The transcription factor STAT3 is activated by interleukin-6-related cytokines and has been implicated as an oncogene; it promotes cell proliferation and is anti-apoptotic. However, in some cases, STAT3 has been shown to be pro-apoptotic, especially in mammary epithelial cells. In this report, we generated SOCS3-deficient murine embryonic fibroblasts (MEFs), in which STAT3 activation is extremely enhanced and prolonged. We found that LIF induces caspase-3 activation and apoptosis of SOCS3-/- MEFs. Exogenous expression of the dominant negative form of STAT3 but not STAT1 suppressed LIF-induced apoptosis of SOCS3-/- MEFs, indicating that STAT3 plays a critical role in apoptosis induction. As shown in mammary gland epithelial cells, expression of the phosphatidylinositol 3-kinase regulatory subunits p50α and p55α was induced in response to LIF in SOCS3-/- MEFs but not in wild-type MEFs, and Akt/protein kinase B activity was substantially reduced in SOCS3-/- MEFs. Furthermore, we found that some of the STAT3 target genes related to apoptosis and proliferation, such as Bcl-2 and cyclin D1, were repressed upon LIF treatment in SOCS3-/- cells. Not only the up-regulation of p50α and p55α but also the repression of cyclin D1 and Bcl-2 in SOCS3-/- MEFs was inhibited by dominant negative STAT3. These data suggest that prolonged activation of STAT3 could induce apoptosis/growth arrest rather than anti-apoptosis and proliferation in certain cases, and SOCS3 is a critical regulator of this balance.

Interleukin-6 (IL-6)2 and its related cytokines, including leukemia inhibitory factor (LIF), oncostatin M, and IL-11, preferentially activate JAK tyrosine kinases and the STAT3 transcription factor (1). STAT3 is activated by phosphorylation on a tyrosine residue in its C terminus. Upon phosphorylation, STAT3 forms homodimer and translocates into the nucleus, where it activates transcription of target genes. STAT3 is persistently phosphorylated in many types of human cancer cell lines and primary tumors, including hepatocellular carcinoma, breast cancer, prostate cancer, head and neck cancer, and several hematologic malignancies, including acute myelogenous leukemia and multiple myeloma (2, 3). Several target genes of STAT3 have been identified, including proteins that are involved in cell survival and proliferation such as Bcl-2, Bcl-xL, Mcl-1, Fas, cyclin D1, cyclin E1, and p21 (4, 5). In addition, other transcription factors, including c-myc, c-jun, and c-fos, are themselves STAT3 targets (6). VEGF and TGFβ have also been shown to be targets of STAT3 and contribute to angiogenesis and fibrosis, respectively (7, 8).

Although STAT3 is generally considered a growth-promoting factor, it has been implicated in the promotion of apoptosis or growth arrest in certain conditions. In mammary glands, epithelial cells undergo apoptosis during involution, and STAT3 has been shown to play an essential role in apoptosis. In conditional knock-out mice lacking the STAT3 gene in epithelial cells, the involution process was strongly delayed (9, 10). Recently, a mechanism of STAT3-induced apoptosis has been proposed. The phosphoinositide-3-OH kinase (PI3K)-Akt/PKB pathway is important for cell survival. During mammary gland involution, LIF induces expression of the two PI3K regulatory subunits p50α and p55α via STAT3, resulting in diminished levels of Akt/PKB activity and eventually leads cells to apoptosis (11).

In M1 myeloid cells, IL-6 or related cytokine-mediated STAT3 activation induces growth arrest, apoptosis, and macrophage differentiation (12–14). STAT3 has been shown to be necessary for the induction of growth arrest and granulocyte differentiation of 32D cells (15). Oncostatin M also activates STAT3 and induces apoptosis and cell cycle arrest in certain tumor cells. Bellido et al. (16) reported that the cyclin-dependent kinase inhibitor p21 is the downstream target of oncostatin M-induced STAT3. Nakayama et al. (17) also reported that the novel oncostatin M-inducible gene OIG37, whose induction is dependent on STAT3, is involved in growth suppression. However, the mechanism of STAT3 regulation of cell proliferation and growth arrest has not been clarified.

Suppressors of cytokine signaling (SOCS) regulate the strength of cytokine signals. Among these, SOCS3 is strongly

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2 The abbreviations used are: IL, interleukin; JAK, Janus kinase; ERK, extracellular signal-regulated kinase; MAP, mitogen-activated protein; PKB, protein kinase B; STAT, signal transducers and activators of transcription; SOCS, suppressors of cytokine signaling; LIF, leukemia inhibitory factor; PI3K, phosphoinositide-3-OH kinase; MEF, murine embryonic fibroblast; IRES, internal ribosome entry site; GFP, green fluorescent protein; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; WT, wild type; RT, reverse transcription; Cre, cyclization recombination.
induced by a variety of cytokines and other stimulators, including IL-6, granulocyte-colony stimulating factor, erythropoietin, leptin, LIF, and lipopolysaccharide (18–22). SOCS3 has a N-terminal kinase inhibitory region that inhibits JAK tyrosine kinase activity as a pseudo-substrate, central SH2 domain, and C-terminal SOCS-box. SOCS3 inhibits JAKs through binding cytokine receptors. Since binding of the SH2 domain of SOCS3 is relatively specific to receptors for STAT3-activating cytokines, such as IL-6, granulocyte-colony stimulating factor, leptin, and LIF, the suppressive effect of SOCS3 has been shown to be relatively restricted to STAT3 (23–26).

SOCS3-deficient mice die as a result of placental defects during embryonic development (27, 28). Embryonic lethality can be rescued by replacing wild-type placental function or crossing with LIF-deficient mice, thus demonstrating the essential role of SOCS3 in placental development and LIF signal suppression. The generation of conditional knock-out mice using the Cre-loxP system revealed that SOCS3 is also essential for the suppression of IL-6/gp130 signaling in macrophages (20, 29). This is consistent with the high affinity binding of SOCS3 to the Tyr-759 region of gp130 (26). STAT3 has been shown to be hyperactivated in SOCS3-deficient cells; however, the effect of STAT3 hyperactivation on cell proliferation and/or apoptosis has not been well investigated.

In this study, we demonstrated that the deletion of the Socs3 gene resulted in hyperactivation of STAT3 in response to LIF and induced apoptosis. The mechanism is partly associated with induction of the PI3K regulatory subunits p50α and p55α, reduced activation of Akt/PKB, and repression of growth-promoting/anti-apoptotic genes, cyclin D1, and Bcl-2. Thus, we suggest that SOCS3 is a critical regulator that could convert STAT3 from an anti-apoptotic messenger to a pro-apoptotic messenger.

**EXPERIMENTAL PROCEDURES**

**Generation of Socs3-deficient MEF—**Socs3fllox/lox (Socs3fl/fl) mice were generated as described previously (30). Murine embryonic fibroblasts (MEFs) were obtained from embryonic day 13.5 Socs3fl/fl embryos by standard protocols. An adenovirus carrying Cre-recombinase (AdCre) was produced as described previously (8). To delete the Socs3 gene, MEFs were infected with AdCre at 500 multiplicity of infection and cultured. We did not observe any SOCS3 expression for at least 6 months. All experiments using these mice were approved by and performed according to the guidelines of the animal ethics committee of Kyushu University, Fukuoka, Japan.

**Retroviral Constructs and Transduction to MEFs—**The SOCS3-IREs-GFP-pMX, STAT1IRES-GFP-pMX, STAT3IRES-GFP-pMX (31), and empty GFP-pMX plasmids were transfected into a packaging cell line, Plat-E, using FuGENE 6 (Roche Diagnostics), and after incubation for 48 h, the culture supernatant was harvested. MEFs were infected with the viruses by adding virus-containing supernatants in the presence of 5 μg/ml Polybrene (Sigma). The infected MEF cells were expanded and sorted by fluorescence-activated cell sorter. GFP-positive cells were used for experiments.

**MTT Assay—**MEFs were plated in a 96-well plate at 1 × 10^3 cells/well 1 day prior to LIF treatment. The cells were left untreated or treated with LIF at the indicated concentration. Three days after treatment, an MTT assay was performed using the Cell Counting Kit-8 (Dojindo) according to the manufacturer’s instructions.

**Detection of Cell Death—**MEFs were plated in a 24-well dish at 5 × 10^3 cells/well 1 day prior to LIF treatment. The cells were left untreated or treated with LIF at the indicated concentration. Three days after treatment, cells were trypsinized and collected with the supernatants, and cell viability was determined by propidium iodide (Sigma) and annexin V (Sigma) staining using flow cytometry.

**Western Blot Analysis—**After stimulation with LIF (10 ng/ml), cells were lysed in lysis buffer (20 mm Tris HCl (pH 7.4), 150 mm NaCl, 0.5% Triton X-100, 100 μm sodium vanadate, 1 mm dithiothreitol, 5 mg/ml leupeptin, 1 mm phenylmethylsulfonyl fluoride), and centrifuged at 15,000 × g at 4 °C for 15 min. Supernatants were boiled in SDS reducing sample buffer and subjected to SDS-polyacrylamide gel electrophoresis and then electroforetically transferred onto polyvinylidene difluoride membranes. After blocking in TBST (20 mm Tris-HCl (pH 7.5), 150 mm NaCl, 0.1% Tween 20) containing 5% dry skim milk, the membranes were probed with primary antibodies overnight at 4 °C. Antibodies used in this study were: anti-phospho-STAT3 (Tyr-705; catalog number 9131), anti-phospho-STAT1 (Tyr-701; catalog number 9171), anti-phospho-Akt (Ser-473; catalog number 4058), anti-Akt (catalog number 9272), and anti-cleaved caspase-3 (Asp-175; catalog number 9661) from Cell Signaling Technology, anti-STAT3 (catalog number sc-482), anti-STAT1 (catalog number sc-346), anti-ERK2 (catalog number sc-154), and anti-Myc (9E10; catalog number 2066) from Santa Cruz Biotechnology, anti-SOCS3 (catalog number 18395) from Immuno-Biological Laboratories, and anti-actin (catalog number A2066) from Sigma. The membranes were extensively washed with TBST and incubated with horseradish peroxidase-conjugated anti-mouse or anti-rabbit antibody for 1 h at room temperature. The membranes were washed twice with TBST for 15 min. Blots were visualized by SuperSignal West Pico chemiluminescent substrate (Pierce).

**RNA Extraction and RT-PCR Analysis—**Total RNA was extracted from MEFs using the TRIzol reagent (Invitrogen). First-strand cDNA was synthesized from 1 μg total RNA with MuLV reverse transcriptase using random hexamers. The cDNA was used as a template for PCR using KOD-Plus DNA polymerase (TOYOBO) according to the manufacturer’s instructions. The following primer pairs were used 5′-GGGTGGCAGGAAAAGGAGG-3′ and 5′-GTTGAGCGTCAAGACCCAGT-3′ (mouse Socs3); 5′-CTGGGCAAGTTCAAGGCGGAACCGT-3′ and 5′-TCTCTGCGTTATTGGCAGACTGGTAG-3′ (mouse Pik3r1-p50α); 5′-GTTACAGTGGCGGGCGCATTAGTTTTA-3′ and 5′-TCTCTGCTGTTATTGGGGACA-CAGGT-3′ (mouse Pik3r1-p50α); 5′-GCCCGCTGTTTTCCAGATTC-3′ and 5′-TCTCTGCTGTTATTGGGACACTGGTAG-3′ (mouse Plakr1-p50α); 5′-ACCAGCTCATCAGCCTAC-3′ and 5′-TCCACACCCCTGTTGCTGA-3′ (mouse G3pdh). The primer pairs for Ccnd1 (cyclin D1), Bcl2, Bcl2L1 (Bcl-xL), and Myc (c-Myc) were previously described (32).
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RESULTS

Generation and Characterization of SOCS3-deficient MEF—To explore the physiological role of SOCS3 in the LIF signaling, we first generated SOCS3-deficient murine embryonic fibroblasts (MEFs) as follows. *Socs3* ^*lox/lox^ MEFs were immortalized by SV40 gene transfection and then infected with adenovirus carrying *lacZ* or *Cre* (8). The *Socs3* gene was almost completely deleted in *Cre*-infected cells, as confirmed by genomic PCR (Fig. 1A), and the gene deletion was stably maintained for more than 6 months. We used *lacZ*-infected MEFs as wild-type (WT) and *Cre*-infected MEFs as SOCS3 ^*del/del* (knock-out) MEFs. As shown in Fig. 1B, LIF treatment induced SOCS3 protein expression in WT MEFs, whereas no SOCS3 protein was detected in SOCS3 ^*del/del* MEFs.

SOCS3 Is Essential for Cell Survival in the Presence of LIF—We looked at the biological consequences of LIF treatment in WT and SOCS3 ^*del/del* MEFs, Cells were cultured in the absence or the presence of LIF for 72 h, and their viability was measured by MTT assay. There was no obvious difference in the growth rate of WT and SOCS3 ^*del/del* MEFs when they were cultured under normal conditions (data not shown); however, SOCS3 ^*del/del* MEFs severely lost their viability in the presence of LIF, whereas WT MEFs showed no such defect (Fig. 2). This proliferation defect was rescued by retroviral transduction of the SOCS3 gene, indicating that the loss of viability of SOCS3 ^*del/del* MEFs upon LIF treatment is due to the lack of SOCS3. Therefore, SOCS3 is required for cell proliferation or survival in the presence of LIF.

SOCS3 ^*del/del* MEFs Undergo Apoptosis in the Presence of LIF—Next we investigated the exact cause of the proliferation defect of SOCS3 ^*del/del* cells in the presence of LIF. Because these cells appeared dead under the microscope, apoptosis was tested by annexin V-fluorescein isothiocyanate and propidium iodide staining 3 days after the addition of LIF in the culture. We found that a significant number of SOCS3 ^*del/del* cells underwent apoptosis in the presence of LIF, whereas few apoptotic cells were observed for WT cells under the same condition (Fig. 3A). Furthermore, cleaved caspase-3, one of the key executioners of apoptosis, was detected in the whole cell extract of SOCS3 ^*del/del* MEFs after 48 h of LIF stimulation (Fig. 3B). Thus, it is concluded that LIF induces apoptosis in SOCS3-deficient MEFs.

Persistent Activation of STAT1 and STAT3 in SOCS3 ^*del/del* MEFs upon LIF Stimulation—LIF binds to a cell surface receptor complex composed of LIF-R and gp130 and activates the JAK/STAT pathway. Upon activation, STAT proteins become phosphorylated on a regulatory tyrosine residue, homodimerize, and translocate into the nucleus to induce the expression of target genes including SOCS family proteins, which in turn down-regulate JAK/STAT signaling. We tested the effect of SOCS3 deficiency on LIF-induced activation of STAT proteins. WT and SOCS3 ^*del/del* MEFs were treated with LIF for 5, 30, and 240 min, and activation of STAT3 and STAT1 was monitored by Western blot with phospho-specific antibodies against STAT3 and STAT1, respectively. As shown in Fig. 4, in WT cells, phosphorylated STAT3 was detected at 5 min after LIF treatment and quickly disappeared by 30 min, coincident with the induction of SOCS3 protein. On the other hand, STAT3 was phosphorylated more strongly upon LIF stimulation in SOCS3 ^*del/del* MEFs, and most notably, phosphorylation of STAT3 was detected over 6 h after stimulation in these cells (Fig. 4). Sustained activation of STAT3 was observed even at 24 and 48 h after stimulation in SOCS3 ^*del/del* MEFs (Fig. 3B). Therefore, SOCS3 is essential for preventing persistent activation of STAT3 upon LIF stimulation. Interestingly, we noticed that enhanced and prolonged phosphorylation of STAT1 was also observed in SOCS3-deficient cells, which is consistent with previous reports for SOCS3 ^*del/del* macrophages (20, 29). These data indicate that SOCS3 normally controls STAT1 activity at appropriate levels as well.

Hyperactivation of STAT3 Is Responsible for LIF-induced Cell Death of SOCS3 ^*del/del* MEFs—As mentioned above, both STAT1 and STAT3 were hyperactivated in response to LIF in
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Since STAT1 is a well known pro-apoptotic factor, we next sought to determine which of these STAT proteins is responsible for LIF-induced cell death in these cells. The Myc epitope-tagged dominant negative form of either STAT1 (STAT1<sup>YF</sup>) or STAT3 (STAT3<sup>YF</sup>) was introduced into SOCS3<sup>−/−</sup> MEFs using a bicistronic retroviral vector pMX carrying IRES-GFP. MEFs were transduced with the retroviral vector, and GFP-positive cells were sorted. Both Myc-STAT1<sup>YF</sup> and Myc-STAT3<sup>YF</sup> were expressed in transduced cells and suppressed LIF-induced hyperactivation of endogenous STAT1 and STAT3, respectively (Fig. 5A). STAT3<sup>YF</sup> also suppressed LIF-induced hyperactivation of STAT1, possibly due to the binding of STAT3<sup>YF</sup> to LIF-R/gp130 complex (33). Alternatively, LIF-induced activation of STAT1 might be in part STAT3-dependent. Then we examined cell viability by the MTT assay and found that STAT3<sup>YF</sup> but not STAT1<sup>YF</sup> suppressed the proliferation defect of SOCS3<sup>−/−</sup> MEFs in the presence of LIF (Fig. 5B). Therefore, hyperactivation of STAT3 is likely to be responsible for LIF-induced cell death of SOCS3<sup>−/−</sup> cells.

Up-regulation of the PI3K Regulatory Subunits p50<sup>H9251</sup> and p55<sup>H9251</sup> and Reduced Akt/PKB Activity Are Associated with LIF-induced Cell Death of SOCS3<sup>−/−</sup> MEFs—It was recently reported that STAT3 induces apoptosis via induction of the PI3K regulatory subunits, p50<sup>H9251</sup> and p55<sup>H9251</sup>, leading to down-regulation of Akt/PKB during mammary gland involution (11). Therefore, we next investigated whether this could account for the mechanism of LIF-induced cell death of SOCS3<sup>−/−</sup> MEFs. WT and SOCS3<sup>−/−</sup> MEFs were stimulated with LIF for different periods of time, and RT-PCR analysis was performed to determine the mRNA levels of PI3K regulatory subunit genes. Expression of p50<sup>H9251</sup> or p55<sup>H9251</sup> was nearly undetectable throughout the duration of the experiment in WT MEFs; however, it was detected as early as 1 h after LIF treatment in SOCS3<sup>−/−</sup> MEFs (Fig. 6A). In contrast, the p85<sup>H9251</sup> subunit was expressed in both WT and SOCS3<sup>−/−</sup> MEFs, and its expression level was not significantly altered upon LIF treatment in both cell types (Fig. 6A). This is consistent with the previous report demonstrating that the expression of p50α or p55α is directly regulated by STAT3 (11). We then examined whether the induction of p50α and p55α upon LIF treatment could be due to the hyperactivation of STAT3 in SOCS3<sup>−/−</sup> MEFs. SOCS3<sup>−/−</sup> MEFs stably trans-
duced with the retroviral vector for SOCS3, STAT3YF, or STAT1YF were treated with LIF for either 30 or 60 min, and RT-PCR was performed. Cells transduced with empty virus were used as a control. We found that either SOCS3 or STAT3YF strongly suppressed the LIF-induced expression of p50 and p55, whereas STAT1YF only weakly did so (Figs. 6B and 7). Therefore, the induction of the PI3K regulatory subunits is mainly mediated by hyperactivated STAT3, and this appears to be the primary mechanism of LIF-induced cell death in SOCS3−/− MEFs since there is an excellent correlation between p50 and p55 induction and the cell death phenotype in these cells upon LIF treatment (Figs. 2, 5B, and 6B).

We next examined the activity of the anti-apoptotic kinase Akt/PKB, as measured by its phosphorylation on Ser-473, in WT, and SOCS3−/− MEFs in response to LIF. Cells were treated with LIF for different periods of time as indicated, and whole cell extracts were Western blotted with antibodies that specifically recognize phospho-Akt(Ser-473) and total Akt. In WT cells, phosphorylation of Akt was readily detectable before stimulation, rapidly elevated upon LIF stimulation, and remained until at least 24 h after LIF stimulation. Despite the lower basal level of Akt phosphorylation in SOCS3−/− cells, LIF treatment increased its phosphorylation to the level seen in WT cells. However, we found that phosphorylated Akt quickly disappeared by 1 h after stimulation, concomitantly with the induction of the PI3K p50α and p55α subunits (Fig. 6, A and C). These results suggest that the hyperactivation of STAT3 induces expression of p50α and p55α, negative regulatory subunits of PI3K, thereby down-regulating Akt/PKB signaling, which eventually results in cell death in response to LIF.

Dysregulation of Cyclin D1 and Bcl-2 in SOCS3-deficient MEFs—Finally, we examined the expression of other STAT3 target genes to demonstrate the functional switch of STAT3 from anti-apoptotic to pro-apoptotic action. SOCS3−/− MEFs stably expressing either STAT3YF or STAT1YF were treated with LIF for either 3 or 24 h, and RT-PCR was performed (Fig. 7). Again, p50α and p55α expression was induced in SOCS3−/− MEFs principally in a STAT3-dependent manner. On the other hand, we found that expression of cyclin D1 and Bcl-2, which have been shown
as targets of STAT3 (34–36), was significantly reduced in SOCS3−/− MEFs upon LIF treatment, and this reduction was suppressed almost completely by STAT3YF but only marginally by STAT1YF. Interestingly, the expression of other STAT3 target genes such as Bcl-xL and c-myc was not altered in WT and SOCS3−/− MEFs, suggesting that gene-specific regulation mechanism(s) may exist. We did not observe strong up-regulation of these STAT3 target genes in LIF-treated WT MEFs under this experimental condition, probably because the induction of these genes occurs rapidly and transiently (see Fig. 6A for SOCS3 expression). Taken together, these results support our notion that the loss of the Socs3 gene can convert STAT3 function from anti-apoptotic/growth-promoting to pro-apoptotic/growth-suppressive.

**DISCUSSION**

STATs are a family of latent transcription factors that mediate signaling by cytokines and growth factors. STAT3 is a pleiotropic transcription factor that is involved in cell proliferation, differentiation, oncogenesis, and inflammation. Interestingly, STAT3 has been shown to possess different functions depending on the cell context. STAT3 usually induces proliferation and prevents apoptosis in many types of cells, whereas it induces growth arrest and apoptosis of certain types of cells, such as mammary epithelial cells. However, the molecular basis for such dual faces of STAT3 has not been clarified. Here, we demonstrate that SOCS3, a negative regulator of JAK/STAT signaling, plays an important role in defining the function of STAT3 in the LIF signaling. We show that LIF induces sustained activation of STAT3 in SOCS3-deficient MEFs, ultimately resulting in apoptosis of these cells. Importantly, restoration of SOCS3 expression by retroviral gene transduction completely rescued this phenotype of SOCS3−/− cells, indicating that the lack of SOCS3 expression is the primary cause of LIF-induced apoptosis in these cells. Furthermore, the SOCS3 deficiency resulted in the altered expression pattern of STAT3 target genes upon LIF stimulation, suggesting that the loss of Socs3 gene can convert STAT3 function from anti-apoptotic to pro-apoptotic.

As seen in mammary epithelial cells during involution (11), we found that LIF induces the expression of the regulatory subunits of P13K p50α and p55α and diminished Akt/PKB activity in SOCS3−/− MEFs. The induction of these PI3K subunits appears to be dependent on STAT3 activity since the dominant negative form of STAT3 could suppress the LIF-induced expression of p50α and p55α, whereas dominant negative STAT1 had only a modest effect. Thus, it seems likely that LIF induces apoptosis by a similar mechanism in SOCS3−/− MEFs and mammary epithelial cells. It is of obvious interest to test
whether the expression level of SOCS3 might be altered during mammary gland involution. Although it has been reported that SOCS3 mRNA is induced rather than suppressed during involution (37), the protein levels of SOCS3 have not been examined yet. It is known that the levels of SOCS3 mRNA and protein are not correlated in several cases and that the level of SOCS3 protein is regulated post-translationally by ubiquitination and phosphorylation (38, 39). We speculate that SOCS3 protein levels might be low in mammary epithelial cells during involution. Alternatively, other protein(s) may interfere with the function of SOCS3 protein, leading to the sustained activation of STAT3 during mammary gland involution.

We and others have previously proposed that SOCS3 is one of the key determinants for the difference between IL-6 and IL-10 (30, 40). In macrophages, STAT3 activation by IL-6 is transient, whereas STAT3 activation by IL-10 is long lasting. In macrophages lacking the SOCS3 gene or carrying a mutation of the SOCS3-binding site (Y759F) in gp130, IL-10 and IL-6 both suppressed lipopolysaccharide-induced TNFα and IL-12 production. SOCS3 protein was strongly induced by both IL-6 and IL-10 in the presence of lipopolysaccharide but selectively inhibited IL-6-mediated, but not IL-10-mediated, STAT3 activation because of selective binding of SOCS3 to gp130 but not to the IL-10 receptor. Therefore, the constitutive activation of STAT3 by IL-10 could suppress the NF-κB and STAT1 pathways, whereas the transient activation of STAT3 by IL-6 is probably pro-inflammatory. Thus, we proposed that the strength and duration of STAT3 activity likely determines the functions of STAT3. This situation resembles the function of ERK/MAP kinase for proliferation or differentiation of neural cells; transient activation of MAP kinase induces proliferation, whereas prolonged activation of MAP kinase induces differentiation.

STAT3 is constitutively activated in many tumor cells, and inhibition of STAT3 often induces apoptosis (2, 3). Apparently, the functions of STAT3 differ between tumor cells and normal cells. SOCS3 expression is often repressed in tumor cells, which could be a mechanism of strong STAT3 activation. However, prolonged activation of STAT3 does not induce apoptosis in most of these cases. One possibility is that p50α or p55α subunits may not be expressed in tumor cells even with the elevated STAT3 activity. If this is the case, other factors may be required for the induction of p50α or p55α in concert with STAT3, and these factors may be missing or inactivated in tumor cells. Alternatively, PI3K activity may be maintained at a high level due to the mutation in PTEN (phosphatase with tensin homology), a negative regulator of PI3K that is frequently mutated in tumors, so that tumor cells can avoid apoptosis potentially induced by constitutively activated STAT3. Further study is necessary to clarify the relationship between STAT3/SOCS3 and the PI3K-Akt/PKB pathways.

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