SOCS1 Silencing Enhances Antitumor Activity of Type I IFNs by Regulating Apoptosis in Neuroendocrine Tumor Cells

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Abstract

IFN-α is commonly used for biotherapy of neuroendocrine carcinomas. However, its antitumor efficacy is often limited due to IFN resistance. In this study, we evaluate the role of suppressor of cytokine signaling protein 1 (SOCS1) in modulating the effects of type I IFNs (IFN-α and IFN-β) in human neuroendocrine BON1 and CM tumor cells. In both cell lines, type I IFNs activated signal transducers and activators of transcription (STAT) and significantly decreased cell viability. However, the effects of IFN-β were significantly more pronounced than those of IFN-α and involved the induction of the intrinsic apoptotic pathway as shown by cleavage of caspase-8, Bid, and caspase-9. Stable overexpression of SOCS1 completely abolished the apoptotic effects of both type I IFNs. In contrast, small interfering RNA (siRNA)–mediated silencing of SOCS1 resulted in strongly enhanced type I IFN signaling as shown by increased and prolonged STAT phosphorylation and stronger induction of apoptosis. Silencing of SOCS1 was associated with down-regulation of basal Bcl-2 and Bcl-XL and up-regulation of basal Bak and Bax, suggesting that reduced SOCS1 expression might lower the threshold of susceptibility to type I IFN–mediated apoptosis by decreasing the ratio of antiapoptotic to proapoptotic molecules. In summary, our results indicate an important role of SOCS1 in IFN resistance of neuroendocrine tumor cells, mediated through negative regulation of type I IFN–induced Jak/STAT signaling. Knocking down SOCS1 by siRNA is a promising new approach to enhance the therapeutict potency of type I IFNs in neuroendocrine tumors. [Cancer Res 2007;67(10):5025–52]

Introduction

Neuroendocrine tumors of the gastroenteropancreatic system are a rare and heterogeneous category of tumors. The majority of neuroendocrine tumors have already metastasized at the time of diagnosis, resulting in a 5-year survival rate of less than 50% (1, 2). IFN-α is used for biotherapy of various tumors, including neuroendocrine tumors (1, 3–5). Although IFN-α generally provides excellent control of hypersecretion-related symptoms, only 10% of patients respond with partial tumor remission (3, 4, 6). Compared with IFN-α, IFN-β has been shown to exhibit more potent growth-inhibitory effects on neuroendocrine BON1 tumor cells in vitro (7).

IFN-α and IFN-β belong to the group of type I IFNs that comprises three major subtypes (IFN-α, IFN-β, and IFN-ω). All type I IFNs display significant structural homology and bind to the same receptor complex. The type I IFN receptor consists of two subunits: IFNAR-1 and IFNAR-2 (8). Specificity of distinct type I IFNs is assumed to be mediated by receptor complex formation of IFNAR-1 and different splice variants of IFNAR-2 (IFNAR-2a, IFNAR-2b, and IFNAR-2c; refs. 7, 9, 10).

Signaling through type I IFN receptor leads to the activation of Janus tyrosine kinases (Jak1 and TYK2), which in turn phosphorylate signal transducers and activators of transcription (STAT) proteins 1 and 2. STAT1 and STAT2 heterodimerize and, together with the accessory factor IFN regulatory factor 9 (p48), form a transcription factor complex that is known as IFN-stimulated gene factor 3 (ISGF3). Finally, ISGF3 translocates to the nucleus and activates the transcription of IFN-stimulated genes, the products of which account for the antiviral, antitumor, and immunomodulatory properties of IFNs (11).

The Jak/STAT signaling cascade is negatively regulated by suppressor of cytokine signaling (SOCS) proteins (12–14). The family of SOCS proteins comprises eight members (SOCS1–7 and CIS), all sharing a central SH2 domain and a COOH-terminal SOCS box motif (15). As their expression is inducible by a variety of cytokines through STAT-dependent mechanisms, SOCS proteins form part of a powerful negative feedback loop of cytokine signaling. SOCS1 and SOCS3 are the most effective inhibitors of Jak/STAT signaling (16, 17). They mediate their action by either interacting with cytokine receptors or directly binding to phosphorylated tyrosine residues of Jak (18, 19). Studies with SOCS1- or SOCS3-deficient mice indicate that SOCS1 is primarily a negative regulator of IFN signaling (20, 21), whereas SOCS3 is primarily a negative regulator of interleukin-6 signaling (22). Increased SOCS1 expression inhibited IFN-mediated antitumor and antiviral effects in a variety of tumor cell lines and was found to correlate with poor response to IFN-α in chronic myeloid leukemia and HCV in vivo (17, 23–27).

Here, we comparatively investigate the effects of SOCS1 overexpression and SOCS1 silencing on type I IFN signaling in neuroendocrine tumor cells. Our findings indicate that SOCS1 has a critical role in regulating the extent of type I IFN response and prove that small interfering RNA (siRNA)–mediated silencing of SOCS1 could be a promising therapeutic strategy to enhance the antitumor effects of type I IFNs in neuroendocrine tumors.

Materials and Methods

Reagents. Human IFN-α was purchased from Roche, and IFN-β1a (Rebi) was kindly provided by Serono, Inc.

Cell culture and stable transfection. Human pancreatic neuroendocrine BON1 tumor cells were kindly provided by R. Göke (Marburg, Germany).
BON1 cells were cultured in DMEM/F12 (1:1) medium (Life Technologies/Invitro) supplemented with 10% FCS (Biochrom), 1% penicillin/streptomycin (Life Technologies), and 0.4% amphotericin B (Biochrom) in a 5% CO₂ atmosphere. Stable BON1 clones were established as described previously (27). Human insulinoma CM cells were kindly provided by P. Pozzilli (Rome, Italy). CM cells were cultured in RPMI (PAA) supplemented with 10% FCS, 1% penicillin/streptomycin, and 0.4% amphotericin B.

**Transfection of siRNA.** BON1 and CM cells were transfected with non-targeted b-Gal siRNA (sense sequence, UUAUGCCGAUCGCGUCACAUU) or SOCS1 siRNA (sense sequence, GCAUCCGCGGCUAAUCUAAU) using DharmaFECT (Dharmacon) following the manufacturer’s protocol. Briefly, cells were seeded in antibiotic-free complete medium and incubated for 24 h before transfection of siRNAs was done. For transfection, 3 μL of DharmaFECT 3 reagent was added to 97 μL Opti-MEM I (Invitrogen) and incubated for 5 min, before 25 μL of 2 μmol/L oligonucleotides and 75 μL Opti-MEM I were added. After 20 min of incubation, the DharmaFECT 3/oligonucleotide mixture was diluted with serum-free DMEM/F12 (1:1) medium and added to BON1 cells in a final concentration of 50 nmol/L.

**RNA isolation and quantitative reverse transcription-PCR.** RNA isolation and quantitative reverse transcription-PCR (RT-PCR) were done as described previously (28), using follow-up primers for amplification: human SOCS1 forward and reverse, 5’-CACGACTCTCCGACATT-3’ and 5’-ACGACCTGAAGGACAGTC-3’, respectively; human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) forward and reverse, 5’-ACCACCTCTCCACCTTTGA-3’ and 5’-CTGTGGCTGATGCAAAATTCTG-3’, respectively. PCR was done using 2.0 units of Platinum Taq DNA Polymerase (Invitrogen) and a final concentration of 1.5 mmol/L MgCl₂ under following conditions: 35 cycles of denaturing at 94°C for 30 s, annealing at 58°C for 30 s, and extension at 72°C for 30 s. Relative expression levels were calculated using the comparative ΔΔCt method and internal GAPDH for normalization.

**Protein extraction and Western blotting.** Protein extraction and Western blotting were done as previously described in detail (27, 29). Primary antibodies used were pSTAT1, Bid, and Bcl-2 (BD Transduction Laboratories); pSTAT2 and STAT2 (Upstate Biotechnology); STAT1 (Santa Cruz Biotechnology); Bcl-xL, caspase-8, caspase-9, caspase-3, and Poly(ADP-ribose) polymerase (PARP; Cell Signaling); Bax and Bak (UBI); and β-actin (Sigma).

**Assessment of cell viability.** BON1 and CM cells were seeded into 96-well plates at a density of 2,500 per well and grown for 24 h. Next, the cells were incubated with various concentrations of IFN-α (10, 100, and 1,000 IU/mL) or IFN-β (1, 5, 50, 100, and 1,000 IU/mL) in complete medium containing 10% FCS. Metabolic activity was measured with Cell Titer 96 AQueous One Solution Cell Proliferation Assay (Promega) 6 days after stimulation according to the manufacturer’s instructions. Following 3 h of incubation with Cell Titer 96 AQueous One Solution Cell Proliferation Assay, absorbance at 492 nm was determined using an ELISA plate reader.

**Quantification of DNA fragmentation and cell cycle analysis.** The rate of apoptotic cell death was quantified by determining DNA fragmentation according to Nicoletti et al. (30). Briefly, cells were incubated for 24 h in a hypotonic buffer (1% sodium citrate, 0.1% Triton X-100, 50 μg/mL propidium iodide) and analyzed by flow cytometry on a FACSCalibur (Becton Dickinson) using CellQuest software (Becton Dickinson). Nuclei to the left of the “G1-peak” containing hypodiploid DNA were considered apoptotic.

**Hoechst staining.** For morphologic assessment of chromatin condensation and DNA fragmentation, cells were fixed with 4% paraformaldehyde in PBS for 20 min followed by washing with PBS. The fixed cells were stained with 250 μg/mL Hoechst 33258 (Sigma) in PBS for 1 h and subsequently examined by fluorescence microscopy.

**Statistical analysis.** Statistical analysis was done using two-tailed Student’s t test. P < 0.05 was considered statistically significant.

**Results.**

**SOCS1 modulates the extent of type I IFN–induced STAT phosphorylation.** To investigate the role of constitutive SOCS1 expression in IFN resistance, we created SOCS1 overexpressing BON1 cell clones. For further experiments, one pCR3.1/SOCS1 clone was selected based on maximal overexpression as determined by quantitative PCR. Compared with the mock-transfected control clone (pCR3.1/mock), SOCS1 mRNA expression was increased ~9-fold in the selected pCR3.1/SOCS1 clone (Fig. 1A). Previous studies reported type I IFNs to induce STAT phosphorylation in several cell lines (31–33). We therefore investigated the ability of IFN-α and IFN-β to stimulate tyrosine phosphorylation of STAT proteins.

Figure 1. SOCS1 overexpression abrogates type I IFN–induced STAT phosphorylation. A, BON1 cells were stably transfected with pCR3.1/SOCS1. Up-regulation of SOCS1 mRNA expression in the selected pCR3.1/SOCS1 clone was verified by quantitative PCR. Each sample was run in triplicate. *** P < 0.001 versus pCR3.1/mock. B, pCR3.1/mock BON1 clones and pCR3.1/SOCS1 BON1 clones were treated with indicated concentrations of IFN-α or IFN-β for 15 min. Protein expression of phosphorylated STAT1 and STAT2 (pSTAT1 and pSTAT2) and total STAT1 and STAT2 was evaluated by Western blot analysis. One representative blot of three experiments.

Cancer Res 2007; 67: (10). May 15, 2007 5026 www.aacrjournals.org

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STAT1 and STAT2 in neuroendocrine tumor cells. In pCR3.1/mock-transfected BON1 cells, treatment with IFN-α or IFN-β dose-dependently induced STAT1 and STAT2 tyrosine phosphorylation within 15 min (Fig. 1B). In comparison, IFN-α- and IFN-β-induced activation of STAT1 and STAT2 was strongly inhibited in pCR3.1/SOCS1 clones (Fig. 1B).

To investigate the comparative effects of increased and reduced SOCS1 expression on type I IFN-mediated Jak/STAT signaling, we identified a siRNA duplex specifically down-regulating SOCS1. The SOCS1 siRNA duplex was efficiently transfected into BON1 tumor cells by DharmaFECT 3 (Dharmacon). Additionally, a non-targeted siRNA (β-Gal) was transfected in each experiment as a control. As verified by quantitative RT-PCR, basal SOCS1 mRNA expression in BON1 cells transfected with SOCS1 siRNA was decreased by ~80% compared with BON1 cells transfected with β-Gal siRNA (Fig. 1B). In addition, we investigated the effects of SOCS1 siRNA transfection on type I IFN-induced SOCS1 mRNA expression. In BON1 cells transfected with β-Gal siRNA, 60 min of incubation with IFN-α (1,000 IU/mL) and IFN-β (1,000 IU/mL) strongly induced SOCS1 mRNA expression ~4- and ~14-fold, respectively. In comparison with the IFN-α- and IFN-β-induced SOCS1 mRNA levels in β-Gal siRNA-transfected BON1 cells, IFN-α- and IFN-β-induced SOCS1 mRNA levels in SOCS1 siRNA-transfected BON1 cells were suppressed by ~60% and ~80%, respectively (Fig. 2A). In β-Gal siRNA-transfected BON1 cells, IFN-α (1,000 IU/mL) and IFN-β (1,000 IU/mL) strongly induced STAT1 and STAT2 phosphorylation, which decreased quickly and was barely detectable after 4 h (Fig. 2B). In contrast, SOCS1 siRNA-transfected BON1 cells showed enhanced and prolonged STAT1 and STAT2 phosphorylation in response to IFN-α and IFN-β, which was still strongly detectable after 4 h (Fig. 2B).

To confirm the critical role of SOCS1 as cause and target of IFN resistance in neuroendocrine tumor cells, we extended our experiments to the human insulinoma cell line CM. CM tumor cells were efficiently transfected with siRNA using DharmaFECT 2 (Dharmacon). Compared with β-Gal siRNA-transfected CM cells, basal as well as IFN-α- and IFN-β-induced SOCS1 mRNA expression levels were significantly decreased by ~45%, ~70%,
and ~ 85% in SOCS1 siRNA-transfected CM cells (Fig. 3A). Similar to the results obtained for BON1 cells, down-regulation of SOCS1 potently enhanced and prolonged type I IFN–induced phosphorylation of STAT1 and STAT2 in CM cells (Fig. 3B).

SOCS1 modulates type I IFN–mediated effects on cell viability. The effects of IFN-α and IFN-β on cell viability of SOCS1 overexpressing and SOCS1-deficient cells were investigated in cell viability assays. Both type I IFNs significantly decreased the number of viable pCR3.1/mock–transfected BON1 clones (Fig. 4A). The antitumor effect of IFN-β was more pronounced than that of IFN-α as shown by a higher maximal reduction of viable cells after 6 days (4 ± 1% and 45 ± 7%, respectively; Fig. 4A). However, the antitumor effects of both type I IFNs were completely abrogated in pCR3.1/SOCS1–transfected BON1 clones (Fig. 4A).

In marked contrast, siRNA-mediated silencing of SOCS1 potently enhanced the loss of BON1 cell viability in response to type I IFNs (Fig. 4B and C). After 6 days of incubation with IFN-α or IFN-β, the maximal reduction of viable SOCS1 siRNA-transfected BON1 cells compared with viable b-Gal siRNA-transfected BON1 cells was strongly increased for both type I IFNs (from 16 ± 6% to 26 ± 5% and 49 ± 8% to 78 ± 5%; Fig. 4B). Additionally, down-regulation of SOCS1 significantly heightened the sensitivity of BON1 cells to very low concentrations of IFN-β (Fig. 4C). As shown in Fig. 4D and E, similar results were obtained for CM cells, suggesting that SOCS1 inhibition might be a general mechanism to increase type I IFNs’ antitumor effects in neuroendocrine tumor cells.

SOCS1 modulates the extent of type I IFN–induced apoptosis. To evaluate the mechanisms underlying the loss of neuroendocrine tumor cell viability, we analyzed whether treatment with IFN-α or IFN-β results in the induction of apoptosis. As assessed by flow cytometry, a 72-h exposure of b-Gal siRNA-transfected BON1 cells to 1,000 IU/mL IFN-α or IFN-β increased the fraction of cells with sub-G1 DNA content (Fig. 5A). Furthermore, Hoechst staining showed cell shrinkage and chromatin condensation (Fig. 5A). Significantly, down-regulation of SOCS1 potently enhanced these hallmarks of apoptosis (Fig. 5A and B).

Figure 3. Down-regulation of SOCS1 enhances and prolongs type I IFN–induced STAT phosphorylation in CM insulinoma cells. A, BON1 cells were transfected with non-targeted b-Gal siRNA or SOCS1 siRNA using DharmaFECT 2. Forty-eight hours later, cells were treated with 1000 IU/mL IFN-α or IFN-β for 60 min. SOCS1 mRNA expression levels in b-Gal- or SOCS1 siRNA-transfected cells were verified by quantitative PCR. Each sample was run in triplicate. ***, P < 0.001 versus untreated control. ++, P < 0.01; ++++, P < 0.001 versus the b-Gal siRNA-transfected comparison group. B, CM cells transfected with b-Gal siRNA or SOCS1 siRNA were incubated with indicated concentrations of IFN-α or IFN-β for 15 min, 2 h, or 4 h. Protein expression of phosphorylated and total STAT1 and STAT2 was evaluated by Western blot analysis. One representative blot of three experiments.
To elucidate the pathways by which BON1 cells undergo type I IFN–induced apoptosis, we analyzed caspase activation by Western blot technique. Treatment with IFN-α slightly induced cleavage of caspase-8, caspase-9, and caspase-3 as well as cleavage and inactivation of PARP (Fig. 5C). However, this effect of IFN-α was markedly enhanced in SOCS1 siRNA-transfected BON1 cells (Fig. 5C). As the caspase-8 substrate Bid provides a link between the extrinsic and the intrinsic apoptotic pathway, we monitored Bid levels after exposure to IFN-α or IFN-β for 24 h. In b-Gal siRNA-transfected BON1 cells, IFN-β caused a slight decrease in Bid protein levels, an effect that was strikingly increased in SOCS1 siRNA-transfected BON1 cells (Fig. 5D). Given the importance of Bcl-2 family members in apoptosis regulation (34, 35), expression of these proteins was examined after treatment with IFN-α or IFN-β for 24 h. In b-Gal siRNA-transfected BON1 cells, IFN-β caused a slight decrease in Bcl-2 and Bcl-xL protein levels, an effect that was strikingly increased in SOCS1 siRNA-transfected BON1 cells (Fig. 5D). Given the importance of Bcl-2 family members in apoptosis regulation (34, 35), expression of these proteins was examined after treatment with IFN-α or IFN-β for 24 h. In b-Gal siRNA-transfected BON1 cells, no major changes in Bcl-2 and Bcl-xL protein levels were noted during treatment with IFN-α and IFN-β, whereas SOCS1 siRNA-transfected BON1 cells exhibited a marked decrease in basal as well as type I IFN–induced Bak expression and also slightly increased basal as well as type I IFN–induced levels of Bax (Fig. 5D).

Down-regulation of SOCS1 affects cell cycle phase distribution. Type I IFNs have been previously shown to induce S-phase accumulation in BON1 cells (7). Consistent with these findings, b-Gal siRNA-transfected BON1 cells responded to both type I IFNs with a slight accumulation of cells in the S phase, which was significant only for IFN-β (Fig. 6A). Moreover, treatment with IFN-α and IFN-β significantly decreased the fraction of cells in G0-G1 phase (Fig. 6A). We next examined whether down-regulation of SOCS1 affected cell cycle phase distribution. Compared with untreated b-Gal siRNA-transfected BON1 cells, untreated SOCS1 siRNA-transfected BON1 cells showed a significantly reduced S-phase fraction as well as a significantly increased G0-G1 phase fraction (Fig. 6B). This finding indicates that down-regulation of SOCS1 results in G0-G1 phase accumulation per se. In addition, treatment of SOCS1 siRNA-transfected BON1 cells with IFN-β strongly induced S-phase accumulation, suggesting that SOCS1 inhibition and type I IFNs might exhibit additive antiproliferative effects by blocking normal cell cycle progression at two different stages.

Discussion

Type I IFNs inhibit proliferation of various cell types by transducing regulatory signals through the Jak/STAT pathway.
IFN-α is commonly used for biotherapy of several malignancies, including neuroendocrine tumors of the gastroenteropancreatic system. However, its antitumor efficacy is often limited due to IFN resistance (1, 3–5). Several overexpression studies have shown that SOCS1 is a critical negative regulator of type I IFN signaling \textit{in vitro} (17, 23, 27, 33). Moreover, increased SOCS1 expression was recently found to correlate with poor antitumor and antiviral response to IFN-α \textit{in vivo} (25, 26). However, the consequences of decreased SOCS1 expression on the effects of exogenously administered type I IFNs have not been characterized. In the present study, we therefore targeted SOCS1 by siRNA to enhance the antitumor effects of type I IFNs on neuroendocrine tumor cells. Transfection of a single SOCS1 siRNA duplex significantly reduced basal as well as IFN-α– and IFN-β–induced SOCS1 mRNA expression levels in BON1 and CM cells. A recent study has shown that bone marrow macrophages from SOCS1−/− mice responded to IFN-α with prolonged tyrosine phosphorylation of STAT1 (21). Consistently, we showed that type I IFN–induced tyrosine phosphorylation of STAT1 and STAT2 in cells transfected with SOCS1 siRNA was markedly increased and prolonged compared with cells transfected with non-targeted siRNA. As a consequence of enhanced and prolonged STAT signaling, SOCS1 silencing significantly amplified the antitumor effects of IFN-α and IFN-β.

The mechanisms by which IFN-α and IFN-β mediate these antitumor activities are only partially understood but can involve apoptosis and interference of normal cell cycle progression (36, 37). In several cancer cell types (including glioma, breast cancer, hepatoma, and neuroendocrine tumor cells), IFN-β has greater antitumor effects than IFN-α via the induction of apoptosis (7, 38–40). In agreement with these studies, we found that in BON1 and CM cells, IFN-β exhibited higher antitumor activity than IFN-α, which was largely mediated by the induction of apoptosis. Exposure of b-Gal–transfected BON1 cells to IFN-β induced modest cleavage of caspase-8, Bid, caspase-9, caspase-3, and PARP. Significantly, siRNA-mediated down-regulation of SOCS1 strongly enhanced this proapoptotic response to IFN-β, indicating that reduced SOCS1 expression facilitates recruitment of the intrinsic apoptotic pathway.

Given the importance of Bcl-2 family members as major regulators of the intrinsic apoptotic pathway (34, 35), we analyzed...
expression levels of antiapoptotic Bcl-2/Bcl-XL and proapoptotic Bak/Bax. Compared with b-Gal siRNA-transfected BON1 cells, SOCS1 siRNA-transfected BON1 cells exhibited a marked decrease in basal protein levels of Bcl-2 and Bcl-XL. Concomitantly, basal protein levels of Bak and Bax were elevated in SOCS1 siRNA-transfected BON1 cells. These findings suggest that reduced SOCS1 expression might lower the threshold of susceptibility to type I IFN–mediated apoptosis by decreasing the ratio of antiapoptotic to proapoptotic molecules.

Despite their proapoptotic properties, type I IFNs can affect all phases of the cell cycle, most commonly by blocking G1-S progression (6, 41–43). In neuroendocrine BON1 tumor cells, IFN-α and IFN-β have been shown to induce S-phase accumulation (7). In our study, we found IFN-α and IFN-β to slightly induce S-phase accumulation and to significantly decrease the fraction of cells in G0-G1 phase. Compared with untreated BON1 cells transfected with b-Gal siRNA, untreated BON1 cells transfected with SOCS1 siRNA showed a significantly reduced S-phase fraction as well as a significantly increased G0-G1 phase fraction. This finding indicates that down-regulation of SOCS1 results in G0-G1 phase accumulation per se. Furthermore, treatment of SOCS1 siRNA-transfected BON1 cells with IFN-β strongly induced S-phase accumulation, suggesting that SOCS1 inhibition and type I IFNs exhibit additive antiproliferative effects by blocking normal cell cycle progression at two different stages.

In summary, this is the first study showing that siRNA-mediated silencing of SOCS1 strongly enhances the antitumor effects of type I IFNs in vitro. Furthermore, Shen et al. have recently reported that siRNA-mediated SOCS1 silencing in murine antigen-presenting dendritic cells strongly enhanced antigen-specific antitumor immunity (44). This important finding suggests that systemic inhibition of SOCS1 might not only enhance the direct antitumor effects of exogenously administered type I IFN but also the general antitumor immune response.

Although the efficacy of systemic RNA interference–mediated silencing of SOCS1 in vivo still has to be proven, several genes have already been successfully silenced after systemic delivery of siRNA in mice and non-human primates (45–47) and future use of this technique in humans seems feasible. Our results indicate that siRNA-mediated silencing of SOCS1 is a powerful tool to overcome IFN resistance and might help to considerably improve the therapy of neuroendocrine tumors and other malignancies.

Acknowledgments

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Figure 6. Down-regulation of SOCS1 affects cell cycle phase distribution. A and B, BON1 cells transfected with b-Gal siRNA (A) or SOCS1 siRNA (B) were incubated with 1,000 IU/mL IFN-α or IFN-β for 72 h. Subsequently, the proportion of cells in G0-G1, S, and G2-M phase was analyzed by flow cytometry. Columns, mean of four independently done experiments. A, significant differences: P < 0.05, for G0-G1 phase fraction of IFN-α–treated cells versus untreated control; P < 0.001, for G0-G1 phase fraction of IFN-β–treated cells versus untreated control; P < 0.05, for S-phase fraction of IFN-β–treated cells versus untreated control. B, significant differences: P < 0.001, for G0-G1 and S-phase fraction of IFN-α–treated cells versus untreated control.

Received 7/12/2006; revised 2/27/2007; accepted 3/1/2007.

Grant support: Deutsche Forschungsgemeinschaft grants BR 1912/5-1 and KFO 128 and Else-Kröner-Fresenius-Stiftung grant P60/05/EKMS 05/62.

Cancer Res 2007; 67: (10), May 15, 2007
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