STAT3 activation [59]. CIS constitutively associates with KIT but its role in KIT signaling is unknown. Since CIS also associates PKCα, PKCβ and PKCθ [76] which play a role in regulation of KIT signaling [45], it is likely that CIS can influence KIT signaling.

11. SOCS proteins in RTK regulated diseases

RTK signaling plays, through activation of multiple signaling cascades, essential roles in regulating cell proliferation, differentiation, maturation, apoptosis and metabolism. In normal cells these signaling pathways are tightly managed by regulatory proteins and mismanagement leads to initiation and development of cancers. SOCS family proteins play important roles in controlling of RTK signaling and thus loss of SOCS proteins may contribute to malignancies. Expression of SOCS family proteins has been reported to be deregulated in many cancers (Table 3). For example, higher SOCS 1, 3, 4 and 7 expression levels were associated with earlier tumor stage and better clinical outcome in breast cancer [77, 78]. The expression of SOCS1 is absence in normal skin or melanocytic nevi while up-regulated in melanoma and related to tumor invasion as well as stage of the disease indicating that SOCS1 has prognostic significance in melanoma [79]. In contrast, the expression of SOCS1 was down-regulated in pancreatic

cancer, acute myeloid leukemia, multiple myeloma, lymphoma and hepatocellular carcinoma [80, 81]. In the latter case, this seems to be explained by aberrant methylation of SOCS1 promoter [23]. It is also evident that SOCS1 is important for preventing chronic inflammation-mediated carcinogenesis. SOCS1 knockout mice develop colon cancer due to hyper activation of STAT1 [82]. A report with a small patient group (19 patients) of classical Hodgkin lymphoma described a loss of function deletion mutation in SOCS1 gene of eight patients that led to nuclear accumulation of activated STAT5 [83]. Inactivating SOCS1 mutation has also been reported in B-cell lymphoma [81]. In contrast, inactivation of SOCS3 specifically in macrophages promotes the expression of the MCP2/CCL8 chemokine and is linked to the reduction of tumor metastases in mice [84].

Expression of SOCS2 was found to be up-regulated in AML, glioblastoma and myeloma [16] but lost in hepatocellular carcinoma [85]. Loss of SOCS2 and SOCS6 expression in hepatocellular carcinoma correlated with aggressive tumor progression and poor prognosis [85], and similar effects are seen in the case of SOCS2 and prostate cancer or breast cancer [77, 86, 87]. Methylation-mediated down-regulation of SOCS3 has been shown to be related to the abnormal cell growth and migration in same disease, which is mediated through loss of regulation of JAK/STAT and FAK signaling [88]. Loss of SOCS3 function results in elevated STAT5 activation leading to higher metastasis in colorectal carcinoma patients [89]. SOCS3 expression is elevated in chronic myeloid leukemia (CML) and confers resistance to interferon α (IFNα) treatment [90] indicating that expression of SOCS3 is associated with disease progression.

Mutations in SOCS genes are a rare event. According to the Cosmic database (http://cancer.sanger.ac.uk/cancergenome/projects/cosmic/) several mutations are present in different SOCS genes (summarized in Fig. S1). However, there is little evidence for recurrent mutations of SOCS genes in cancer although the regions containing SOCS genes are often amplified or deleted in tumors. The clinical correlations are in most cases unknown. One report describes a loss of function mutation in SOCS3 (F136L) in a Japanese myeloproliferative disorder patient cohort [91]. The SOCS3-F136L mutation was found in 3 out of 127 patients and 2 out of 160 healthy donors. In addition to the mutation in the SOCS3 gene, hypermethylation of the SOCS1, SOCS2 and SOCS3 genes or deletion of the SOCS2 gene was reported in patients with myeloproliferative disorder [92-95].

Activation of RTKs induces SOCS expression (Table 1) which may negatively regulate mitogenic signaling. However, recent findings suggest that RTKs can bypass this negative regulation. For instance, SOCS1 cooperates with oncogenic FLT3 in development of hematopoietic malignancies by other means than degrading FLT3 [26]. Tyrosine phosphorylation of SOCS3 on Y204 and Y221 residues blocks interaction with Elongin C and directs SOCS3 to degradation. Thus, this suggests a mechanism whereby tyrosine kinases can escape negative feedback control from SOCS3 by phosphorylation on specific tyrosine residues [96].

It is clear from these data that SOCS family proteins may play dual roles in cancer, either promoting or inhibiting growth, depending on tumor type. While these proteins generally controls receptor signaling by destabilizing receptor or its substrate proteins, they can also cooperate with receptors in mediating mitogenic signaling. In some cases receptors can escape negative regulation by phosphorylation on specific tyrosine residues as demonstrated in the case of SOCS3 [96].

Since SOCS proteins play a role in INSR signaling, it is likely that this family of proteins might play a regulatory role in type II diabetes. INSR signaling can be disrupted by multiple SOCS proteins (discussed above). Overexpression of SOCS1 or SOCS3 in mice results in systemic insulin resistance in mice through the negative regulation of IRS phosphorylation [29, 97, 98]. Furthermore, SOCS1 or SOCS3 over expression significantly increased plasma insulin levels and perturbed the glucose-lowering

effect in insulin tolerance tests. Interestingly, a polymorphism in the non-coding 5' region of the SOCS2 gene is associated with type II diabetes in a Japanese patient population [99]. Whether these effects are related with defects in insulin receptor signaling or insulin secretion remain to be established.

Both FLT3 and KIT receptors are involved in rheumatoid arthritis, [100-102] and expression of several SOCS proteins has been reported to be deregulated in this disease [103, 104], suggesting a possible role of SOCS proteins in RTK–mediated arthritis. Up-regulation of CIS and SOCS can be connected to rheumatoid arthritis [103, 104].

12. Conclusions

Although SOCS proteins have been extensively studied in respect to the cytokine signaling, accumulating data also suggest an important role of SOCS proteins in RTK signaling (Table 2). In general SOCS proteins interact with activated RTKs. This association is mediated through the SH2 domain of SOCS proteins and phosphotyrosine residues of activated receptors. Similar to other SH2 domains, the SH2 domains of the SOCS family display sequence preference at +1 and +3 positions C-terminal to the phospho-tyrosine. In many cases SOCS show propensity to bind sequences containing pY-Hydrophobic-Hydrophobic-Hydrophobic residues, but many exceptions have also been observed (Table 4). SOCS3 shows preference to the motifs pY-(SAVYF)-Hydrophobic-(V/I/L)-Hydrophobic-(H/V/I/Y) [105]. SOCS6 and SOCS7 display preference to phospho-peptides containing pY-V- Hydrophobic-Hydrophobic [65]. SOCS2 has higher affinity for the motifs pY-(I/L/V)-(I/L)-(I/V)-I and SOCS4 has higher affinity for the motifs pY-(I/L/V)-X-(I/V/S/T/G)-X [60]. Signaling through RTKs is tightly regulated by interacting proteins and very often SH2-domain containing proteins are involved in this regulation [4-7, 106-108]. Since SH2 domain of different SOCS proteins displays specificity to the different pY consensus sequence it is expected that SOCS proteins display a degree of selectively in regulation of RTK signaling. Therefore, depending on binding sites SOCS proteins might play different roles, which are probably predicted by secondary SOCS interacting proteins or other competing RTK binding partners.

In some cases SOCS proteins can recruit signaling proteins to the receptor by acting as an adaptor. While the N-terminal part recruits signaling proteins in some cases, the C-terminal SOCS-box has the ability of associating with Elongin B/C and Cullins 2/5 to assemble E3-ubiquitin ligases. Therefore association of SOCS protein to RTKs can be regulated at different levels. In several cases SOCS proteins mediate ubiquitination of the receptor or associated proteins and many cases ubiquitination is followed by degradation. Thus it is often the case that SOCS proteins negatively regulate receptor signaling (Fig. 3), but other outcomes are also possible. For example, mono-ubiquitination can lead to receptor internalization, while Lys63 linked poly-ubiquitination can serve to assemble multi-protein complexes. Therefore, defining the nature of the ubiquitination events promoted by RTK bound SOCS would be essential to better understand their functional role in the regulation of growth factor signaling. Importantly, additional ubiquitin ligases such as CBL or F-box culling ring ligases are also known to bind and modulate RTK activity and so far little is known on how the activity of these different E3 ligases are coordinated in time and space and whether they have independent, redundant, antagonistic or cooperative functions in RTK signaling. Thus, our knowledge of the function of SOCS proteins in RTK regulation is in its infancy and further research is needed to understand the mechanism of action of these important physiological regulators.

The ability of SOCS proteins to associate with multiple RTKs, which are very often mutated or overexpressed in multiple cancer and also associated with various common diseases, opens up a possibility to use this family of proteins as a therapeutic agent. Since, in most of cases SOCS proteins play an inhibitory role in receptor downstream signaling, in theory overexpression or stabilization of

selective SOCS proteins could be beneficial in diseases driven by RTK activation. One can also envision that measurements of SOCS protein levels could serve as biomarker for selection of therapies aiming at inhibition of specific RTKs. Therefore, a better understanding of the roles of SOCS proteins on RTK signaling can certainly be beneficial for researchers and clinicians in the development of effective drugs against many aggressive diseases.

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Figure legends:

Fig 1. SOCS family proteins: SOCS family is consist of eight SH2 and SOCS box domains containing proteins. SOCS4 - SOCS7 proteins have comparatively a longer N-terminal uncharacterized region. SOCS4 and SOCS5 possess a unique SOCS domain. Eight SOCS proteins are further subdivided into four group.

Fig 2. RTK regulation by SOCS family proteins: SH2 domain of SOCS family can interact with phospho-tyrosine residue of activated receptor. Then SOCS proteins recruit Elongin B, Elongin C, Rbx1 and Cullin complex and transfer ubiquitin to the receptor resulting in receptor degradation.

Fig 3. SOCS proteins in regulation of RTKs: SOCS family proteins associates with RTKs in response to respective ligand stimulation. This association is mediated through SH2 domain of SOCS and phosphotyrosine residues of receptor. Activation of receptors lead to transcriptional activation of SOCS and which in turn then negatively regulates receptor signaling by SOCS-box mediated ubiquitination and degradation of receptor. SOCS proteins also have ability of recruiting signaling proteins through N-terminal proline rich region. This interaction probably accelerates receptor signaling.

Table 1: Up-regulation of SOCS expression by RTKs:

RTK	**Ligand**	**SOCS**	**Reference**
FGFR3	FGF	SOCS1	[36, 57]
		CIS	[75]
		SOCS3	[36, 57]
KIT	SCF	SOCS1	[25]
		SOCS6	[18]
FLT3	FLT3-ligand (FL)	SOCS1	[26]
		SOCS6	[17]
		SOCS2	[16, 26]
INSR	Insulin	SOCS6	[66]
		SOCS7	[73]
EGFR	EGF	SOCS3	[58]
		SOCS4	[59]
		SOCS5	[59]
PDGFR	PDGF	SOCS3	[58]

Table 2: Regulation of RTKs by SOCS proteins:

SOCS	RTKs	Ligand dependent	Regulation	Reference
SOCS1	KIT	Yes	Negative regulation of mitogenic signaling	[25]
	FLT3	Yes	Negative regulation of mitogenic signaling	[25]
	PDGFR	Yes		[25]
	CSF1R	Yes	Negatively regulates cell proliferation	[25] [30]
	INSR	Yes	Down-regulation of INSR substrate IRS1 and IRS2	[28] [29]
	EGFR	No	Blocks EGF induced STAT1 and STAT3 activation	[35]
	FGFR3	No	Blocks FGF induced STAT1 activation but accelerates MAPK activation	[36]
	IGF1R	Yes		[34]
	AXL			[33]
	MET	Yes	Blocks HGF-induced MET, GAB1 and ERK1/2 phosphorylation	[32]
SOCS2	IGF1R	Yes		[34]
	FLT3	Yes	Negatively regulates FL-induced ERK1/2 signaling and FLT3-ITD induced STAT5 phosphorylation, colony formation and cell proliferation	[16]
	EGFR	Yes	Blocks EGF-induced STAT5 phosphorylation	[48]
	INSR			[44]
SOCS3	INSR	Yes	Negatively regulates INSR signaling by down-regulating INSR substrate IRS1 and IRS2	[52] [28] [29]
	IGF1R	Yes		[54, 55]
	EGFR	No	Blocks EGF-induced STAT1 and STAT3 phosphorylation	[35]
	FGFR3	No		[36, 57]
	PDGFR?		No effect	[58]
SOCS4	EGFR		Reduces EGFR expression	[59, 60]
	KIT			[18]
SOCS5	EGFR		Negatively regulates EGFR signaling by accelerating receptor degradation	[59, 61]
	KIT			[18]
SOCS6	INSR	Yes	Blocks AKT and ERK1/2 phosphorylation	[67]
	FLT3	Yes	Negative regulates ERK1/2 and p38 activation as well as cell proliferation	[17]
	KIT	Yes	Negative regulates ERK1/2 and p38 activation	[18]
SOCS7	EGFR	Yes		[60]
	INSR	Yes	Negatively regulates insulin signaling	[73]
	IGF1R?	Yes	Negatively regulates IGF1 signaling	[74]
CIS	EGFR		Negatively regulates EGF-induced STAT3 activation	[59]
	KIT			[18]

Table 3: SOCS expression in cancer:

SOCS	Cancer type	Expression	Role	Reference
SOCS1	Hepatocellular carcinoma	Down-regulation		[23]
	Breast cancer	Up-regulation		[77]
	Breast cancer	Up-regulation	Higher disease free survival and overall survival	[78]
	Prostate cancer	Down-regulated in Patients after Androgen Ablation Therapy and up-regulated in Recurrent Cancer		[109]
	Melanoma	Up-regulation	Related to disease progression	[79]
SOCS2	Hepatocellular carcinoma	Down regulation	Related to aggressive tumor progression	[85]
	Breast cancer	Up-regulation		[77]
	Prostate cancer	Up-regulation		[86, 87]
SOCS3	Hepatocellular carcinoma	Down-regulation	Promotes cell growth and migration	[88]
	Breast cancer	Up-regulation	Higher disease free survival and overall survival	[78]
	Breast cancer	Up-regulation		[77]
SOCS4	Breast cancer	Up-regulation		[78]
SOCS6	Hepatocellular carcinoma	Down-regulation	Related to aggressive tumor progression	[85]
	Lung squamous cell carcinoma	Down-regulation	Poor prognosis	[64]
	Prostate cancer	Down-regulation		[86]
SOCS7	Breast cancer	Up-regulation	Higher disease free survival and overall survival	[78]
CIS	Breast cancer	Up-regulation	Increase proliferation through ERK activation	[77]

Table 4: Known SOCS binding sites in RTKs:

SOCS	Receptor	Species	Ref	pY	+1	+2	+3	+4	+5
SOCS1	CSF1R	Mouse	[30]	697	K	N	I	H	L
				721	V	E	M	R	P
SOCS2	FLT3	Human	[16]	589	F	Y	V	D	F
				919	I	I	M	Q	S
SOCS3	INSR	Human	[52]	960	L	F	L	R	K
SOCS4	EGFR	Human	[60]	1092	I	N	Q	S	V
SOCS6	KIT	Human	[65]	568	V	Y	I	D	P
	FLT3	Human	[17]	589	F	Y	V	D	F
				591	V	D	F	R	E
				919	I	I	M	Q	S
Consensus					Hydro	Hydro	Hydro		

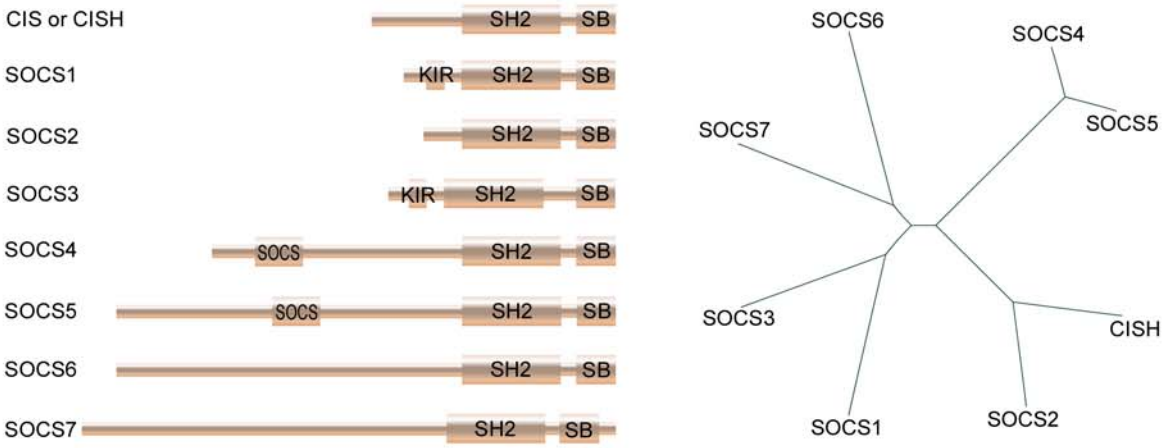
CIS or CISH
SH2
SB
SOCS1
KIR
SH2
SB
SOCS2
SH2
SB
SOCS3
KIR
SH2
SB
SOCS4
SOCS
SH2
SB
SOCS5
SOCS
SH2
SB
SOCS6
SH2
SB
SOCS7
SH2
SB
SOCS6
SOCS4
SOCS5
SOCS7
SOCS3
CISH
SOCS1
SOCS2

Figure 1

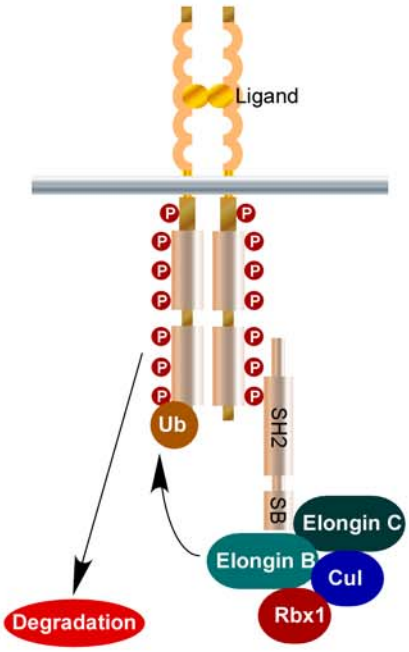

Figure 2

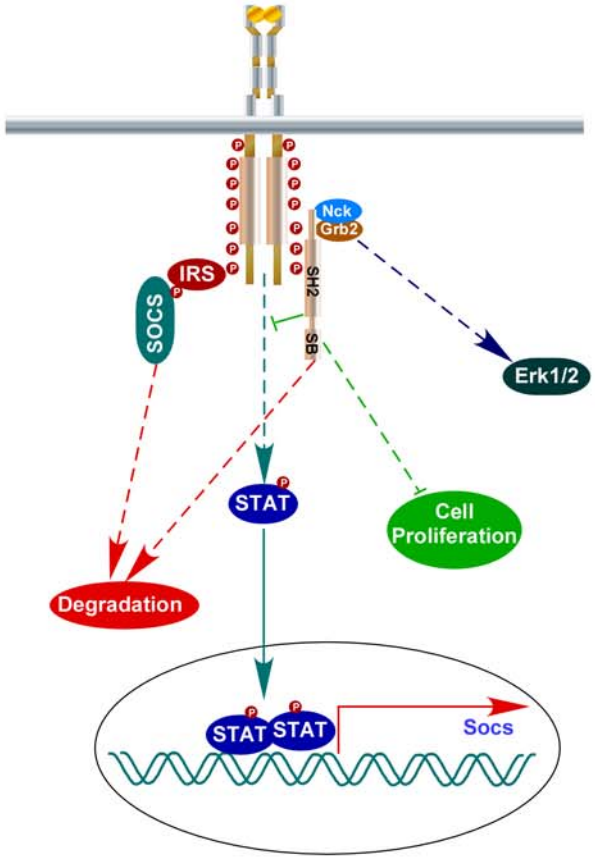
Nck
Grb2
IRS
SOCS
SH2
SB
Erk1/2
STAT
Cell
Proliferation
Degradation
STAT
STAT
Socs

Figure 3

SOCS proteins in regulation of receptor tyrosine kinase signaling

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Supplementary materials

Figure S1: Mutation in SOCS proteins according to the Cosmic database.

http://cancer.sanger.ac.uk/cancergenome/projects/cosmic/