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# Suppressors of cytokine signalling-3 and -1 in human carcinogenesis

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## 1. ABSTRACT

The role of suppressors of cytokine signaling (SOCS)-3 and -1 has been investigated in various cancers. These proteins have been identified as endogenous controllers of activation of Janus kinase/signal transducers and activators of transcription factors pathway factors under physiological conditions and in disease. SOCS-3 expression is lost in several cancers due to epigenetic mechanisms, mostly promoter methylation. In liver, lung, and squamous head and neck cancer, and several hematological malignancies SOCS-3 acts as a classic tumor suppressor. In prostate cancer, SOCS-3 effects are cell type-dependent. It prevents apoptosis in androgen receptor-negative cells. However, in androgen-sensitive cells, it could act as a negative feedback factor for androgenic regulation. Melanoma cells which overexpress SOCS-3 confer a growth advantage. SOCS-1 is in most cancers a tumor suppressor which may inhibit expression of cyclin-dependent kinases and cyclins. In general, the mechanisms responsible for the different effects of SOCS in cancer cell lines have to be further investigated. The results discussed in the present review may have an impact on personalized approaches in cancer medicine.

## 2. INTRODUCTION

Inflammatory cytokines are implicated in the development and progression of various cancers. In many scientific publications, it was demonstrated that cytokines which are expressed either in tumor cells or in adjacent fibromuscular stroma have an eminent role in stimulation of proliferation, prevention of cell death, and facilitation of cellular events that lead to metastatogenesis. Both autocrine and paracrine cytokine loops have been described in human cancer. There is a strong evidence showing that interleukin-6 (IL-6) is up-regulated in cancer tissue and regulates apoptosis and angiogenesis (1). IL-6 is a pleiotropic cytokine which may either inhibit proliferation of cells or act as a survival factor. Various strategies for pharmacological anti-IL-6 intervention have been worked out. Most notably, researchers used the monoclonal anti-IL-6 antibody siltuximab (CNTO 328) in several experimental and clinical studies in myeloma and in prostate cancer (2). In addition to pharmacological agents which antagonize IL-6 effects, there is an interest in expression and function of endogenous suppressors of cytokine signaling in oncology. These proteins are negative feedback regulators of cytokine signaling which under normal conditions prevent a

**Table 1.** Overview of important functions of SOCS proteins in human cancers

| SOCS protein | Function | Cancer |
|---|---|---|
| SOCS-3 | Protection from apoptosis in androgen-insensitive cells | Prostate |
| SOCS-3 | Inhibition of androgen-regulated proliferation and secretion | Prostate |
| SOCS-3 | Growth-inhibitory response | Lung, liver, head and neck, lymphoma, glioblastoma multiforme |
| SOCS-3 | Inhibition of migration, bFGF antagonism | Liver, prostate |
| SOCS-3 | Enhancement of response to chemotherapy | Thyroid |
| SOCS-1 | Cell cycle | Prostate |
| SOCS-1 | Enhancement of histone deacetylase inhibitors | Colon |

permanent stimulation of the major cytokine signaling pathway, i.e. that of Janus kinases (JAK) and signal transducers and activator of transcription factors (STAT). Constant activation of STAT3, if not inhibited, could most probably cause a chronic inflammation in numerous tissues in which cytokine receptors are expressed.

In the present review, the focus will be on suppressors of cytokine signaling (SOCS), since they are the most frequently investigated controllers of cytokine function. Other negative regulators, such as protein inhibitors of activated STAT (PIAS), have been studied less frequently in experimental oncology. In addition, SOCS2, which is implicated in the regulation of the growth hormone/STAT5 pathway, may also have a role in modulation of cellular events in human cancers.

## 3. EPIGENETIC CHANGES IN THE PROMOTER OF THE SUPPRESSOR OF CYTOKINE SIGNALING-3 GENE IN HUMAN TUMORS

The members of the SOCS family (seven SOCS proteins and CIS) have a similar structure; they contain the N-terminal domain, the SH2 domain in the middle, and the conserved C-terminal SOCS Box domain (3,4). The SH2 domain may interact with JAK kinases or cytokine receptors thus inhibiting a cellular response to cytokines. The SOCS Box interacts with elongins and other proteins thus being responsible for proteosomal degradation of associated proteins. SOCS-1 and -3 have also a kinase inhibiting region. The promoter region of the human SOCS-3 gene is approximately 1.1 kbp and is responsive to growth factors and interferon gamma (5). In most studies in oncology, the role of SOCS-1 and -3 has been investigated. For understanding the specific role of SOCS proteins, it is important to note that in many malignant cells the presence of phosphorylated STAT3 is causatively related to malignant progression and tumor aggressiveness (6). Therapeutic attempts have been developed in order to reduce activation of this pathway in tumors in which STAT3 is an established oncogene.

Some of the most important functions of SOCS proteins in cancer are summarized in Table 1. SOCS-3 and -1 proteins were initially described as tumor suppressors and their function was elucidated in lung cancer. It was demonstrated that SOCS-3 expression is lost in lung cancer cell lines and clinical samples and that forced expression of that inhibitor of cytokine signaling yields a reduced tumor growth and diminished phosphorylation of STAT3 (7). It was shown that the mechanism being responsible for the down-regulation of SOCS-3 expression is promoter hypermethylation in CpG islands. The treatment of cells with the demethylating agent 5-aza-2´-deoxycytidine led to restoration of expression of SOCS-3 in lung cancer. Similar changes in methylation of SOCS-3 were reported for head and neck squamous cell tumors (8). SOCS-3 mRNA and protein down-regulation was detected by quantitative real-time PCR and immunohistochemistry, respectively. The same methylation pattern was also observed in lymph node metastases. Similarly as in lung cancer, 5-aza-2´-deoxycytidine treatment yielded higher expression levels of SOCS-3 in head and neck cancer. When SOCS-3 was overexpressed, growth inhibition and diminished expression of STAT3 and its target proteins bcl-2 and bcl-xL were noted. On the basis of these results, a possible gene therapy approach with the aim to increase the expression of SOCS-3 in tumor cells may be developed in the future. Another example of tumor suppressive function of SOCS-3 are the results obtained in hepatocellular carcinoma models (9). In that tumor, phosphorylated STAT3 promotes and SOCS-3 inhibits growth. Again, SOCS-3 expression was reduced as a consequence of promoter hypermethylation which seems to be a common mechanism in human cancers. Suppressive role of SOCS-3 could be explained as a result of physical interaction with focal adhesion kinase (FAK), which is of crucial importance for migration of tumor cells. Thus, SOCS-3 decreases FAK expression and phosphorylation (9). In the above mentioned study, the authors were able to demonstrate direct implications of modulation of SOCS-3 levels on STAT3 phosphorylation. The results obtained in cell lines were in concordance with those obtained in cancer specimens. SOCS-3 deletion may occur early in liver carcinogenesis, thus facilitating tumor development in patients suffering from hepatitis (10). In an experimental model of concavalin A-mediated hepatitis, SOCS-3 deletion protected against liver injury. In another tumor of the gastrointestinal tract, chloangiocarcinoma, sustained IL-6 signaling and up-regulation of the oncogene Mcl-1 were observed as a result of SOCS-3 gene promoter methylation (11). Interestingly, forced SOCS-3 expression led to cellular sensitization to substances which induce apoptosis.

Methylation of the promoter of the SOCS-3 gene was observed in 35% of patients diagnosed with glioblastoma multiforme and was associated with an unfavourable clinical outcome (12). In glioblastoma multiforme clinical specimens, it was found that the expression of PIAS3 is also lost (13). The regulation of PIAS3 and SOCS in this tumor may be heterogenous. Interestingly, it was found that PIAS3 overexpression causes a reduced expression of SOCS-3. With regard to PIAS3 expression, it seems that there are differences

between tumor cells *in vitro* and clinical specimens. In primary cultures obtained from the patients, the expression of PIAS3 was preserved, although it is not known whether this was sufficient to exert physiological effects. Inhibition of expression of PIAS3 by siRNA yielded increased proliferation of glioblastoma cells. In addition, PIAS3 expression was associated with reduced expression of matrix metalloproteinase 9, thus suggesting a mechanism by which PIAS3 participates in regulation of invasive behaviour of this central nervous system tumor. Consistently with tumor suppression function of PIAS3 in glioblastoma multiforme, PIAS3 overexpression in lung tumors yielded enhanced sensitivity to chemotherapeutic drugs (14). This inhibition was also associated with the antagonistic effect on activation of Akt. Interestingly, an increased sensitivity to chemotherapy was specific for PIAS3 since it was not observed upon elevation of levels of SOCS-3. Taken together, these data strongly suggest that PIAS3 has an important tumor suppressive function.

An anti-tumor effect *in vitro* and *in vivo* was reported when adenoviral transfer of SOCS-3 was performed in malignant pleural mesothelioma (15). Iwahori and associates showed that the therapeutic effect is associated with a cell cycle arrest and is due to interactions with several signaling pathways, including inhibition of STAT3, FAK, and mitogen-activated protein kinases. Consistent with other data in which the anti-tumor role of SOCS-3 was documented, exogenous expression of SOCS-3 potentiated the effects of chemotherapy agents taxol and doxorubicin in thyroid cancer (16). Thyroid tumors were found to express low levels of endogenous SOCS proteins. Importantly, phosphorylation of Akt was also reduced in cells in which SOCS-3 was delivered by means of lentiviral transfection, thus suggesting the complexity of regulation of signaling pathways by SOCS in cancer. This benefitial effect on Akt leads to inhibition of expression of anti-apoptotic Bcl-xL in thyroid neoplasms. The effects of SOCS-3 overexpression on responsiveness to chemotherapy agents in thyroid cancer were also confirmed *in vivo* (16). It is important to note that SOCS overexpression may lead to attenuation of function of several other cytokines, such as IL-4. IL-4 is known as a potent pro-survival cytokine which may be in particular involved in regulation of stem or tumor initiating cells in a paracrine or autocrine manner.

In contrast to the studies mentioned above in which SOCS-3 and pSTAT3 are frequently inversely correlated, concomitant expression of SOCS-3 and phosphorylated STAT3 was observed in cutaneous T cell lymphoma cell lines (17). It seems that in this disease the negative feedback loop described above does not exist. Although functional implications of aberrant expression of SOCS-3 in lymphoma have not been completely clarified, it seems that it decreases the sensitivity of cells to treatment with interferon alpha. In this context, it is of interest to mention that SOCS-3 or -1 repress the expression of the antiviral proteins 2,5-OAS and MxA induced by interferon-alpha (18). This effectiveness of this cytokine was demonstrated in therapy of lymphoma. Thus, SOCS-3 in this case does not act as a tumor suppressor since it protects the tumor cells from growth inhibition caused by interferon-alpha (19). Coexpression of SOCS-3 and STAT3 was also seen in anaplastic large cell lymphoma (20). In contrast, SOCS-3 expression was low in Philadelphia-negative chronic myeloproliferative disorders as a result of promoter hypermethylation (21). Aberrant SOCS3 methylation was reported in several multiple myeloma cell lines and in clinical specimens. SOCS-3 methylation was associated with shortened patients' survival (22). Collectively, these data show that SOCS-3 may have different functions in hematopoietic cell tumors.

It has been generally accepted that chronic colon inflammation contributes to carcinogenesis. As expected, overexpression of SOCS-3 in colon carcinogenesis *in vitro* yielded inhibition of STAT3 phosphorylation, activation of nuclear kappa B, and led to a reduced number of tumors (23). In case of colon epithelium, cytokine-induced expression of SOCS-3 may represent a mechanism by which uncontrolled cellular proliferation is prevented. This process may be dysregulated in cancer.

## 4. MULTIPLE FUNCTIONS OF SOCS-3 IN CARCINOMA OF THE PROSTATE

Comprehensive studies on SOCS-3 were carried out in human prostate cancer. This experimental work is of particular interest because of a multitude of cytokine effects in cancer. The presence of SOCS-3 was detected in prostate cancer cell lines in which there was no STAT3 expression (PC-3) or phosphorylation (DU-145) (24). In the subsequent studies, causal relationship between SOCS-3 expression and STAT3 phosphorylation in prostate was demonstrated (25). SOCS-3 down-regulation by siRNA was followed by IL-6-induced phosphorylation of STAT3, which was not observed in untreated DU-145 cells. In LNCaP cells, which express a mutated androgen receptor (AR) and respond to a number of steroids and anti-hormones, SOCS-3 expression is lost. However, SOCS-3 could be induced in the LNCaP cell line after treatment with 5-aza-2´-deoxycytidine. In clinical specimens obtained from prostate cancer, SOCS-3 expression increased in comparison to benign prostate (24). Higher levels of SOCS-3 mRNA were also observed in unmethylated cases of prostate cancer versus benign tissue in another study (26).

There may be several interactions between signaling pathways of ILs and androgen in prostate cancer. Functions of SOCS-3 in prostate cancer may depend on the presence or absence of the androgen receptor (AR). In cells in which the AR is expressed, such as LNCaP, androgenic hormones increased the expression of SOCS-3 which subsequently diminished AR-regulated proliferation and differentiation (27). For example, the secretion of well-known AR target prostate-specific antigen was down-regulated after forced expression of SOCS-3. Thus, in these conditions SOCS-3 may have a potentially important negative feedback role in prostate cancer. Importantly, the changes in androgenic regulation of cellular events caused by SOCS-3 were observed without addition of exogenous IL-6. SOCS-3 is also regulated by estrogen receptor in

breast and liver cancer cells. Functional implications of this regulation in steroid-responsive tissues have not been reported yet (28, 29). Interestingly, the SOCS-3 gene promoter is up-regulated by prostaglandin E2 in breast cancer cells independently of STAT (30). Collectively, these findings imply that SOCS-3 and probably other SOCS proteins may have functions in cells which are not influenced by inflammatory cytokines and do not reflect alterations in the activation status of STAT. In cell lines in which the AR is not detectable constitutive SOCS-3 expression at mRNA and protein level was documented (24,25). In those cells, it was demonstrated that SOCS-3 depletion leads to an increased apoptosis. In PC3 cells, SOCS-3 was shown to inhibit the anti-proliferative and pro-apoptotic effect of cAMP (24). Thus, those results in prostate cancer show that SOCS-3 may have both tumor suppressor and tumor promoter roles, depending on cellular context. SOCS-3 anti-apoptotic effects could be explained by its up-regulation of the Bcl-2 oncogene. SOCS-3 and Bcl-2 may influence expression of each other in cancers. In a previous study, it was shown that Bcl-2 up-regulates the expression of SOCS-3 in B cells and in lymphoma (31). Another important anti-apoptotic function of SOCS-3 in prostate cancer was revealed in the presence of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and resveratrol. Resveratrol, contained in fruit and red wine, is considered a chemopreventive agent in prostate cancer (32). With regard to clinical relevance of these findings, it was shown that SOCS-3 is expressed at a high level in cells from prostate cancer patients who failed endocrine- and chemotherapy (25).

The concept that SOCS-3 has different functional roles depending on the cellular context is further supported by our data which revealed that this negative regulator of cytokine signaling is also implicated in the control of biological effects of basic fibroblast growth factor (bFGF), which influences proliferation, migration, and angiogenesis. bFGF acts through several signaling pathways to which p44/p42 mitogen-activated protein kinases belong. In prostate cancer cells, bFGF activation of that pathway was antagonized by SOCS-3, as evidenced in siRNA and overexpression experiments (33). Thus, SOCS-3 expression in prostate cancer has an impact on cellular migration and its function may be investigated in future *in vivo*.

Similarly as in prostate cancer, constitutive expression of SOCS-3 was also reported for melanoma (34). It was found that ten melanoma cell lines SOCS-3 are mRNA-positive. Nine cell lines do not express high levels of SOCS-3 protein and the mechanisms being responsible for the differences between mRNA and protein expression have not been clarified yet. Functional insights into SOCS-3 effects in melanoma were obtained with 1286 cells which are normally insensitive to a growth-inhibitory effect of IL-6. Suppression of SOCS-3 expression has re-established the growth-inhibitory role of IL-6. In a subgroup of patients with melanoma, however, there was a higher expression of the SOCS-3 protein. In a recent study, the researchers have utilized an autologous model of B16F10 melanoma cells and found that lack of SOCS-3 is associated with prolonged animal survival and a fewer number of lung and liver metastatic nodules (35). The results of that study support the contention that SOCS-3 should be specifically targeted in selected human neoplasms. Constitutive expression of SOCS-3 in melanoma cells was associated with the development of resistance to interferon gamma (36), consistent with previous findings demonstrating resistance to interferon alpha in chronic myelogenous leukemia cells (37).

## 5. SOCS-1 REGULATES CELL CYCLE IN CANCER

In several cancers, the role of SOCS-1, an inhibitor of STAT3 and -6 signaling, has been addressed. Collectively, the results show that SOCS-1 is an important tumor suppressor. Interestingly, it is highly expressed in prostate cancer although it inhibits prostate cancer cell growth (38). Inhibition of SOCS-1 expression by specific siRNA leads to an increased expression of cyclin-dependent kinases and cyclins in androgen-insensitive prostate cancer cells. On the basis of a high expression of SOCS-1 in prostate cancer, one can conclude that SOCS-1 may have some presently unrecognized functions in prostate cancer. Although SOCS-1 is considered a tumor suppressor, it is not required for inhibition of proliferation of LNCaP cells by IL-6.

There may be several further implications of SOCS1 limitations of nuclear factor kappa B signaling within the cell nucleus (39). SOCS-1 can also directly interact with the tumor suppressor p53 thus regulating cellular senescence (40). SOCS-1 expression is induced by the histone deacetylase inhibitor trichostatin A in colon cancer cells thus representing a mechanism by which this drug induces a cell cycle arrest and antagonizes tumor growth (41). In tumors in which STAT3 acts as an oncogene, decreased expression of SOCS-1 contributes to tumor progression and is a consequence of epigenetic changes (42-44). Methylation of the SOCS-1 gene promoter in dormant tumor cells leads to an increased resistance to apoptosis induced by imanitib (45). SOCS-1 methylation was reported also in squamous head and neck carcinoma as a determinant of response to IL-6 or epidermal growth factor (46). Although no prognostic significance of methylation of SOCS-1 was observed in hepatocellular cancer, it seems that SOCS-1 methylation is negatively associated with hepatitis B virus infection in patients (47). In liver cancer, SOCS-1 acts as a tumor suppressor similarly to SOCS-3 (48). Another mechanism by which SOCS-1 is inactivated in cancer are mutations as described in Hodgins lymphoma (49). In concordance with the observations showing a tumor suppressor role of SOCS-1, use of SOCS-1-mimetic peptide in prostate cancer cell lines can lead to a growth inhibition of those cells through antagonism of STAT3 activity (50).

Further evidence for the tumor suppressor role of SOCS-1 in cancer was obtained in studies in which it was shown that restoration of its expression significantly diminished the number of brain metastasis of melanoma (51). SOCS-1 is responsible for regulation of matrix metalloproteinase 9 and angiogenic growth factors bFGF

and vascular endothelial growth factor. In contrast, Li and associates have reported on an increased expression of SOCS-1 in eight melanoma cell lines derived at different stages of tumor development (52). The reasons for the differences between these two studies have not been yet clarified. However, no functional studies were performed by those authors.

In glioblastoma multiforme, the effects of SOCS-3 and -1 were compared simultaneously in terms of sensitivity to radiation therapy (53). Similar sensitization effects were observed when SOCS-1 was overexpressed and SOCS-3 blocked.

In line with the previous observations, SOCS-1 silencing in neuroendocrine tumors has been associated with the enhancement of therapeutic response to interferons alpha and beta (54). In those conditions, several anti-apoptotic genes were down-regulated.

## 6. PERSPECTIVE

Collectively, the data summarized in the present review point to the differential regulation of cellular events by SOCS-3 and -1 in human cancers. The explanations for multiple effects of these proteins in cancer are missing and more work is needed to clarify tumor suppressive and tumor promoting roles of SOCS in cancer. This may be achieved either if appropriate *in vivo* models are developed or novel partner proteins of SOCS discovered. Interactions between SOCS proteins and nuclear factor kappa B or p53 recently discovered may stimulate new research approaches which will lead to a better understanding of SOCS function. Importantly, different SOCS effects in cancer could be considered as a part of a more personalized approach in cancer medicine. In summary, it became clear that modulation of SOCS expression may be considered as a part of multitarget therapy in cancers and individual factors leading to alterations in SOCS expression should be also investigated in the future.

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