

SOCS2: physiological and pathological functions

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1. ABSTRACT

Suppressors of cytokine signalling (SOCS) proteins are modulators of cytokine and growth factor signalling whose aberrant regulation has been linked to a variety of inflammatory and neoplastic diseases. SOCS proteins are able to act as substrate-recruiting component of E3-ubiquitin ligase complexes and target interacting proteins for degradation. At least some of the family members can also directly inhibit tyrosine kinases such as Janus Kinases (JAK). The most studied family members, CIS, SOCS1, SOCS2 and SOCS3 are important regulators of the JAK-STAT pathway. Here, we focus on SOCS2 and review its biological function as well as its implication in pathological processes. Furthermore, we take advantage of the known crystal structures of SOCS2 to discuss the potential effects of a selection of SOCS2 mutations that were identified in tumour tissues.

2. INTRODUCTION

Cytokines include a large family of glycoproteins that govern important biological processes such as proliferation, differentiation, immunity and haematopoiesis (1). These glycoproteins are important mediators of cell-cell communication. They are secreted by cells upon environmental stimuli in order to forward information to neighbouring cells bearing the appropriate receptor on their surface. The message from the cell surface is then rapidly transferred to the nucleus using different signalling cascades. The most important one in regard to cytokines is the Janus kinase and signal transducer and activator of the transcription (JAK-STAT) pathway. However, it seems obvious that cytokine actions have to be stringently controlled in both magnitude and duration. Indeed, aberrant cytokine signalling has been associated with many diseases, including several cancers,

disorders in haematopoiesis and autoimmune diseases. A number of key regulatory proteins, such as the protein inhibitors of activated STATs (PIAS), the Src-homology 2 (SH2) -containing protein tyrosine phosphatases (SHPs) and the protein family of suppressors of cytokine signalling (SOCS) control cytokine responses.

SOCS proteins, which emerged since their discovery in 1999 as the main regulator of cytokine signalling, are rapidly induced upon JAK/STAT signalling by activated signal transducer and activator of transcription factors (STATs) to negatively regulate cytokine signalling via a classical feedback loop (2). The SOCS family consists of 8 proteins, namely SOCS1-SOCS7 and cytokine-inducible SH2-containing protein (CIS). Each of these proteins has a central SH2 domain, an amino-terminal domain of variable length and sequence, and a carboxy-terminal 40 amino-acid region called SOCS box (2). The SOCSs can interact with a series of signalling intermediates through the binding of their SH2 domain to phosphorylated tyrosine residues, particularly those on cytokine receptors and JAKs, leading to the blockade of the signal (2). Indeed, due to the SOCS box they can act as ubiquitin ligases for associated proteins and target them for proteasomal degradation. Within the E3 ligase complex composed of Rbx1/2, cullin5, elonginBC and SOCS2, SOCS2 thus constitutes the substrate recognizing component (3–5). The function of each member of the SOCS proteins is somewhat difficult to analyse as these proteins may reciprocally regulate each other (6,7) due to their ubiquitin E3 ligase activity. Therefore their role in diverse functions has to be interpreted with caution. Here in this review we will focus on SOCS2 and its main involvement in biological processes as well as disease paradigms.

3. BIOLOGICAL FUNCTIONS OF SOCS2

SOCS2 is induced by diverse kinds of cytokines that activate STAT5. The most important ones include GH, PRL, EPO, GM-CSF, G-CSF, IL-1, IL-2, IL-3, IL-4, IL-6, IL-15, CNTF, IFN- α , IFN- γ , LIF and insulin (8–12). SOCS2 has been shown to be able to regulate several signalling pathways among them those induced by GH, PRL, LIF, IL-2, IL-3, IL-6, EGF and IGF-1 (6,10,11,13–15). However, as some of these findings rely on overexpression experiments, their physiological relevance is not entirely clear. SOCS2 is predominantly involved in the ubiquitination of target proteins, including receptors such as GHR and also diverse signalling proteins (16).

SOCS2 has mainly been associated with growth hormone (GH) signalling and is thereby involved in cell growth (9,17,18). Mice lacking the SOCS2 gene are indistinguishable from their littermates at birth. However, by three weeks they display accelerated growth and phenotypically resemble GH transgenic mice and humans with elevated levels of GH, resulting in adult mice that are 30 to 40 percent larger than wild-type mice (9,17). Their gigantism is related to uncontrolled responses to growth hormone that results in increased bone length (9,18,19). This phenotype is reversed when GH is genetically inactivated, demonstrating that SOCS2 is a key negative regulator of GH-induced overgrowth (18). Besides, SOCS2^{-/-} mice display an enlargement of internal organs that is also seen in IGF-1 transgenic mice. Altogether, these observations indicate an essential role for SOCS2 in controlling growth by GH and/or IGF-I. SOCS2 is primarily thought to inhibit STAT5 phosphorylation in response to GH signalling. Hepatocytes derived from SOCS2^{-/-} mice have prolonged STAT5a and STAT5b phosphorylation in response to GH and absence of STAT5 abrogated the overgrowth phenotype observed in SOCS2^{-/-} mice (19). The mechanism by which SOCS2 regulates STAT5 activation is still not completely elucidated. However, it appears to involve the competitive binding of SOCS2 to the STAT5 and SHP2 binding sites on the GH receptor (15,19,20).

Strikingly, depending on the expression level, SOCS2 can either act as an enhancer or suppressor of growth signalling (21,22). At low levels, SOCS2 inhibits several signalling cascades such as GH, prolactin and interleukins whereas at high levels, SOCS2 restores or even increases responsiveness to these growth factors (6,7,10,21). This dualistic effect, which is specific to SOCS2, is also reported *in vivo*: SOCS2 transgenic mice do not show any sign of apparent growth retardation but rather are larger than wild-type littermates (22). Thus, SOCS2 has opposite effects, which are likely to be dependent on its cellular concentrations. SOCS2 may bind to different tyrosine-phosphorylated residues with different affinities. Alternatively, the capacity of

SOCS2 to potentiate signalling may be due to its ability, at high concentrations, to compete with endogenous SOCS1 and/or SOCS3 consequently blocking their inhibitory effects. Indeed, SOCS2 can enhance GH, IL-2 and IL-3 signalling by accelerating degradation of other SOCS members (6,21). Along this line, acromegalic patients have increased risk of colonic polyps. It is thought that high levels of SOCS2 are responsible for SOCS1 degradation resulting in a reduced negative feedback loop on GH signalling, thus favouring a hyperplastic polyp phenotype (23). In contrast, using SOCS2^{-/-} mice, Kiu *et al* have shown that SOCS2 is not a physiological regulator of SOCS3 expression and action in primary haematopoietic cells (24). A recent study by Kazi and colleagues demonstrates that SOCS2 associates with activated receptor tyrosine kinase FLT3 through phosphotyrosine residues, thereby increasing FLT3 ubiquitination and degradation. This leads to a reduction in Erk1/2 and STAT5 activation and decreased FLT3-ITD-mediated cell proliferation (25). Further mechanistic studies need to be conducted in order to shed light on the exact mechanism of SOCS2-mediated regulation.

4. SOCS2 FUNCTION IN THE IMMUNE SYSTEM

Over the last years it has become increasingly evident that SOCS proteins have important roles in the maintenance of homeostasis and resolution of inflammatory processes. Indeed, SOCSs are likely to be involved in differentiation of cells of the innate and adaptive immunity thereby helping to shape the inflammatory response (26,27). An increasing number of recent studies suggest that SOCS proteins participate to pattern recognition receptor (PRR) signalling. In particular, SOCS2 is upregulated upon TLR activation and regulates IL-1 β and IL-10 (28). The same group shows in a more recent paper that SOCS2 is induced in monocyte-derived dendritic cells upon TLR8 and NOD signalling thereby controlling the release of pro-inflammatory mediators from DCs (29). Furthermore, TLR4 signalling in monocyte-derived DCs induces type I interferon, which in turn activates SOCS2 via STAT3 and STAT5 (30). Furthermore, human NK cell function is regulated by SOCS2 (31). Besides innate immunity, SOCS proteins are also key regulators of T cell differentiation. SOCS proteins are involved in balancing T helper cell polarization, which seems to rely on their capacity to regulate JAK and STAT activation (32–34). Indeed, SOCS1 and SOCS3 promote Th17 cell differentiation by inhibiting Th1 differentiation (35,36) whereas differentiation of Th2 cells is regulated by SOCS3 (36). SOCS2 was recently shown to play a major role in atopic Th2 cell-associated allergic immune responses by inhibiting the development of Th2 cells (33). Along this line, SOCS2^{-/-} mice show uncontrolled Th1 cell mediated responses to *Toxoplasma gondii* leading to mortality, suggesting increased proinflammatory responses to

infection (37). Accumulating evidence suggests that Foxp3⁺ Tregs do not always stably express Foxp3 and may repolarize to other lineages (38–40). Interestingly, SOCS2 is highly expressed in inducible Tregs leading to prevention of IL-4-induced Foxp3⁺ iTreg instability (41). Foxp3⁺ Treg cells are essential in establishing tolerance at mucosal surfaces and in regulating type 2 responses (42–44). As SOCS2 is on one side able to inhibit Th2 differentiation and on the other side to ensure iTreg stability, SOCS2 may be an ideal therapeutic target for Th2-biased diseases.

SOCS2 is involved in several inflammatory disorders. SOCS2 and CIS were shown to be downregulated in osteoarthritis whereas no change in SOCS1 and SOCS3 expression could be observed (45). It seems that the lack of SOCS2 expression may contribute to the disease as IL-1 beta and TNF alpha cytokines were increased along the progression of the disease. Systemic lupus erythematosus (SLE) as well as rheumatoid arthritis (RA) patients with an active form of the disease displayed similar SOCS2 levels in peripheral blood mononuclear cells (PBMCs) compared to normal individuals (46). In contrast, peripheral blood T cells displayed an increase in SOCS2 levels in RA patients (47). Interestingly a TNF-alpha blocking agent decreased SOCS2 transcript levels in RA patients suggesting that TNF alpha may play a role in the regulation of SOCS2 gene expression in PBMCs (46). SOCS2 and SOCS3 are also elevated in the skin of patients with psoriasis or allergic contact dermatitis (48). Although SOCS2 is ubiquitously expressed by human pancreatic islets (49,50), SOCS2^{-/-} mice do not exhibit alterations in glucose metabolism and auto-immune mediated cell death of pancreatic beta cells (51). SOCS2 has also been associated with type-2 diabetes (52). Along this line, constitutive expression of SOCS2 in beta cells leads to hyperglycemia and glucose-intolerance through an impaired insulin secretion (53) and could thus predispose to diabetes.

5. SOCS2 FUNCTION IN THE CNS

Some SOCS members have been shown to interact with the GTPase activating protein p120 RasGAP to enhance Ras activation (54) and the guanine nucleotide exchange factor Vav (55,56), raising the hypothesis that SOCS proteins could also play a role in the CNS (57). Among SOCS family members, SOCS2 is highly expressed in the CNS during development from embryonic day 14 to postnatal day 8 in the mouse (58). Results from genetic studies unravelled a role for SOCS2 in neuronal differentiation and neurite outgrowth. SOCS2^{-/-} mice display a 30 percent reduction in NeuN positive cells in the cortex and a slight decrease in interneurons whereas astrocytes were bigger in size compared to wild type mice (59). Overexpression of SOCS2 in SOCS2-transgenic mice seems to predominantly affect interneurons and neuronal connectivity in the cortex (60)

and leads to increased survival of neurons generated during adult hippocampal neurogenesis, which correlated with improved performance in a hippocampal-dependent cognitive task (61). Expression of SOCS2 was able to reverse the inhibitory effect that GH plays on the differentiation of neuronal progenitors into neurons by controlling expression of a neurogenic transcription factor, Neurogenin-1 (62). Goldschmit and colleagues could show that SOCS2 induces neurite outgrowth in PC12 cells (a model for neuronal-like differentiation) and cortical neurons via an EGF receptor/cSrc/STAT5 dependent pathway (63,64). As overexpression of SHP2 abrogates neurite outgrowth and EGFR phosphorylation, SOCS2 might mediate these effects by competing with SHP2 for binding EGFR and thereby blocking phosphatase activity (63). Furthermore, a recent study reports that SOCS2 is able to regulate the TrkA neurotrophin receptor leading to enhanced pAKT and pERK1/2 signalling which results in increased neurite outgrowth (65). SOCS2 is also involved in EPO-driven neuronal differentiation (66). Thus, in the CNS, SOCS2 is possibly not primarily regulating GH signalling. Along this line, a study by Kasagi *et al* showed that intravenous administration of recombinant human GH in rats or GH-stimulated neurons did increase SOCS3 and CIS mRNA levels whereas SOCS2 transcript level were unchanged in hypothalamic neurons (67). Furthermore, GH did neither change expression levels of SOCS2 (62) nor reverse the SOCS2-driven neurite outgrowth in neurospheres (68). It is not clear whether SOCS2 is involved in neuronal activity. Whereas neurons derived from neurospheres of SOCS2 transgenic mice appeared more complex, with increased number of neurites and outgrowth, basic electrophysiological analysis demonstrated that these neurons were of comparable immature neuronal phenotype as the wild-type neurons (68). Thus, although SOCS2 expression can regulate neuronal morphology, it appears to have little effect on neuronal ion channel expression and neuronal activity.

Importantly, only few reports have addressed the role of SOCS2 in the diseased CNS. One study proposes that cAMP increases cytokine-induced regenerative responses in the injured retina by suppressing SOCS-mediated negative feedback on cytokine signalling (69). However, the effect in that study seems to be driven by SOCS3. A recent report by Choi and colleagues shows that SOCS2 levels are upregulated in the rat hippocampus after brain ischemia (70). Co-labelling studies with glial and neuronal marker showed that SOCS2 was majorly present in glial cells as well as a small subset of immature neurons. Thus, the author's claim that SOCS2 seems to be involved in glial reactions and possibly may contribute to adult hippocampal neurogenesis after an ischemic insult (70). A recent study highlights SOCS2 as a potential target for schizophrenia (71). Further studies need to determine to which extent SOCS2 may be involved in neuronal

replacement after CNS injury. Using genetic mouse models in combination with diverse models of CNS damage may shed light on the involvement of SOCS2 in the regeneration process after CNS injury.

6. SOCS2 IN CANCER

Over the last years, SOCS proteins have emerged as potential tumour suppressor-like proteins (72,73). Among SOCS family members, SOCS1 and SOCS3 have been widely studied. SOCS1 and SOCS3 have been shown to suppress cell growth and their expression is frequently down-regulated in human cancers (74). Low SOCS2 gene expression has been associated with breast, pulmonary, hepatocellular and ovarian cancers (75–80). On the contrary, SOCS2 is described to be highly expressed in bone marrow cells from patients with chronic myeloid leukaemia (CML) and thus hypothesized to be involved in advanced stages of CML (81). However, a recent study by Hansen and colleagues demonstrates that development and progression of BCR/ABL1-induced CML as well as normal hematopoietic stem cell function is not dependent on SOCS2 (82).

Loss of SOCS2 in breast cancer is a crucial step towards deregulation of the cell cycle, resulting in a growth-promoting effect with an inverse correlation between SOCS2, cyclin A and Ki67 (76,79). The expression of SOCS2 decreases with higher tumour grade in breast cancer (83). Along this line, patients with high SOCS2 expression had an improved survival rate and high SOCS2 expression proved to be an independent predictor for good prognosis in breast cancer (79,83). Genetic variation on the JAK/STAT/SOCS signalling pathway has been associated with breast-cancer mortality (84).

In HCC SOCS2 down-regulation is significantly correlated to advanced TNM staging and appears to be a prognostic marker (77). SOCS2 might have a more complex role in prostate cancer. A low SOCS2 expression in primary prostate tissue is reported to be associated with an increased incidence of metastasis after radical prostatectomy and SOCS2 mRNA levels decrease during prostate cancer progression (85,86). Further studies show that SOCS2 expression is enhanced in tumour tissue compared with benign tissue (78,87). Zhu *et al* report that upregulation of SOCS2 correlates to lower Gleason Score (GS), absence of metastasis and longer disease-free survival time (78). In contrast, others report that SOCS2 positively correlates with GS and disease progression and those patients with high SOCS2 expression are more likely to experience tumour relapse (86,87). These conflicting results could either be explained by varying expression patterns within different ethnic groups that were analysed in those studies or alternatively could reflect the presence of diverse

subgroups of tumour cells expressing varying amounts of SOCS2. Furthermore, SOCS2 also seems to have a dual effect in prostate cancer as described in normal non-oncogenic signalling cascades. Hoefer *et al.* reported that SOCS2 acts as an accelerator whereas Iglesias-Gato *et al.* show an antagonistic effect of SOCS2 on oncogenic proliferative signals (86,87). Future studies need to clarify these opposite effects.

Limited data about the role of SOCSs in CRC prompted us to investigate their expression patterns as well as their clinical significance in CRC (88). By integrating different datasets covering more than 600 CRC patients as well as normal controls into one meta-analysis the power to detect biologically relevant signals specific for CRC is increased. Furthermore the biomarker identified is more likely to suit as a universal biomarker, discarding any possibility of specificity due to ethnic differences. This bioinformatic analysis, which was validated in a second patient cohort, identified SOCS2 as biomarker for CRC. Most importantly, SOCS2 has a prognostic value in early CRC (88). Along the same line, a gene signature including PIM1, CISH, ID1 and SOCS2 is able to identify patients with high JAK-pSTAT5 activity in hematologic malignancies which may benefit from treatments targeting JAK-STAT signalling (89). Importantly, it has been shown that the disruption of one allele of SOCS2 in GH transgenic mice leads to an increase in colon and jejunal crypt proliferation, thus favouring the formation of hyperplastic and lymphoid polyps in the colon (90). SOCS2 deletion in mice promotes the spontaneous development of intestinal tumours driven by mutations in the APC/beta-catenin pathway (91). Along this line, it has been shown that forced overexpression of SOCS2 inhibits proliferation of the Caco-2 colon cancer cell line (92). These findings provide evidence that SOCS2 normally limits tumour growth and strongly supports its tumour suppressive potential.

One possible mechanism that explains down-regulation of SOCS proteins in cancer is methylation in their gene promoter region. CpG islands of the SOCS2 gene were shown to be hypermethylated in endometrial cancer (93), 6.5 percent of glioblastoma patients, 14 percent of primary ovarian cancers (75), 43–63 percent of melanoma (94,95) and 25 percent of CRC patients (88). However, methylation of SOCS2 could be found neither in human breast cancer patients (75) nor in pulmonary adenocarcinoma, (80). Inconsistent results regarding SOCS2 methylation have been described in myeloproliferative neoplasms (MPNs) (96–98). A recent throughout analysis reports absence of SOCS2 methylation in MPN (99). Along this line, we and others have highlighted the care with which methylation studies should be performed (88,99). While most studies rely for their methylation analysis only on a low number of CpG sites it should strongly be recommended to perform whole promoter analysis as this provides much more

accurate results on the methylation pattern. Moreover, it is absolutely mandatory to analyse methylation in both the tumour and its normal counterpart tissue as methylation of both samples would on one hand indicate that methylation is tissue-specific and not cancer-specific and on the other hand that epigenetic regulation of the transcription is not mediated by methylation.

7. STRUCTURE-FUNCTION ASPECTS FOR SOCS2 SNPS REPORTED IN TUMOR TISSUES

For SOCS2, a couple of SNPs have been associated with disease states. One study by Qin *et al.* reported that 9 percent of patients with myeloproliferative neoplasms do have a T→C polymorphism in the exon 15 of the SOCS2 gene (100). Furthermore, an evolutionary-based haplotype analysis of haplotype-tagging SNPs followed by a “sliding window” haplotypic analysis indicated SNPs that mapped to the 5' region of the SOCS2 gene to be associated with type-2 diabetes with high statistical significance (50). Undoubtedly, SNPs within non-coding regions of the gene (such as the promotor region) may significantly affect the expression levels of SOCS2. Here, we will focus on SNPs that lead to missense mutations within the coding region and discuss their potential implications for SOCS2 function.

Although a number of SNPs can be found in the different databases, not much is known about their potential effects on SOCS2 structure and function. The solved structures of the SOCS2/elonginBC complex (101) and of the SOCS2/elonginBC/Cullin5 complex (102) have recently shed light on the protein interfaces that determine the interactions between these components of the SOCS2 E3-ligase complex (Figure 1A). In the E3 ligase complex, SOCS2 directly interacts with elonginC and Cullin5 with elonginC being the major interaction partner for SOCS2 (102). Another report by Bullock *et al.* (103) identified the consensus motif for the phosphotyrosine motif recognized by the SOCS2 SH2 domain ((VIL)-X-D-pY-(VIL)-(IL)-(VI)). These studies thus also provide a detailed view of the SH2 domain binding pockets and the residues involved in the recognition of phosphotyrosine motifs of substrates. Figure 1A shows the overall structure of the SOCS2/elonginBC/Cul5, including the important binding interfaces between SOCS2 and its direct interaction partners (elonginC and cullin5). The solved structures of SOCS2 also highlight the large interfaces between the SOCS2 extended SH2 domain (ESS), the SH2 domain and the SOCS-box, which are important for the orientation of the different domains (101-103). Figure 1B highlights the main SOCS2 residues which are involved in the mentioned interfaces. In addition it indicates the location of the SOCS2 SNPs within the sequence. These 21 SNPs, which have been identified in different tumour tissues (104) are also represented in Table 1. The table includes information on

the localization of the different SNPs within SOCS2, as well as their potential effects as deduced from the solved crystal structures of SOCS2. The potential effects for two of the mutations are impossible to predict as they are located in the N-terminal region which was not included in the crystal structures. For others their location in the structure (e.g. exposure at the protein surface) and/or the conservation of some of the physico-chemical properties of the mutated amino-acid make it difficult to reach clear conclusions concerning their impact. However, the potential effects of other SNPs can be predicted with much more confidence and we will discuss the 5 most important ones in more detail. Figure 2 highlights important predicted changes in contacts (Figure 2A) or surface potentials (Figure 2B) for the SNPs I72N, N94D, R96Q, C167R and P184L.

The mutated residue C167 is located on the longest of the three helices of the SOCS-Box (helix H1) and is part of the large hydrophobic interface which connects to the alpha4-helix of elonginC. The resulting four helix bundle is very similar to the one formed between the von Hippel-Lindau (VHL) tumor suppressor and elonginC, which are also forming an E3-Ligase complex further including elonginB, cullins and Rbx proteins (101,105). C167 is absolutely conserved among all SOCS proteins as well as in VHL, where it corresponds to C162 in helix H1 (105). Importantly, C162 constitutes a mutation hotspot in VHL as is it among the six most frequently mutated residues identified in the VHL syndrome (106). This disease is characterized by a genetic predisposition to develop tumours in various tissues and mutations impairing the tumour suppressor function of VHL seem to be causative for the disease (107). In the VHL/elonginC interface, C162 is one of the three most important residues for the interaction (105) and the SOCS2 structure reveals that C167 is similarly important for the interaction. Panel (a) of Figure 2A depicts the interactions of C167 with surrounding residues. Panel (b) illustrates that an arginine residue at this position would require much more space (besides introducing a positive charge into the hydrophobic interface) and thereby disrupt crucial interactions in the SOCS2/elonginC interface. Similarly to the C162 mutations in VHL, the C167R mutation in SOCS2 would thus certainly dramatically impair the assembly of the SOCS2 E3-ligase complex.

Proline 184 and arginine 186 of SOCS2 are the only residues which interact with cullin5 (102). Although this interaction is rather minor, it seems to synergistically contribute to the formation of the SOCS2/elonginBC/cullin5 complex. The feature which is determinant for the significant contribution of this interface to the overall binding affinity is an interaction between P184 of SOCS2 and W53 of cullin5 (Figure 2A-c) (102). In fact, this ring-to-ring stacking interaction seems to determine the specificity of SOCS2 towards cullin5 instead of cullin2. Most interestingly, it has been shown

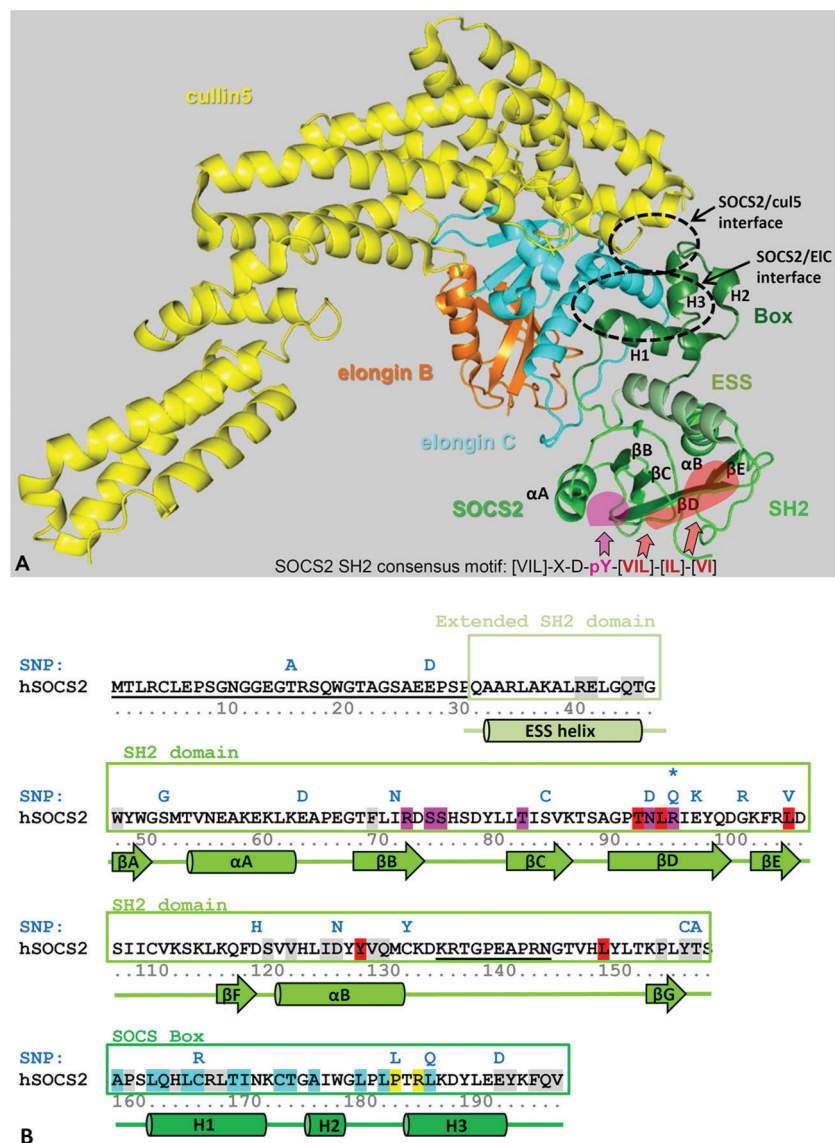


Figure 1. Important interfaces in the SOCS2/elonginBC/cullin5 complex. (A) Solved structure of the SOCS2/elonginBC/cullin5 structure highlighting the interfaces between SOCS2 and elonginBC and cullin5, respectively (dotted circles) (101-103). The binding sites for the different residues of pY-motifs recognized by the SOCS2 SH2 domain are also indicated. (B) Sequence of human SOCS2 highlighting the location of the investigated SNPs. The different SOCS2 domains are indicated by green squares and the secondary structure is given below the sequence. Residues which were not resolved in the crystal structure are underlined. SOCS2 amino acids involved in the different interactions are coloured as follows: blue: SOCS2/elonginBC interface; yellow: SOCS2/cullin5 interface; grey: interface between the SOCS2 box, ESS and SH2 domain; magenta: residues contacting the phosphotyrosine residue of bound pY-motifs; red: residues involved in the recognition of positions +1 to +3 of bound pY-motifs.

that mutation of the corresponding residue in the SOCS1 box (N197) to proline shifts the SOCS1 binding specificity from cullin2 to cullin5 (4). Furthermore, P184 of SOCS2 corresponds to a valine residue in VHL, which is known to interact with cullin2 instead of cullin5 (4,108). In light of this, it can be postulated that the SNP leading to the P184L mutation (Figure 2A-d) will most likely affect cullin5 binding and also impair the formation of the SOCS2/elonginBC/cullin5 complex. In fact it is possible that it will shift the specificity of SOCS2 from cullin5 to cullin2.

Figure 2B highlights the effects of mutations within the SH2 domain of SOCS2. The mutation I172N affects a residue which is located within the hydrophobic core of the SH2 domain and also contributes to the ESS/SH2 interface by contacting L43 within the ESS (Figure 2B-a). As I172 makes extensive van-der-Waals contacts its mutation will most likely affect the stability of the SH2 domain and may thus affect the SH2 domain function.

Two of the reported SNPs, N94D and R96Q, directly affect the recognition of phosphotyrosine motifs

Table 1. Localization and possible effects of the SOCS2 SNPs reported in the COSMIC database (104)

Mutation (CDS)	Mutation (amino acid)	Mutation ID (COSM)	Tumour tissue	Localisation	Function	Predicted potential effect of the mutation
c. 46A>G	p.T16A	COSM1562415	Large intestine	N-term.	Nd	/
c. 84G>T	p.E28D	COSM944525	Endometrium	N-term.	Nd	/
c. 154A>G	p.S52G	COSM95257	Lung	SH2 domain; AA-loop	Exposed	/
c. 192G>T	p.E64D	COSM1513314	Lung	SH2 domain; AB-loop	Exposed	/
c. 215T>A	p.I72N	COSM116078	Ovary	SH2 domain; beta C-strand	Participates in SH2 hydrophobic core	SH2 structural stability and function
c. 254C>G	p.S85C	COSM459338	Cervix	SH2 domain; beta B-strand	Contributes to hydrogen bond network within the pY binding pocket	Minor effect on SH2 pY recognition is possible
c. 280A>G	p.N94D	COSM4045432	Stomach	SH2 domain; beta C-strand	Contributes to hydrogen bond network of the pY binding pocket	modifies the positive charge of the SH2 pY binding pocket and strongly affects pY recognition
c. 286C>T	p.R96*	COSM1582843	Stomach	SH2 domain; beta C-strand	Central R residue responsible for pY recognition	Loss of SH2 and Box functions due to protein truncation
c. 287G>A	p.R96Q	COSM277418	Endometrium	SH2 domain; beta C-strand	Central R residue responsible for pY recognition	Loss of SH2 domain function
c. 287G>A	p.R96Q	COSM277420	Large intestine	SH2 domain; beta C-strand	Central R residue responsible for pY recognition	Loss of SH2 domain function
c. 292G>A	p.E98K	COSM944527	Endometrium	SH2 domain; beta C-strand	Exposed	/
c. 304G>A	p.G102R	COSM4045433	Stomach	SH2 domain; DE-loop	Exposed	Minor
c. 316T>G	p.L106V	COSM3764448	CNS	SH2 domain; beta E-strand	Hydrophobic pocket involved in SH2 substrate binding	Effect on SH2 substrate recognition
c. 358G>C	p.D120H	COSM695391	Lung	SH2 domain; FB-loop	Exposed	/
c. 379G>A	p.D127N	COSM277419	Large intestine	SH2 domain; alpha B-helix	Participates in SH2/box interface	Minor
c. 398G>A	p.C133Y	COSM944528	Endometrium	SH2 domain; BG-loop	Participates in SH2 hydrophobic core	May affect conformational mobility of the BG-loop and substrate recognition
c. 470A>G	p.Y157C	COSM3955308	Lung	SH2 domain	Participates in SH2/box interface	May affect SH2/box interdomain orientation and protein stability
c. 472A>G	p.T158A	COSM1513313	Lung	SH2 domain	Participates in SH2/box interface	Minor
c. 499T>C	p.C167R	COSM315490	Lung	SOCS box; H1-helix	Important residue in box/elonginC interface	Affects elonginC binding
c. 551C>T	p.P184L	COSM1706033	Skin	SOCS box; H2H3-loop	Crucial for box/Cul5 binding	Affects Cul5 and elonginC binding
c. 560T>A	p.L187Q	COSM695389	Lung	SOCS box; H3-helix	Participates in box/elonginC interface	Minor effect on elonginC binding
c. 579A>C	p.E193D	COSM168180	Large intestine	SOCS box; H3-helix	Exposed	/

by the SH2 domain. The mutated residues are located in the phosphotyrosine binding pocket (Figure 2B-c), which also contains the central arginine R73. The positively charged surface (coloured in blue in Figure 2B-c) of the

PY-binding pocket is crucial for the recognition of the negatively charged pY residue. Figure 2B-d illustrates that the N94D mutation dramatically affects the surface potential of the pY-binding pocket and creates a negative

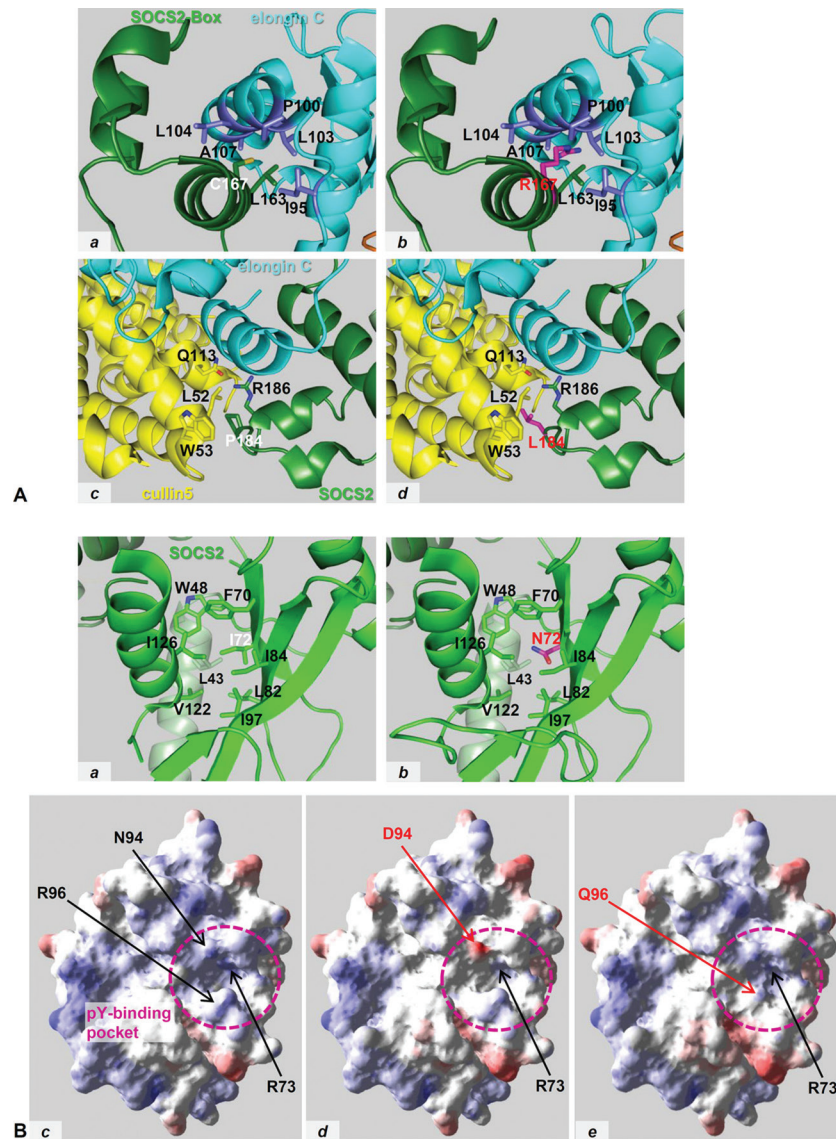


Figure 2. Location of the amino acids mutated in 5 selected SOCS2 SNPs. (A) SNPs in the SOCS2/elonginBC (panels a and b) and SOCS2/cullin5 interfaces (panels c and d). The non-mutated wild type residues (C167 and P184) are labelled in white and the side chains of important interacting residues in the wild-type structures are represented as rod models. Mutated residues (R167 and L184) are highlighted in red. (B) SNPs within the SH2 domain of SOCS2. Panels a and b: wild-type and mutant situation for the SNP corresponding to the I72N mutation. Panels c, d and e: electrostatic potential maps of the surface of the wild-type SH2 domain (panel c) as well as the mutants N94D (panel d) and R96Q (panel e). Red and blue regions indicate negative and positive charges, respectively.

charge, concomitantly reducing the effect of the positive charge of R73. This mutation will thus dramatically reduce the affinity of phosphotyrosine recognition by the SH2 domain and disrupt SH2 function. A similar effect can be predicted for the mutation R96Q. In many SH2 domains, phosphotyrosine binding is mediated via two basic residues, the central arginine residue and a second arginine of lysine residue flanking the pY-binding pocket. In SOCS2, this second residue is arginine 96 at the betaD6 position. Figure 2B (c vs. e) illustrates that the R96Q mutation reduces the positive charge in the

pY pocket and will most likely significantly impair the recognition of pY-motifs by the SH2 domain.

To conclude, the solved crystal structures of the SOCS2 containing complexes allow predicting the effects of reported SNPs and show that several of them can dramatically affect specific functions of the SOCS2 protein. Detailed structure-function studies of such SNPs would give a better understanding of the molecular mechanisms that lead to or support tumour development.

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