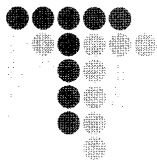

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Decreased Expression of the Suppressor of Cytokine Signaling 6 in Human Hepatocellular Carcinoma

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Abstract

Suppressors of cytokine signaling (SOCS) proteins were originally identified as negative feedback regulators of cytokine signaling and include the Janus kinase/Signal transducer and activator of transcription (JAK/STAT) pathways. Recent studies have shown that SOCS proteins negatively regulate the receptor tyrosine kinase (RTK) pathway including the insulin receptor (IR), EGFR, and KIT signaling pathways. In addition, SOCS1 and SOCS3 have been reported to have anti-tumor effects in human hepatocellular carcinoma (HCC). However, it is uncertain whether other members of the SOCS family are associated with tumor development and progression. In this study, to investigate whether SOCS6 is aberrantly regulated in HCC, we examined the expression level of SOCS6 in HCC by Western blot analysis and immunohistochemical staining. The results showed that SOCS6 was down-regulated in all examined HCCs compared to the corresponding normal tissues. In addition, expression of SOCS6 was observed in the cytoplasm of most normal and precancerous tissue, but not in the HCCs by immunohistochemical staining. This is first report to demonstrate that SOCS6 is aberrantly regulated in HCC. These findings suggest that underexpression of SOCS6 is involved in hepatocarcinogenesis, and SOCS6 may play a role, as a tumor suppressor, in HCC development and progression.

Keywords: SOCS6, Down-regulation, Hepatocellular carcinoma

Hepatocellular carcinoma (HCC) is the fifth most common neoplasm worldwide, and in some areas of the world it represents the primary cause of cancer related death[1]. Any agent leading to chronic liver injury, and eventually cirrhosis, is considered an oncogenic agent; hepatitis B and hepatitis C as well as alcohol are common oncogenic agents. However, the molecular progression to HCC is not well understood[2]. Recent studies have found that genetic alterations of the tumor suppressor genes or oncogenes such as p53, pRb, β-catenin, and AXIN1 are involved in hepatocarcinogenesis[3-6]. However, the frequency of mutations found in these genes appears to be very low in HCCs. Furthermore, it is not clear how these genetic changes are associated with the clinical features of HCC patients. Therefore, further study is needed to understand the molecular events associated with HCC and their clinical correlates.

Suppressors of cytokine signaling (SOCS) proteins are a group of negative feedback regulators of cytokine signaling and include the Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathways[7]. The SOCS family consists of eight members: SOCS1-7, and CIS (cytokine-inducible SH2-containing protein) that have a conserved Src-homology 2 (SH2) domain and C-terminal SOCS box and a variable N-terminal region[7]. The SH2 domain facilitates the SOCS protein interaction with phosphorylated target proteins such as JAK; the SOCS box is thought to act as a binding domain for an E3 ubiquitin ligase complex[8-10]. Recent studies have shown that SOCS proteins can act as negative regulators of receptor tyrosine kinase (RTK) signaling including the insulin receptor (IR), EGFR, and KIT[11-13]. However, the regulation of RTK by SOCS proteins remains poorly understood.

One member of the SOCS family, SOCS6 does not regulate the JAK/STAT pathway like other SOCS

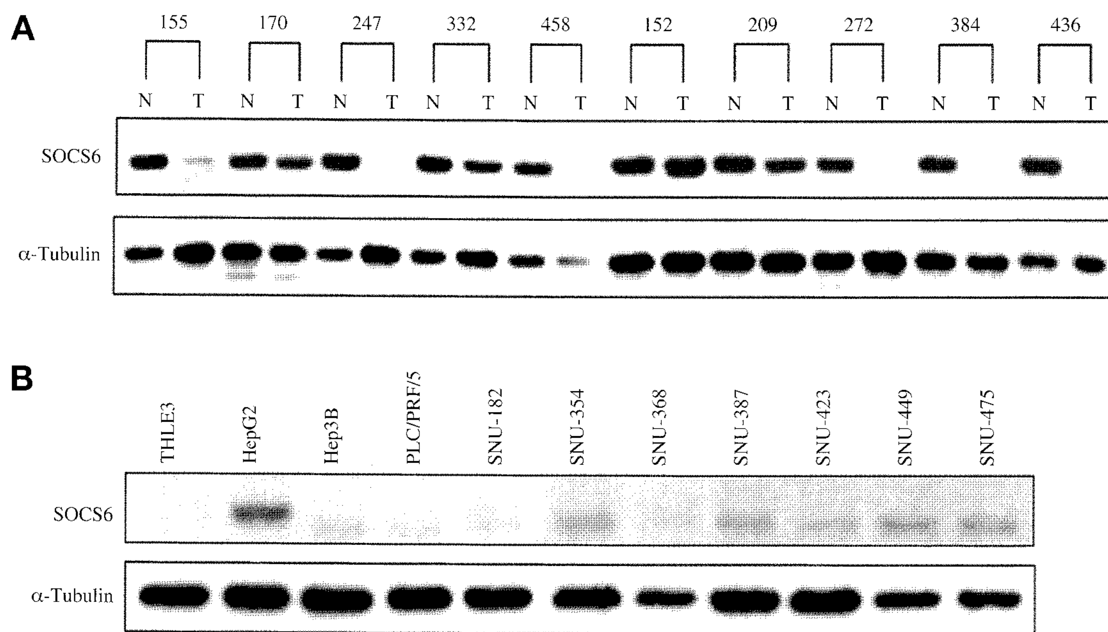


Figure 1. Western blot analysis of SOCS6 in HCC tissues Results of the Western blot analysis are shown in black with a white background (N, normal; T, tumor). α-Tubulin were used as internal controls in the Western blot analysis. The experiments were repeated three times.

proteins, such as SOCS1-3 and CIS[14]; it induces proteasomal degradation of target proteins by binding to the E3 ubiquitin ligase, heme-oxidized IRP2 ubiquitin ligase-1(HOIL-1) through the SH2 domain and SOCS box[15]. SOCS6 has been shown to attenuate IR signaling including the activation of ERK1/2, AKT and IRS-1[16]. It has been reported that SOCS6 interacts with KIT and negatively regulates stem cell factor-mediated KIT signaling[13]. These results suggest that SOCS6 is not an inhibitor of cytokine signaling, but rather RTK signal transduction.

There is some evidence that SOCS proteins are implicated in human cancers. SOCS1 and SOCS3 were silenced by promoter methylation in HCC, and when restored, they inhibited the growth of human hepatocellular carcinoma cells[17,18]. However, evaluation of SOCS6 has not been performed to date. Therefore, we investigated the regulation of SOCS6 in human HCCs using Western blot and immunohistochemistry analysis to determine whether SOCS6 acts as a tumor suppressor in hepatocarcinogenesis.

SOCS6 Was Down-regulated in HCC

To investigate the aberrant regulation of SOCS6, we performed western blot analysis on 10 selective pairs of HCC and their corresponding normal tissue. As shown in Figure 1A, expression of SOCS6 was significantly down-regulated in 9 samples out of the 10 HCCs compared to the corresponding non-cancerous tissue in the same patient. In addition, endogenous expression of the liver cancer-derived cell lines resulted in a very low or non-detectable level of expression by Western blot analysis, except for the HepG2 cells. The immunohistochemical staining analysis showed strong positive cytoplasm in normal hepatocytes, but was negative or weak positive in the HCCs (Figure 2).

Discussion

The results of this study showed down-regulation of SOCS6 in HCCs. Previous studies have reported that SOCSs, especially SOCS1 and SOCS3, were down-regulated and accompanied activation of the JAK/STAT pathway in HCC; however, the level of the SOCS6 protein was unchanged in the normal and tumor samples[17-19]. In addition, hepatocarcinogenesis was induced in SOCS3-deficient mice[20]. These data suggest that SOCS1 and SOCS3 are implicated in the development and progression of HCC by acting as tumor suppressors. In contrast to these results, our data showed down-regulation of SOCS6 in hepatocarcinogenesis. As shown in Figure 1, the SOCS6 protein was reduced in most of the tumor samples by both Western blot analysis and immunostaining (Figures 1 and 2).

The activity of SOCS6 in cancer-related signal trans-

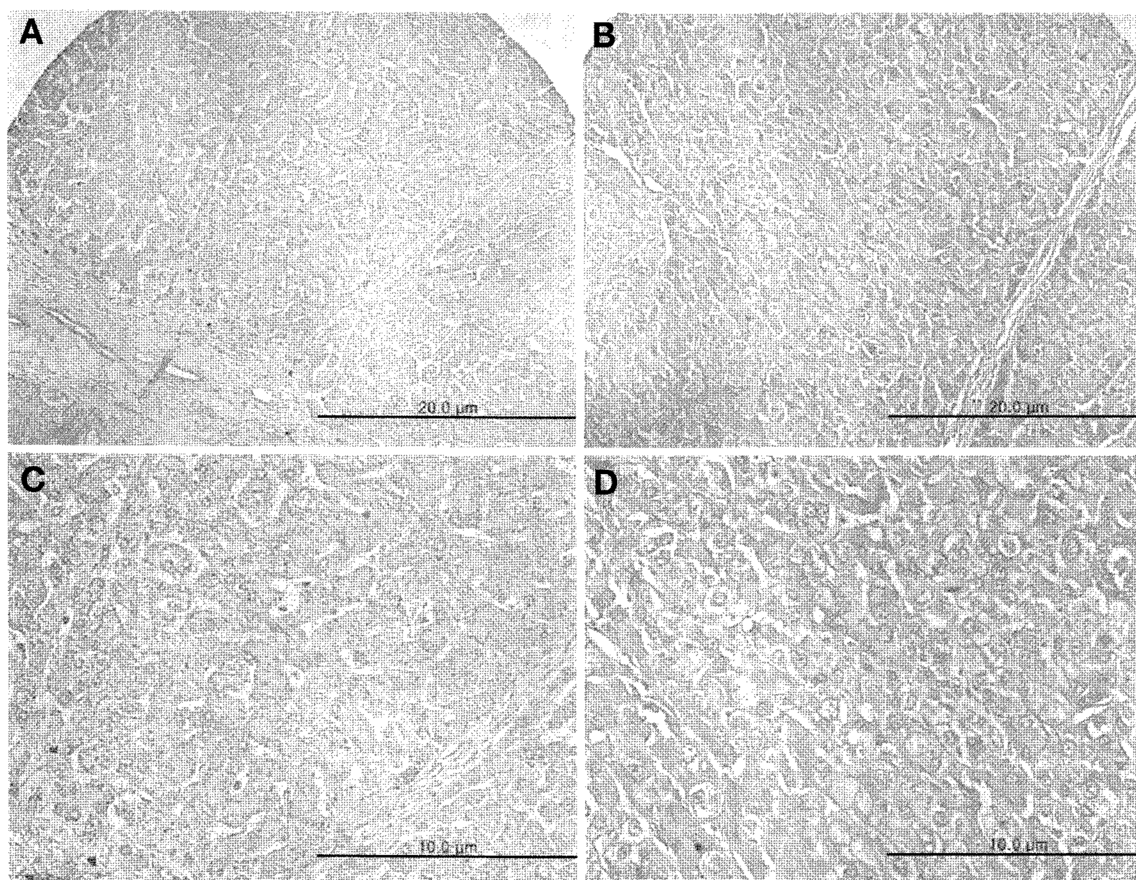
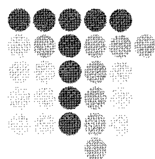


Figure 2. Immunohistochemical staining of SOCS6 in HCC tissues Representative images of immunostaining of SOCS6 in normal and HCC tissue samples-The cytoplasm of normal liver tissue shows strong intensity for SOCS6 antibody (A, C), but shows negative or weak positive results in the HCCs (B, D). Magnifications, ×200 (A, B), ×400 (C, D).

duction pathways has not been extensively studied. Previous data suggested that SOCS6 is a negative regulator of stem-cell-factor induced KIT receptor signaling upstream of ERK1/2 and p38 activation, without alteration of KIT expression[13]. The multikinase inhibitory antitumor agent, Sorafenib, has been shown to inhibit tumor angiogenesis and induce apoptosis of the hepatocellular carcinoma cell line, PLC/PRF/5[21]. Given the above information, SOCS6 was thought to be associated with tumor suppressing effects via the regulation of KIT receptor signaling. However, further study is needed to determine whether SOCS6 acts as an anti-tumor regulator in tumor development and progression.

The half life of SOCS proteins demonstrates that it is associated with both protein-protein interactions and phosphorylation[15]. Interaction between the SOCS box and Elongin B/C complex can rescue SOCS1 from proteasomal degradation[10]. By contrast, the RING-finger containing protein TRIM8/GERP interacts with SOCS1 and destabilizes it[22]. However, Elongin C interacts with and stabilizes SOCS3; but this binding was shown to be interrupted by phosphorylation of SOCS3[23]. Consistent with these findings, several studies have suggested that SOCS6 can be degraded by the proteasome. When transfected to CHO cells, SOCS6 was destabilized 1 hr after induction by insulin treatment; this degradation was partially inhibited by the proteasome inhibitor, LLnL[24]. In another report, SOCS6 was shown to be rapidly broken down without other proteasome inhibitors, MG132 and epoxomicin[15]. Therefore, one possible mechanism involved in the regulation of SOCS6 protein dysregulation appears to be proteasomal degradation.

Other SOCS family members promote degradation of various proteins by the proteasome. SOCS1 facilitates turnover of the TEL-JAK oncogene in a SOCS-box dependent manner[25]. SOCS3 can cause receptors such as CD33 and sialic acid-binding immunoglobulin-like lectin (Siglec) 7 to be destroyed, which is dependent on the proteasome, and block receptor-mediated responses[26,27]. Furthermore, SOCS6 was transiently



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Genomic and Proteomic Profiling of the Cadmium Cytotoxic Response in Human Lung Epithelial Cells

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Abstract

Microarray and proteomic expression patterns in response to cadmium exposure were analyzed in human lung epithelial cells. Among 35,000 genes analyzed by cDNA microarray, 228 genes were up-regulated and 99 genes were down-regulated, based on a fold change cut-off value of $\geq$2. Combining two-dimensional gel electrophoresis and matrix-assisted laser desorption/ionization-time-of-flight-mass spectrometry (MALDI-ToF-MS), 25 of 629 protein spots showed fold changes in expression $\geq$2 (17 up-regulated, 8 down-regulated). After comparing the cDNA microarray and proteomic analyses, only transglutaminase 2, translation elongation factor 1 alpha 1, and glyceraldehyde-3-phosphate dehydrogenase showed overlapping signals in the cDNA microarray and proteomic analyses, whereas the remaining differentially expressed proteins showed large discrepancies with respect to mRNA expression.

Keywords: Genomics, Proteomics, Cadmium, Human lung epithelial cells

Cadmium (Cd^{2+}) is a ubiquitous industrial and environmental pollutant that bioaccumulates in the upper levels of the food chain, including humans[1-3]. Considering its current rate of release into the environment, cadmium content in the human body is likely to increase in the future[4]. Cadmium has been shown to be an effective inducer of apoptosis, and also affects cell proliferation, differentiation, gene expression, and signal transduction. Moreover, cadmium can promote the generation of reactive oxygen species (ROS), inhibit DNA repair and DNA methylation, and disrupt E-cadherin-mediated cell-cell adhesion[5,6]. The inhibition of DNA repair represents a mechanism by which cadmium enhances genotoxicity and promotes tumor initiation. The major mechanisms of cadmium-induced gene expression include the modulation of signal transduction pathways via enhanced protein phosphorylation and the activation of transcription and translation factors[7]. Cadmium has also proven to be toxic in many organs, including the lung, kidney, liver, testis, brain, bone, and blood. Cadmium is a known lung carcinogen and has been implicated in the development of other pulmonary diseases, including emphysema and interstitial fibrosis[8]. Cadmium is classified as a category I carcinogen by the International Agency for Research on Cancer and the US National Toxicology Program.

The identification of genes that are differentially expressed in response to toxic cadmium exposure would provide a better understanding of its mechanism of action and facilitate the search for sensitive and specific biomarkers of cadmium exposure and susceptibility. However, mRNA expression does not necessarily predict the level of corresponding protein expression and does not account for post-translational modification. Thus, although gene microarrays offer insight into the expression of numerous genes within a cell type, proteomic analysis is required to determine global protein expression in the cell. In this study, genomic and proteomic expression profiles were compared to identify genes and proteins altered by cadmium exposure[9]. Our results may assist in the selection of biomarkers for cadmium exposure at the gene and protein levels.

Cadmium Cytotoxicity in Human Lung Epithelial Cells

The MTT assay was used to determine the relative cell viability of human lung epithelial cells (NCI-H292) following exposure to a range of concentrations of cadmium chloride for different exposure times. Cadmium chloride reduced cell viability in a concentration-dependent manner. As shown in Figure 1, exposure to 40 μM cadmium chloride for 12 h resulted in an approximate 25% decrease in cell viability. This concentra-

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