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SHORT COMMUNICATIONS AND CASE REPORTS

Suppressors of cytokine signaling as tumor repressors. Silencing of SOCS3 facilitates tumor formation and growth in lung and liver

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Summary

The suppressors of cytokine signaling (SOCS) are inhibitors of cytokine signaling that function via the Janus kinase (JAK)/signal transducers and activators of transcription (STAT) pathway. Eight SOCS (SOCS1-SOCS7 and CIS /cytokine-inducible SH2-domain) proteins with similar structures have been identified. Cytokines bind to specific sites on the extracellular domains of their cognitive receptor, causing receptor dimerization. This allows the recruitment of JAKs to the receptors, which then cross-phosphorylate each other before phosphorylating the receptor on key tyrosine residues. STAT molecules bind to these phosphorylated docking sites, are in turn phosphorylated, dimerized, and enter the nucleus where they initiate transcription. Some of the genes transcribed by these factors include the SOCS genes. The SOCS proteins then act to negatively regulate activated receptor complexes by inactivating JAKs or blocking recruitment sites for STATs and also may target signaling complexes for ubiquitination and degradation. Lung cancer and hepatocellular carcinoma (HCC) are associated with abnormalities of the JAK/STAT pathway. In conclusion, determining the importance of SOCS family in health and disease will no doubt aid to the development of novel therapeutic strategies in human carcinogenesis.

Key words: activators of transcription (STAT), hepatocellular carcinoma, Janus kinase (JAK) signal, JAK/STAT pathway, lung cancer, suppressors of cytokine signaling (SOCS), tumor repressors

Introduction

SOCS are inhibitors of cytokine signaling that function via the JAK/signal transducers and STAT pathway. Eight SOCS proteins (SOCS1-SOCS7 and CIS) containing proteins with similar structures have been identified so far (discovered in the late 1990s) (Figure 1) [1]. SOCS family members, however, have distinct mechanisms of inhibition of JAK/STAT signaling. Abnormalities of the JAK/STAT pathway are associated with cancer, including lung cancer and HCC [2-5].

Mechanisms of suppressors of cytokine signaling induction and action

Pioneer research has documented that SOCS proteins are induced by cytokines that use JAK/STAT signaling pathways. Cytokines bind to specific sites on the extracellular domains of their cognitive receptor, causing receptor dimerization. This allows the recruitment of JAKs to the receptors, which then cross-phosphorylate each other before phosphorylating the receptor on key tyrosine residues. STAT molecules bind to these phosphorylated docking sites, are in turn phosphorylated, dimerized, and enter the nucleus where they initiate transcription. Some of the genes transcribed by these factors include the SOCS genes. The SOCS proteins then act to negatively regulate activated receptor complexes by inactivating JAKs or blocking recruitment sites for STATs and also may target signaling complexes for ubiquitination and degradation (Figure 2). Each cytokine binds to a specific receptor that spans target cell membranes and induces dimerization or oligomeriza-

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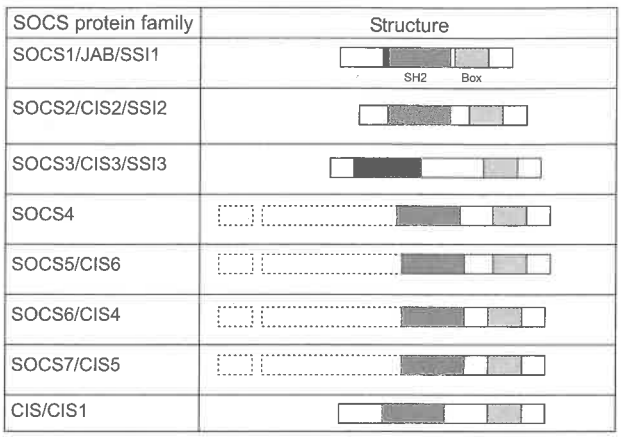


Figure 1. The plethora of the SOCS protein family. The kinase inhibitory region of SOCS1 and SOCS3 is indicated in black.

tion, causing conformational changes in receptor structure. These receptor modifications allow for the recruitment of JAKs, enzymes that bind to the receptors and phosphorylate specific tyrosine residues on themselves and on the intracellular portion of the receptors. Tyrosine phosphorylated receptors then serve as docking sites for signaling proteins that contain Src homology 2 (SH2) domains or phosphotyrosine binding domains. Such signaling proteins include RAS and phosphatidylinositol 3-kinase that, being activated by cytokine receptors, are also associated with signal transduction by receptor tyrosine kinases that mediate the actions of growth factors such as the epidermal growth factor (EGF) or insulin-like growth factors (IGFs). In addition, a family of transcription factors known as STATs bind to activated cytokine receptors via SH2 domains and are key-mediators of cytokine action. Once bound,

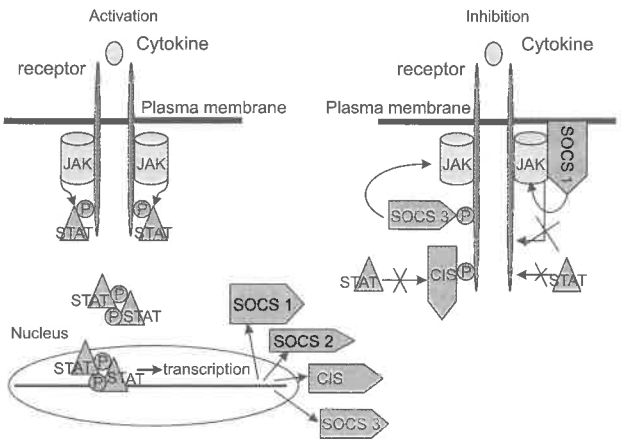


Figure 2. SOCS1 binds to the JAKs and inhibits catalytic activity, SOCS3 binds to JAK-proximal sites on cytokine receptors and inhibits JAK activity, and CIS blocks the binding of STATs to cytokine receptors.

the STATs themselves become a substrate target of the JAKs and are phosphorylated on key tyrosine residues. Activated STATs dissociate from the receptor and form homo- or heterodimers with other STAT molecules. These paired molecules cross the nuclear membrane and bind to DNA sequences in the promoter regions of target genes that are selective for particular STAT family members and mediate the transcription of cytokine-regulated genes [6].

Dysregulated JAK-STAT signaling and methylation-mediated silencing of SOCS-1, SOCS-2 or SOCS-3 genes in lung and liver tumors

Lung cancer is the leading cause of cancer death in the world, but the molecular mechanisms for its development have not been well characterized.

He et al. focused on the restoration of SOCS-3 expression which resulted in the downregulation of STAT3 activation, induction of apoptosis and growth suppression in human lung cancer cell lines [3]. Recently, He and colleagues cloned the functional 5' promoter region of the human SOCS-3 gene and showed that this region is highly conserved in murine and rat SOCS-3 promoters. They also found that the wild type SOCS-3 promoter construct has significantly greater activity in human non small-cell lung cancer (NSCLC) cell lines than in normal cells in accordance with STAT3 deregulation in these cells. Finally they have confirmed that frequent hypermethylation of the functional SOCS-3 promoter correlates with its transcription silencing in NSCLC cell lines and primary lung cancer tissue samples.

Seki et al. [7] have described 15 cases (19% of 79 examined in total) with tumor-specific increase of STAT3 phosphorylation and 51 (65%) cases with STAT3 phosphorylation proportional to its expression. In addition, exogenous expression of either Janus kinase binding protein (JAB) or dominant negative STAT3 in lung carcinoma cell lines led to the suppression of STAT3 phosphorylation and the drastic reduction of anchorage-independent growth of the cells.

Ogata et al. [8] analyzed the roles of an inhibitor of STAT (protein inhibitor of activated STAT [PIAS] 3) in the development of lung cancer. Treatment with an inhibitor of phosphatidylinositol 3-kinase (LY294002) retarded the growth of human lung cancer cells and rendered them more sensitive to chemotherapeutic agents. Inhibition of PIAS3 with small interfering RNA, nevertheless, led cancer cells to accelerate cell proliferation, deteriorate chemosensitivity, and augment Akt phosphorylation.

HCC is a quite common human cancer. Many

efforts have been made to discover aberrant expression control in HCC, however the specific molecular mechanisms involved in hepatocarcinogenesis remain to be determined. Nagai et al. [9] have shown that expression of the SOCS-1 gene was markedly reduced in half of 8 HCCs analyzed. Yoshikawa and colleagues identified aberrant methylation in the CpG island of SOCS-1 that correlated with its transcription silencing in HCC cell lines. The incidence of aberrant methylation was 65% in the 26 human primary HCC tumor samples analyzed [10].

Okochi et al. have described that aberrant methylation of the SOCS-1 gene was detected in 30 of 50 (60%) HCC specimens. They also concluded that SOCS-1 may be a novel tumor suppressor, and its aberrant methylation may be a key event for HCC transformation of cirrhotic nodules [11]. Hoshida and coworkers examined SOCS-1 gene methylation in more than 200 patients with chronic liver disease and found that the severity of liver fibrosis is strongly correlated with SOCS-1 gene methylation. The authors concluded that these findings suggest that SOCS-1 contributes to protection against hepatic injury and fibrosis, and may also protect against hepatocarcinogenesis [12].

In recent trials the available data indicate that SOCS-3 negatively regulates cell growth and cell motility by inhibiting JAK/STAT and FAK signalings in HCC cells [13]. Moreover, STAT3 enhances hepatic fibrosis through the upregulation of transforming growth factor (TGF)-beta1 expression, and SOCS-3 prevents this process [14]. Finally, the ubiquitous activation of Ras and JAK/STAT pathways in HCC suggests the potential use of Ras and JAK/STAT inhibitors and demethylating agents as therapeutic modality for human HCC [15], while deletion of the SOCS-3 gene in hepatocytes promotes the activation of STAT3, resistance to apoptosis, and an acceleration of proliferation, resulting in enhanced hepatitis-induced hepatocarcinogenesis [16].

Conclusions and future implications

Pioneer researchers have shown that more than 20% of all malignancies are initiated by persistent inflammation. DNA damage and malignant transformation may be induced from inflammatory cells (possible mediators: nitric oxide, reactive oxygen species, prostaglandin E2). Transcription 3 (STAT3) activation in tumor cells contributes to an expansion of tumor cells by promoting cell proliferation, survival, angiogenesis and tissue remodeling. An important mechanism for constitutive STAT3 activation is by SOCS-3 silencing. Also DNA damage and the resulting emergence of malignant cells may implicate the SOCS-1 silencing, which results in strong and persistent STAT1 activation. Thus, determining the importance of SOCS family in health and disease will no doubt aid to the development of novel therapeutic strategies in human carcinogenesis.

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