Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 335 (2005) 168–174
BBRC
www.elsevier.com/locate/ybbrc

Estrogen receptor-α regulates SOCS-3 expression in human breast cancer cells

Jason Matthews [a,*], Tova Almlöf [a], Silke Kietz [a], Jörg Leers [a], Jan-Åke Gustafsson [a,b]

[a] Department of Biosciences at Novum, Karolinska Institutet, Novum, S-14157 Huddinge, Sweden
[b] Department of Medical Nutrition, Karolinska Institutet, Novum, S-14186 Huddinge, Sweden

Received 6 July 2005
Available online 25 July 2005

Abstract

The suppressor of cytokine signalling (SOCS) protein family negatively regulates cytokine action. In this study, we investigated the effects of estrogen (E2) on SOCS-3 expression in T47D and MCF-7 human breast cancer cells. Real-time PCR analysis of E2-treated T47D cells revealed a ligand and time-dependent increase in of SOCS-3 mRNA levels. Cloning of a 1.7 kb fragment of the human SOCS-3 5′ flanking sequence, and subsequent analysis of potential transcription factor-binding sites identified an incomplete ERE motif located −1493 to −1489 upstream of the start site. Transient transfection of the cloned fragment in MCF-7 cells showed that both E2 and genistein treatment caused an increase in reporter gene activity, which was inhibited by co-treatment with ICI 182,780. Chromatin immunoprecipitation analysis revealed an E2 and time-dependent recruitment of ERα to the E2 responsive region of the human SOCS-3 promoter. In summary, this study shows that ERα directly regulates human SOCS-3 promoter activity in human breast cancer cells, thus modulating cytokine activity.

Keywords: Estrogen receptor; SOCS-3; Cytokine; STAT; Chromatin immunoprecipitation; Nuclear receptor; Promoter cloning

Cytokines mediate their effects through interactions with cell membrane receptors resulting in changes in cellular homeostasis. Ligand binding to cytokine receptors causes activation of the Janus kinases (JAKs) and subsequent phosphorylation of signal transducers and activators of transcription (STATs) [1]. Phosphorylated STATs dissociate from the receptor and form homo- or heterodimers. STAT dimers then translocate to the nucleus, where they bind their cognate DNA response elements within the promoter of target genes initiating transcription [2]. The negative regulation of cytokine-induced signalling pathways involves a number of proteins. There are three different classes of negative regulators of cytokine action: the SH2-containing protein tyrosine phosphatase I (SHPI), the protein inhibitors of activated STATs (PIAS), and the suppressors of cytokine signalling (SOCS). The SOCS-protein family consists of SOCS1–7 and cytokine-inducible SH2 containing protein (CIS) and their expression is induced by a number of cytokines and hormones, such as estrogen (E2) [3].

The biological actions of estrogens are mediated by binding to one of two specific estrogen receptors (ERs), ERα or ERβ, which belong to the nuclear receptor superfamily, a family of ligand-regulated transcription factors [4]. Both ERs are widely distributed throughout the body, displaying distinct but overlapping expression patterns in a variety of tissues [4,5].

Recently, E2 has been demonstrated to up-regulate SOCS-2, but not SOCS-1 or SOCS-3 expression in human embryonic kidney (HEK293) cells [6], whereas the expression of both SOCS-2 and SOCS-3 was increased in liver in response to E2 [7]. These data suggest that the regulation of SOCS proteins exhibits cell-type and species variations. The promoter regions of SOCS-2 and SOCS-3 have been cloned and contain several

* Corresponding author. Fax: +46 8 7745538.
E-mail address: jason.matthews@biosci.ki.se (J. Matthews).

0006-291X/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2005.07.057

STAT elements [8]. A functional estrogen responsive element (ERE) is present in the murine SOCS-3 (mSOCS-3) promoter [7]. A portion of the human SOCS-3 (hSOCS-3) promoter has also been cloned, and this sequence also contains several STAT elements, but no ERE motif [9]. However, only a 1.1 kb fragment of the hSOCS-3 promoter has thus far been reported and upstream regulatory elements remain to be identified. Although the expression of SOCS-3 can be regulated by ERs, it remains to be determined whether this regulation occurs at the transcriptional level.

In this study, we investigated the effects of E2 on SOCS-3 expression in T47D and MCF-7 human breast cancer cells. We report the cloning of a 1.7 kb fragment of the hSOCS-3 5′ flanking sequence. Subsequent analysis of potential transcription factor-binding sites identified an ERE motif located −1493 to −1489 upstream of the start site. Transient transfection of the cloned fragment in MCF-7 human breast carcinoma cells showed that both E2 and genistein treatment caused an increase in reporter gene activity, which was inhibited by co-treatment with the pure antagonist ICI 182,780. E2 treatment of T47D human breast cancer cells resulted in a temporal increase of SOCS-3 mRNA levels. Furthermore, chromatin immunoprecipitation (ChIP) analysis revealed the time-dependent recruitment of ERα to the E2 responsive region of the hSOCS-3 promoter, suggesting that ER directly regulates SOCS-3 promoter activity thus providing another mechanism by which E2 can modulate cytokine activity.

Materials and methods

Chemicals and biochemicals. 17β-Estradiol, dihydrotestosterone, 4-hydoxytamoxifen, ICI182,780, and genistein were from Sigma (St. Louis, MO). Antibodies used for ChIP included, for ERα, H-184 (Santa Cruz Biotechnology, Santa Cruz, CA). All other chemicals and biochemicals were of the highest quality available from commercial sources.

RNA isolation and real-time PCR. T47D cells were seeded in 6-well plates and grown in phenol red-free DMEM supplemented with 5% DCC-FCS for 2 days prior to treatment with ligands. RNA was isolated using RNAeasy spin columns (Qiagen). One microgram of the extracted RNA was pre-treated with DNaseI for 15 min at room temperature, and then reverse transcribed using random hexamer primers and SuperScriptII (Invitrogen). Real-time PCR was performed with 1 μl of the cDNA synthesis reaction using SYBR green (Invitrogen). For the SOCS3 mRNA, the primers were 5′-GAAGATCC CCCTGGTGTTGA-3′ and 5′-TTCCGACAGAGATGCTGAAGA GT-3′.

Cloning of the human SOCS-3 promoter. Cloning of the hSOCS-3 promoter was done by querying the Entrez human genomic databases with the mRNA sequence of hSOCS3. A match was found to BAC clone number clone RP11-806H10 (GenBank Accession No. AC061992). Subsequent computational analysis using the transcription factor-binding site identification software Genomatrix MatInspector [10] revealed a putative E2 responsive motif located at −1504 to −1486. A 1.7 kb fragment of the hSOCS-3 5′ regulatory region was PCR amplified using primers 5′-CAAAGAGCTCAGTGGGCCGAG GCTGGGTAG-3′ and 5′-CAAAAGATCTGGCGCACGGAGCCA GCGTGGATCTG-3′; the underlined sequences represent restriction enzymes *Sac*I and *Bgl*II, respectively. The amplified sequence was TA-cloned into pGEMTeasy (Promega), excised using *Sac*I and *Bgl*II and subcloned into pGL3 basic creating pGL3-hSOCS-3, which contained −1673 to −1 of the hSOCS-3 promoter. Mutation of the ERE motif (pGL3-mutERE) was done using PCR mutagenesis with pGL3-hSOCS3 as template and the primers 5′-CGGGCCGCCTGAATTC CCGCAGTT-3′ and 5′-AACTGCGGGAATTCAGGCGGCCCG-3′. The plasmid containing the deletion of the incomplete ERE site pGL3-delERE was generated by digesting pGL3-hSOCS3 with *Sac*I and *Xho*I. The ends were filled in using Klenow and the restriction enzyme sites were lost after blunt end ligation, producing pGL3-delERE encompassing −808 to −1 of the hSOCS-3 promoter. All plasmids were sequenced to ensure that only the desired mutations were generated.

Transient transfection. MCF-7 human breast carcinoma cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Invitrogen), supplemented with 5% fetal calf serum (FCS) (Invitrogen), 2 mM L-glutamine, and 1% penicillin/streptomycin, and cells were maintained at 37 °C containing 5% CO_2. For transient transfection experiments, cells were seeded in 6-well plates in phenol red-free DMEM supplemented with 5% dextran-coated charcoal (DCC)-treated FCS 24 h before transfections. Cells were transfected with Lipofectamine 2000 according to the manufacturer's recommendations (Invitrogen). All reactions included 10 ng pCH110-β-Gal (Pharmacia) to normalize for transfection efficiency. After transfection, cells were treated with ligands for 24 h before luciferase (Biothema, Dalarö, Sweden) and β-galactosidase assays were performed (Tropix, Bedford, MA).

Chromatin immunoprecipitation. ChIP assays were performed essentially as previously described [11]. ChIP DNA (5 μl) was amplified by PCR with primers 5′-CCGCTGCCTGGCTGTGGGGTAG-3′ and 5′-CTTCGGGAGAGCGGGCAGTTC-3′ for hSOCS-3 promoter and 5′-CAAAGAGCTCATGGTCACCCACAGCAAGTTTCCC-3′ and 5′-TTAAAGCGGGGCATCGTACTGGT-3′ for hSOCS-3 mRNA region.

Results

Human SOCS-3 is an estrogen responsive target gene

SOCS-3 was identified as an estrogen responsive gene from preliminary experiments using membrane arrays (Clontech) probed with RNA isolated from exposed T47D cells (data not shown). Real-time PCR was then used to verify the result from the membrane array experiments, and confirmed the E2-dependent increase in the expression of SOCS-3, and showed that SOCS-3 mRNA was strongly induced after 1 h of E2 treatment, but rapidly returned to basal levels after 3 h (Fig. 1). In addition, no induction was seen after 6 h, suggesting that SOCS-3 mRNA levels are tightly regulated. This temporal induction pattern of SOCS-3 mRNA is similar to that reported by others. SOCS-3 mRNA was rapidly induced after 1 h exposure to erythropoietin in 32D cells, which was markedly reduced after 3 h and not detectable after 8 h of treatment [12].

Cloning of a 1.7 kb fragment of the human SOCS-3 promoter

To date only a 1.1 kb fragment of the human SOCS-3 (hSOCS-3) 5′ genomic sequence has been reported [9]. The sequence is very GC-rich and contains several

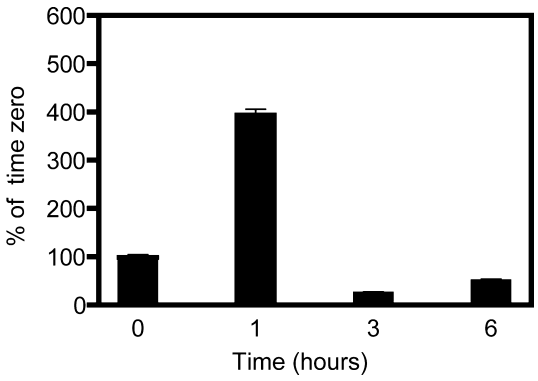

Fig. 1. Estrogen and time-dependent increase in SOCS-3 mRNA in T47D cells exposed to 10 nM estradiol. RNA isolated from exposed cells was reverse transcribed as described in Materials and methods. Expression levels of SOCS-3 mRNA were determined using real-time PCR and normalized to 18S ribosomal RNA.

putative STAT-binding sites; however the sequence does not contain an identifiable ERE motif. In a genome-wide screen for high affinity ERE-binding sites some functional elements are located as far as 10 kb from the transcriptional start site [13], and an ERE motif is present in both the mSOCS-3 and rat SOCS-3 promoters. Therefore, we wanted to clone a larger fragment of the hSOCS-3 promoter to check for upstream EREs, that may be important in mediating its E2 responsiveness. We then queried the human genomic database at NCBI with the SOCS-3 mRNA sequence and identified BAC clone number clone RP11-806H10 (GenBank Accession No. AC061992), which represented a sequence of human chromosome 17 and contained the entire SOCS-3 coding sequence and its 5′ genomic sequence, confirming previously reported findings [9]. Using computational methods to search for transcription factor-binding sites [10], we identified an incomplete ERE at position −1504 to −1486, and designed PCR primer pairs to amplify a 1.7 kb fragment of the 5′-flanking sequence (Fig. 2). The sequence is identical to the overlapping region in the GenBank Accession No. AC061992 sequence. The hSOCS-3 5′-flanking sequence is very GC-rich and was difficult to amplify using conventional polymerase. However, using PCR amplification systems specifically designed to amplify GC-rich templates significantly improved amplification efficiency, producing a single amplified product, whereas *taq* DNA polymerase or other similar enzymes were unable to efficiently amplify the sequence (Fig. 3A).

Estrogen responsiveness of the human SOCS-3 promoter

To study the promoter activity we cloned the 1.7 kb fragment of the 5′-flanking region into the pGL3 basic vector upstream of the luciferase reporter gene. This

```
-1673
AGTGGGCCGA GGCTGGGTAG CTGCCACGAA GGGGCGTCCC CTGCAGCCCT GGGTCTCCCC TCTGGAATCT
GCCCGCAGGT GACTGTCGCA CGTCTCCAAC CTCCGGCTCC CGGGTCTGAC CCCAGCCCCG CTGCCTGGCT
GTGGGGTAGC CCCACTTCCC TTTCGGCTGG CCCGGGCCGC CTGACCCGCA GTTGGGCCCT CGGGGAGGCC
CGGGTGGCGG CGAGGGGGTG TCCCCGCGGG GTCCGGGAAA AGGGGAAGGG GAACCGGGAG GCTCTCCAGG
TCGGCCTCCT AGAACTGCCC GCTCTCCCGA AGCGGCGCCC CCGCCTCTGC CAGAAATCAG CCTTCTTAGA
AGGGAGGGGG GTGGGGGGAA AGTGTGAATG AGAAGTTGGG GGCGGAGCGC GCGGCGGGGA GGGGCCGCTG
CCAGGAACGC GCCGCCGGGG CTGGCGCCGC GCCCACCGGC CGCCTCGGCC GCCTCTCGTC GCGCTTTGTC
TCCGCGCGCG TCCCTCCCGG TCCCTGCCCC TGCTCGCGGC CCGCCCTCGG CGCCCGCGGC CCCTCCCTCA
CCCTCCGCGC TCAGCCTTTC TCTGCTGCga gtagtgacta aacattacaa gaaggccggc cgcgcagttc
caggaatcgg ggggcggggc gcggcggccg cctatatacc cgcgagcgcg gcctccgcgg cggctccgac
ttggactccc tgctccgctg ctgccgcttc ggccccgcac gcagccagcc gccagccgcc cgcccggccc
agctcccgcc gcggcccctt gccgcggtcc ctctcctggt cccctcccgg ttggtccggg ggtgcgcagg
gggcagggcg ggcgcccagg ggaagctcga gggacgcgcg cgcgaaggct cctttgtgga cttcacggcc
gccaacatct gggcgcagcg cgggccaccg ctggccgtct cgccgccgcg tcgccttggg gacccgaggg
ggctcagccc caaggacgga gacttcgatt cgggaccagg taggaaggag gagcgcggcg tggggagggg
tctcgctcag tcccgggagc ttttcccggt ttcccctccc cttcccgggt cattcccggc agggaggtga
cgaggtaggg gcagagcgga tggaagccgg agatcccagg ttcccggaat actccggctg gggccttcgg
gcttctcctg tcccctccct acccccgtgc ctcgggtttc tccctccgtc cacaccgccc ggggctactg
gactgagcgg cgcccaggca gtcccggggg cccttctcct gctccccacc cggccacact cctggagacc
taacttccgc gcgcgagttt cccacgctgc gcccttgcag tgcgcgcctg ggaaggggct gcccggggcc
accctgccgg caggggcggg agccgtgcgg gctccgtgag gcgcctggat cggagcgcgg gcccaggaga
gggccccccgg ggcagtgggt gccccagtcg ctcggcgaag gcaggggagc cggggcgggc cgggcgcgct
ggagggttcc gggcactcaa cgcgctcgcg ccttcctctc cgcagccccc cgggatgcgg tagcggccgc
tgtgcggagg ccgcgaagca gctgcagccg ccgccgcgca gatccacgct ggctccgtgc gccatg
                                                                      +1
```

Fig. 2. Nucleotide sequence of the 5′-flanking region of the human SOCS-3 gene. The start codon ATG is boxed and has been defined as +1. An incomplete ERE motif is shown in bold and the 5′ end of the sequence is shown as −1673. The primers used in ChIP assays are underlined. The cloning of the sequence is described in Materials and methods. The sequence similar to a shorter clone of hSOCS-3 promoter is shown in lower case [9]. This sequence is identical to a segment of the human genomic database sequence (GenBank Accession No. AC061992) and BAC clone RP11-806H10.

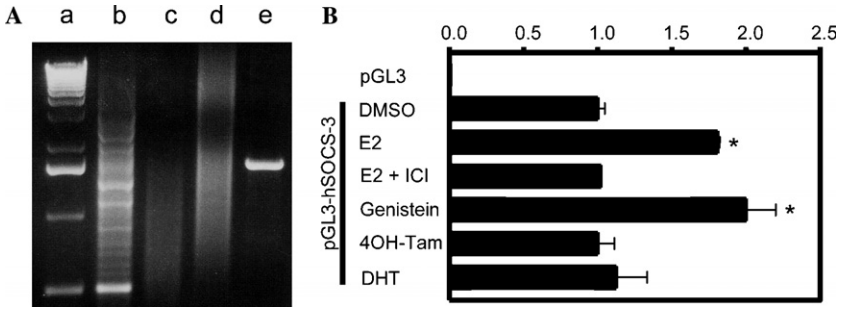

Fig. 3. PCR amplification and estrogen-induced activation of hSOCS-3 promoter. (A) PCR amplification of the GC-rich 1.7 kb fragment of the human SOCS-3 promoter (a) 1 kb ladder, (b) *taq* DNA polymerase (Roche), (c) *pfu* DNA polymerase (Roche), (d) expand DNA polymerase (Roche), and (e) GC-rich (Roche). (B) Transient transfected MCF-7 cells were treated with 10 nM E2, 100 nM genistein, 100 nM 4OH-tamoxifen 1 mM dihydrotestosterone (DHT) or cotreated with 10 nM E2 + 100 nM ICI182780 for 24 h. Luciferase reporter gene activity is expressed as fold-induction of ligand over solvent. Reporter gene activity significantly ($P < 0.05$) greater than DMSO-treated control is indicated by an asterisk.

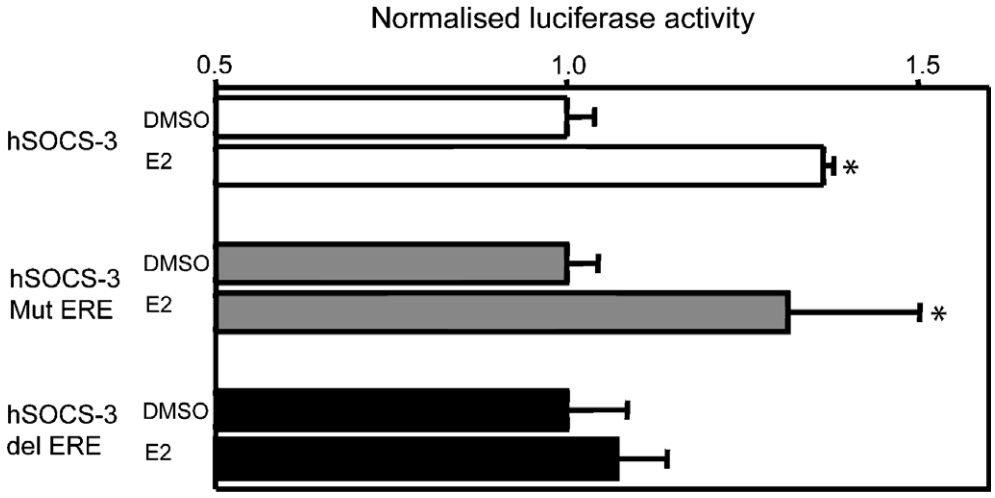

Fig. 4. Importance of the ERE in the hSOCS-3 promoter in mediating E2 responsiveness. Transfection of the hSOCS-3 mutant and deletion clones into MCF-7 cells was performed as described in Materials and methods. Luciferase activity has been normalized to β-galactosidase activity and expressed as fold-induction over DMSO control. These data are from two independent experiments, and the results shown are the means and standard deviations of three independent measurements. Reporter gene activity significantly ($P < 0.05$) greater than DMSO-treated control is indicated by an asterisk.

plasmid was then transfected into MCF-7 human breast cancer cells and treated with various ligands. The 1.7 kb fragment cloned into the pGL3-basic vector displayed high basal luciferase activity, which was approximately 100-fold higher compared to that of the empty vector arbitrarily set to unity (Fig. 3B). Treatment with 10 nM E2 resulted in a significant increase in luciferase activity, which was inhibited by co-treatment with 100 nM of the pure antagonist ICI182,780. In addition, treatment with 100 nM of the partial agonist genistein also resulted in a significant increase in luciferase activity, whereas treatment with either 4-hydroxytamoxifen or dihydrotestosterone did not.

In order to verify the importance of the incomplete ERE motif in mediating the estrogen responsiveness of the SOCS-3 promoter, transient transfection reporter assays were done using a reporter plasmid containing a mutated ERE sequence and one in which the ERE sequence was deleted. The data presented in Fig. 4 show that deletion but not mutation of the ERE sequence abolished E2 responsiveness, suggesting that sequence elements surrounding the putative ERE motif also contribute to the E2 regulation of SOCS-3. Taken together, these data demonstrate that the hSOCS-3 promoter contains an estrogen responsive region in which the incomplete ERE sequence is important but not critical in mediating this response.

In vivo recruitment of estrogen receptor-α to the human SOCS-3 promoter

To confirm the involvement of ERα in the regulation and recruitment to the human SOCS-3 promoter in vivo, we performed ChIP assays on chromatin isolated from MCF-7 cells exposed to 10 nM E2. Following protein cross-linking, shearing of the chromatin, and

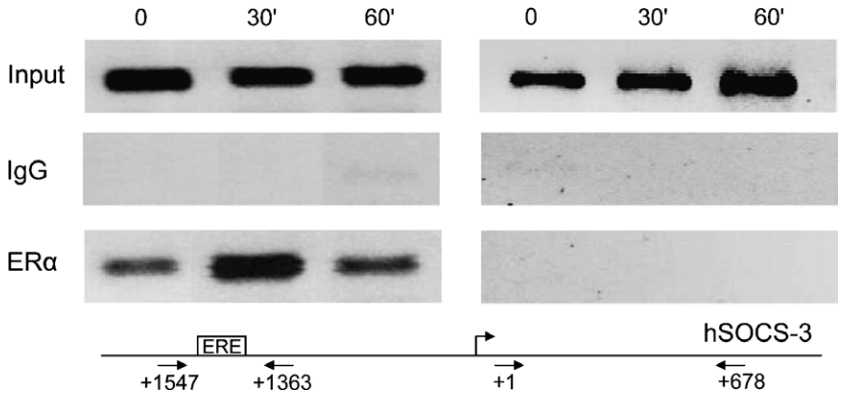

Fig. 5. Recruitment of ERα to the hSOCS-3 promoter. ChIP analysis of human MCF-7 cells shows the estrogen and time-dependent recruitment of ERα to the incomplete ERE motif in the human SOCS-3 promoter. ChIP assays were performed as described in Materials and methods. These data represent two independent experiments.

immunoprecipitation with antibodies that recognize ERα, a fragment of the human SOCS-3 promoter containing the putative ERE half site was amplified by PCR. ChIP analysis showed the time-dependent recruitment of ERα to the incomplete ERE motif, which was evident after 30 min and decreased after 60 min of E2 treatment (Fig. 5). The recruitment profile of ERα to the hSOCS-3 promoter also agreed with the rapid and transient induction of E2-induced SOCS-3 expression shown in Fig. 1. No recruitment of ERα was observed to the SOCS-3 coding sequence. These data demonstrate the direct recruitment of ERα to the hSOCS-3 promoter, and suggest that the E2-dependent regulation of SOCS-3 expression is via ERα recruitment to this estrogen response region.

Discussion

In this study, we describe the cloning of a 1.7 kb fragment of the hSOCS-3 promoter, its regulation by E2 and the identification of a putative estrogen responsive region. The E2 regulation of the hSOCS-3 promoter is supported by similar findings for the mSOCS-3 promoter performed using liver and hepatocarcinoma cell lines [6]. In addition, we demonstrate using ChIP the time and E2-dependent recruitment of ERα to the E2 responsive region of the hSOCS-3 promoter.

Using sequence information obtained by searching human genome databases, we cloned a 1.7 kb 5′ genomic region of the hSOCS-3 promoter, which contained a newly identified ERE sequence at position −1504 to −1486. The mSOCS-3 promoter has been shown to contain an incomplete ERE sequence at nucleotides −1366 to −1352 that contributes to its regulation by E2 [6], and a similar sequence is present in the rat SOCS-3 promoter (unpublished observations). Similar to an earlier report describing the cloning and characterization of a short fragment of the human SOCS-3 promoter [9], we found that the ~1.7 kb 5′ genomic region of the hSOCS-3 promoter displayed significant basal activity. The promoter activity has been reported to be dependent on STAT-binding sites, and the high basal level might be the result of constitutively active STAT proteins that are present in a variety of human tumors [14–16]. These functionally important STAT-binding sites have been proposed to contribute to SOCS-3 acting as a negative autoregulator of its own gene expression [8]. In this model, SOCS-3 expression is stimulated by a STAT-3 dependent a pathway, whereas SOCS-3 is a negative regulator of STAT-3 activation. The rapid return of SOCS-3 mRNA to basal level is most likely the result of this negative autoregulation.

The high basal activity of the hSOCS-3 promoter was further increased by E2 treatment, a level similar to that reported for the mSOCS-3 promoter [7]. Previous in vitro and in vivo studies of ER null mice have shown that this effect is mediated by ERα and not ERβ [7]. Deletion but not mutation of an incomplete ERE motif abolished the E2 regulation of the hSOCS-3 promoter, which suggests that E2 regulation is via an E2 responsive region rather than being mediated via a single ERE motif. ChIP assays confirmed the E2 and time-dependent promoter occupancy of ERα to the E2 responsive region of hSOCS-3 in human breast cancer cells. ERs regulate gene expression in two ways; via the classical pathway through direct DNA-binding via EREs or via the non-classical pathway by protein–protein interactions with other transcription factors, such as activating protein-1 (AP-1) [17], as well as nuclear factor-κB, and stimulating protein-1 (Sp1) [18]. Several AP-1 and Sp1 sites are present in the E2 responsive region surrounding the ERE motif. ERα as been shown to interact with AP-1 and Sp-1 factors and full activation of the E2 responsive pS2 promoter requires both the ERE and AP-1 elements [19]. Therefore, one cannot exclude the importance of these and other transcription factors in mediating the stimulatory affects of E2 on SOCS-3 expression.

The stimulation of SOCS-3 expression by E2 may be important in the regulation and activity of other cytokines, such as interleukin-6 (IL-6), since SOCS-3 is known to play an important role in IL-6 signalling [20]. E2 is an important negative regulator of interleukin-6-dependent transcription [21]. The development of postmenopausal osteoporosis is related to an increase in IL-6 activity, which may be a result of reduced E2 levels [22,23]. Although there are confounding reports on the ability of ERα to directly interact with STAT-3 and consequently inhibit the expression of IL-6 [6,24], ERα has been shown to directly interact with both IL-6 and NF-κB [25,26]. The E2 stimulation of the hSOCS-3 promoter presented here and elsewhere for the mSOCS-3 promoter [7] supports the general findings that ERα exerts indirect negative regulatory effects on IL-6 activity. In addition to regulating IL-6 activity, SOCS-3 modulates the activities of many other important signalling pathways such as leukemia inhibitory factor, interferon-γ, growth hormone, and insulin signalling [6,27,28]. Thus the E2 regulation of SOCS-3 gene expression may have significant physiological consequences through the potential modulation of several important cytokines that are involved in autoimmune, inflammatory, and metabolic disorders.

Acknowledgments

The authors thank all members of the Receptor Biology Unit for their assistance and helpful discussions during this study. This study was supported by a postdoctoral fellowship from the David and Astrid Hageléns Foundation (to J.M.) and by grants from the Swedish Cancer Fund and KaroBio AB.

References

[1] M.B. Davidson, Effect of growth hormone on carbohydrate and lipid metabolism, Endocr. Rev. 8 (1987) 115–131.
[2] J. Finidori, Regulators of growth hormone signaling, Vitam. Horm. 59 (2000) 71–97.
[3] D.L. Krebs, D.J. Hilton, SOCS: physiological suppressors of cytokine signaling, J. Cell Sci. 113 (Pt. 16) (2000) 2813–2819.
[4] K. Pettersson, J.-Å Gustafsson, Role of estrogen receptor beta in estrogen action, Annu. Rev. Physiol. 63 (2001) 165–192.
[5] J.F. Couse, K.S. Korach, Estrogen receptor null mice: what have we learned and where will they lead us? Endocr. Rev. 20 (1999) 358–417.
[6] K.C. Leung, N. Doyle, M. Ballesteros, K. Sjogren, C.K. Watts, T.H. Low, G.M. Leong, R.J. Ross, K.K. Ho, Estrogen inhibits GH signaling by suppressing GH-induced JAK2 phosphorylation, an effect mediated by SOCS-2, Proc. Natl. Acad. Sci. USA 100 (2003) 1016–1021.
[7] G.M. Leong, S. Moverare, J. Brce, N. Doyle, K. Sjogren, K. Dahlman-Wright, J.-Å Gustafsson, K.K. Ho, C. Ohlsson, K.C. Leung, Estrogen up-regulates hepatic expression of SOCS-2 and -3 in vivo and in vitro, Endocrinology (2004).
[8] C.J. Auernhammer, C. Bousquet, S. Melmed, Autoregulation of pituitary corticotroph SOCS-3 expression: characterization of the murine SOCS-3 promoter, Proc. Natl. Acad. Sci. USA 96 (1999) 6964–6969.
[9] B. He, L. You, K. Uematsu, M. Matsangou, Z. Xu, M. He, F. McCormick, D.M. Jablons, Cloning and characterization of a functional promoter of the human SOCS-3 gene, Biochem. Biophys. Res. Commun. 301 (2003) 386–391.
[10] K. Quandt, K. Frech, H. Karas, E. Wingender, T. Werner, MatInd and MatInspector: new fast and versatile tools for detection of consensus matches in nucleotide sequence data, Nucleic Acids Res. 23 (1995) 4878–4884.
[11] J. Matthews, B. Wihlen, J. Thomsen, J.-Å Gustafsson, Aryl hydrocarbon receptor-mediated transcription: ligand-dependent recruitment of estrogen receptor alpha to TCDD responsive promoters, Mol. Biol. Cell 25 (2005) 5317–5328.
[12] A.G. Jegalian, H. Wu, Regulation of Socs gene expression by the proto-oncoprotein GFI-1B: two routes for STAT5 target gene induction by erythropoietin, J. Biol. Chem. 277 (2002) 2345–2352.
[13] V. Bourdeau, J. Deschenes, R. Metivier, Y. Nagai, D. Nguyen, N. Bretschneider, F. Gannon, J.H. White, S. Mader, Genome-wide identification of high-affinity estrogen response elements in human and mouse, Mol. Endocrinol. 18 (2004) 1411–1427.
[14] R. Buettner, L.B. Mora, R. Jove, Activated STAT signaling in human tumors provides novel molecular targets for therapeutic intervention, Clin. Cancer Res. 8 (2002) 945–954.
[15] L.B. Mora, R. Buettner, J. Seigne, J. Diaz, N. Ahmad, R. Garcia, T. Bowman, R. Falcone, R. Fairclough, A. Cantor, C. Muro-Cacho, S. Livingston, J. Karras, J. Pow-Sang, R. Jove, Constitutive activation of Stat3 in human prostate tumors and cell lines: direct inhibition of Stat3 signaling induces apoptosis of prostate cancer cells, Cancer Res. 62 (2002) 6659–6666.
[16] T. Bowman, R. Garcia, J. Turkson, R. Jove, STATs in oncogenesis, Oncogene 19 (2000) 2474–2488.
[17] K. Paech, P. Webb, G.G. Kuiper, S. Nilsson, J.-Å Gustafsson, P.J. Kushner, T.S. Scanlan, Differential ligand activation of estrogen receptors ERalpha and ERbeta at AP1 sites, Science 277 (1997) 1508–1510.
[18] B. Saville, M. Wormke, F. Wang, T. Nguyen, E. Enmark, G. Kuiper, J.-Å Gustafsson, S. Safe, Ligand-, cell-, and estrogen receptor subtype (alpha/beta)-dependent activation at GC-rich (Sp1) promoter elements, J. Biol. Chem. 275 (2000) 5379–5387.
[19] T. Barkhem, L.A. Haldosen, J-Å Gustafsson, S. Nilsson, pS2 Gene expression in HepG2 cells: complex regulation through crosstalk between the estrogen receptor alpha, an estrogen-responsive element, and the activator protein 1 response element, Mol. Pharmacol. 61 (2002) 1273–1283.
[20] B.A. Croker, D.L. Krebs, J.G. Zhang, S. Wormald, T.A. Willson, E.G. Stanley, L. Robb, C.J. Greenhalgh, I. Forster, B.E. Clausen, N.A. Nicola, D. Metcalf, D.J. Hilton, A.W. Roberts, W.S. Alexander, SOCS3 negatively regulates IL-6 signaling in vivo, Nat. Immunol. 4 (2003) 540–545.
[21] S.T. Pottratz, T. Bellido, H. Mocharla, D. Crabb, S.C. Manolagas, 17 beta-Estradiol inhibits expression of human interleukin-6 promoter–reporter constructs by a receptor-dependent mechanism, J. Clin. Invest. 93 (1994) 944–950.
[22] M.K. Lindberg, M. Erlandsson, S.L. Alatalo, S. Windahl, G. Andersson, J.M. Halleen, H. Carlsten, J.A. Gustafsson, C. Ohlsson, Estrogen receptor alpha, but not estrogen receptor beta, is involved in the regulation of the OPG/RANKL (osteoprotegerin/receptor activator of NF-kappa B ligand) ratio and serum interleukin-6 in male mice, J. Endocrinol. 171 (2001) 425–433.

[23] V. Poli, R. Balena, E. Fattori, A. Markatos, M. Yamamoto, H. Tanaka, G. Ciliberto, G.A. Rodan, F. Costantini, Interleukin-6 deficient mice are protected from bone loss caused by estrogen depletion, EMBO J. 13 (1994) 1189–1196.

[24] T. Yamamoto, T. Matsuda, A. Junicho, H. Kishi, F. Saatcioglu, A. Muraguchi, Cross-talk between signal transducer and activator of transcription 3 and estrogen receptor signaling, FEBS Lett. 486 (2000) 143–148.

[25] B. Stein, M.X. Yang, Repression of the interleukin-6 promoter by estrogen receptor is mediated by NF-kappa B and C/EBP beta, Mol. Cell. Biol. 15 (1995) 4971–4979.

[26] S. Kurebayashi, Y. Miyashita, T. Hirose, S. Kasayama, S. Akira, T. Kishimoto, Characterization of mechanisms of interleukin-6 gene repression by estrogen receptor, J. Steroid Biochem. Mol. Biol. 60 (1997) 11–17.

[27] C.J. Auernhammer, V. Chesnokova, C. Bousquet, S. Melmed, Pituitary corticotroph SOCS-3: novel intracellular regulation of leukemia-inhibitory factor-mediated proopiomelanocortin gene expression and adrenocorticotropin secretion, Mol. Endocrinol. 12 (1998) 954–961.

[28] C.M. Steppan, J. Wang, E.L. Whiteman, M.J. Birnbaum, M.A. Lazar, Activation of SOCS-3 by resistin, Mol. Cell. Biol. 25 (2005) 1569–1575.