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# Suppressor of cytokine signaling 1 modulates invasion and metastatic potential of colorectal cancer cells

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ABSTRACT

Suppressor of cytokine signaling (SOCS) 1 is an inducible negative regulator of cytokine signaling but its role in human cancer is not completely established. Here we report that, while SOCS1 is expressed in normal colonic epithelium and colon adenocarcinomas, its level decreases during progression of colon adenocarcinomas, the lowest level being found in the most aggressive stage and least differentiated carcinomas. Forced expression of SOCS1 in metastatic colorectal SW620 cells reverses many characteristics of Epithelial–Mesenchymal Transition (EMT), as highlighted by the disappearance of the transcription factor ZEB1 and the mesenchymal form of p120ctn and the re-expression of E-cadherin. Furthermore, miRNA profiling indicated that SOCS1 also up-regulates the expression of the mir-200 family of miRNAs, which can promote the mesenchymal–epithelial transition and reduce tumor cell migration. Accordingly, overexpression of SOCS1-induced cell morphology changes and dramatically reduced tumor cell invasion *in vitro*. When injected in nude mice, SOCS1-expressing SW620 cells induced metastases in a smaller number of animals than parental SW620 cells, and did not generate any adrenal gland or bone metastasis. Overall, our results suggest that SOCS1 controls metastatic progression of colorectal tumors by preventing the mesenchymal–epithelial transition (MET), including E-cadherin expression. This pathway may be associated with survival to colorectal cancer by reducing the capacity of generating metastases.



## 1. Introduction

The suppressors of cytokine signaling (SOCS) belong to a family of adaptor proteins that negatively regulate cellular signaling. The SOCS proteins contain a central SH2 domain that interacts with phosphorylated tyrosines (e.g. tyrosines residues located in the intracellular region of cytokine receptors, which become phosphorylated upon cell stimulation). In line with this notion, SOCS1 was originally described as an inhibitor of the signaling mediated by the cytoplasmic

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tyrosine kinases JAK (Janus kinase) (for reviews see Ilangumaran and Rottapel, 2003; Valentino and Pierre, 2006). In addition, the SOCS proteins contain a C-terminal SOCS box that interacts with regulators of the protein ubiquitylation machinery, Elongin B/C and Cullin5 (Kamura et al., 2004; Mahrour et al., 2008). Accordingly, SOCS1 plays a critical role in the proteasomal degradation of its binding partners (Zhang et al., 1999). Through these two modes of action, SOCS1 participates in the regulation of multiple cell functions. The neonatal lethality observed in *socs1* knockout mice (Alexander et al., 1999) highlights the physiological importance of this protein.

SOCS1 expression is tightly regulated by several mechanisms. SOCS1 is primarily regulated at the transcriptional level. Indeed, upon cytokine cell stimulation, active JAK kinases and downstream Signal Transducers and Activators of Transcription (STAT) proteins induce the expression of *SOCS* genes. Besides, the SOCS1 promoter is actively repressed by the nuclear proteins GFI-1B (Jegalian and Wu, 2002), Ets-1 (Travagli et al., 2004) and Sp2 (Letourneur et al., 2009). The *SOCS1* gene can also be regulated by methylation of the CpG islands located in the translated exon 2 (Yoshikawa et al., 2001). Stabilization of SOCS1 proteins by proteasome inhibitors suggests that cells may also regulate SOCS1 levels through the proteasome pathway (Zhang et al., 1999).

Expression of SOCS1 is often de-regulated in cancer cells. The *SOCS1* gene is methylated in human hepatocellular carcinoma (Yoshida et al., 2004; Yoshikawa et al., 2001) or colorectal tumors (Fujitake et al., 2004; Lin et al., 2004). In colon cancer, *SOCS1* gene methylation is mainly associated with the CpG island methylator phenotype (CIMP) subclass (Shen et al., 2007), and represents one of the nine high-ranking CIMP-predicting markers (Weisenberger et al., 2006). Besides, analysis of genetic variations in colorectal cancers indicated that single nucleotide polymorphisms (SNPs) in genes belonging to the JAK/STAT signaling pathway, including two in the *SOCS1* gene, were associated with cancer survival (Slattery et al., 2013).

Beyond being mere diagnostic/prognostic markers, genetic and epigenetic events occurring at the *SOCS1* locus may also have a functional relevance in cancers. On one hand, several studies have characterized *SOCS1* as a tumor suppressor gene. *socs1*$^{-/-}$ (Tg) mice, in which lethality conferred by the lack of SOCS1 is bypassed through expression of ectopic SOCS1 in T and B cells, spontaneously developed colorectal carcinomas at 6 months of age (Hanada et al., 2006). Of note, *socs1*$^{-/-}$ Tg mice treated with anti-IFN-$\gamma$ antibody did not develop such tumors, suggesting that chronic inflammation was a critical determinant for the development of these colorectal tumors. *In vitro* studies with human hepatocellular carcinoma-derived cell lines indicated that restoration of SOCS1 expression leads to growth-suppressing activity (Yoshikawa et al., 2001). Similarly, SOCS1 expression was found to be reduced in melanoma-derived tissues that had metastasized to the brain, as compared to the corresponding melanoma tumors (Huang et al., 2008). Thus, these studies suggested that SOCS1 has an important, protective role in cancer development. On the other hand, SOCS1 has been also identified as a progression marker of human melanoma. Indeed, in human melanoma, Li et al. (2004) indicated that the level of SOCS1 protein correlates with tumor invasion and stage of diseases. Specifically, melanocytes of the normal skin or melanocytic nevi lack SOCS1 protein expression while melanoma cells express SOCS1 (Li et al., 2004). The later finding suggests that the exact role of SOCS1 in tumor progression is likely to depend on the type of cancer considered.

Here we addressed the role of *SOCS1* in colorectal cancer (CRC). We report that while SOCS1 is well expressed in CRC, its expression level decreases with the aggressiveness of the tumors. By manipulating SOCS1 levels in colorectal tumor cells, we show that this protein controls the EMT process, and the tumor cell invasiveness and metastatic potential.

## 2. Material and methods

### 2.1. Plasmids

The pcDNA3-myc-m*Socs1*-expressing plasmid is a generous gift from Yasukawa et al. (1999). Site-specific mutations in this plasmid were performed using the quick change site-directed mutagenesis kit (Stratagene) and the oligonucleotides described in Table S1. For the ΔSOCS-box mutant, a stop codon was introduced at position 760 (NM_001271603.1), just before the SOCS-box-encoding sequence. Those plasmids were used for SW620 cell transfection. For retroviral infection of SW620, HT29 or HCT116 cells, the mutated SOCS1-encoding sequences were subcloned into the pBabe vector in-between the BamH1 and XhoI restriction sites. For each construct, the SOCS-encoding region and the cloning junctions were verified by sequencing.

Silencing of ectopic SOCS1 expression was performed through lentiviral infection using a PLKO vector (TRCN0000067419) from OpenBioSystems, in which the targeting sequence of the short hairpin (mature antisense) was CGCATCCCTCTTAACCCGGTA. Silencing of CDH1 expression was performed using GIPZ lentiviral CDH1 ShRNA (CDH1 ShRNA-1-GFP: #V3LHS_346823: AAAATTTCCAATTTCATCG; CDH1 ShRNA-2-GFP: #V2LHS_14834: ATAATAAAGACACCAACAG) from OpenBioSystems. The pMD2.G (VSV-G) and pCMVdeltaR8.74 plasmids, as well as the lentiviral construct carrying an expression cassette containing the firefly luciferase and the green fluorescent protein (GFP) genes separated by an internal ribosomal entry site (pMEGIX-Luc plasmid), were kindly provided by Dr J.L. Villeval (UMR 1009; Villejuif, France) (Morgenstern and Land, 1990).

### 2.2. Viruses production and transduction of cells

Replication-defective lentiviral particles were produced by transient transfection of 293T cells with the above-mentioned plasmids, together with the pMD2.G (VSV-G) and pCMVdeltaR8.74 plasmids, according to standard protocols (Naldini et al., 1996). The day before transduction, cells were plated at low cell density (30,000 cells/well) in a 24-well dish. Viruses were added to cells for 24 h. To obtain stable cell lines in a polyclonal background, the GFP-positive luciferase-expressing cells (when applicable) were sorted for green fluorescence using a MoFlo cell sorter (Beckman–Coulter, Miami, FL) and maintained under selective pressure in the presence



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