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Suppressor of cytokine signaling 1 suppresses muscle differentiation through modulation of IGF-I receptor signal transduction ☆,☆☆

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Abstract

Suppressor of cytokine signaling (SOCS) 1 was initially identified as an intracellular negative feedback regulator of the JAK-STAT signal pathway. Recently, it has been suggested that SOCS1 affects signals of growth factors and hormones. One of them, SOCS1, is also known to be involved in auto-regulation of IRS-1-mediated signaling. However, the mechanism(s) of SOCS1 induction by insulin-like growth factor (IGF)-I and a role of SOCS1 on IGF-I receptor-mediated signaling are not clarified. Here, we investigate SOCS1 on muscle differentiation. We found that muscle differentiation was suppressed in SOCS1 stable transformant C2C12 myoblasts, while it was promoted in SOCS1-deficient myoblasts. Additionally, SOCS1 augmented MEK phosphorylation and reduced Akt phosphorylation induced by IGF-I. Then, SOCS1 stable transformant C2C12 myoblasts, infected with adenovirus bearing constitutively active Akt, have the ability to differentiate again. Collectively, these findings suggest that SOCS1 suppresses muscle differentiation through negative feedback regulation of IGF-I receptor-mediated signaling.
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The suppressors of cytokine signaling (SOCS), also known as the signal transducer and activator of transcription (STAT)-induced STAT inhibitor (SSI) family or the cytokine-inducible src homology (SH) 2 domain-containing protein (CIS) family, are a group of intracellular proteins consisting of eight members. The only four to have been studied to any extent, SOCS1, SOCS2, SOCS3, and CIS, have been shown to work as negative feedback regulators of cytokine signaling [1,2].

Recent evidence has shown that SOCS can affect not only cytokine signals but also other signals evoked by growth factors and hormones [3–5]. The growth enhancement observed in SOCS2-deficient mice with normal GH levels suggests a suppressive effect of SOCS2 on the GH signaling pathway [6,7] while the accelerated lobuloalveolar development in the mammary gland of SOCS1/IFNγ-double deficient mice suggests a role for SOCS1 as a negative regulator of prolactin signaling [8].

The increase in insulin sensitivity observed in SOCS1-deficient mice demonstrates the inhibitory action of SOCS1 on insulin signaling [9], and SOCS3 has also been reported to have an inhibitory effect on insulin sig-

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☆☆ *Abbreviations:* SOCS, suppressor of cytokine signaling; IGF, insulin-like growth factor; IFN, interferon; PI3K; phosphatidylinositol 3-kinase.
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naling [10]. A number of mechanisms for the inhibition have been proposed. Among these are that SOCS1 suppresses the insulin signaling pathway at both levels of IRS-1 phosphorylation by interaction with IRS-1 and inhibition of insulin-induced-JAK activation, while SOCS3 suppresses sole IRS-1 phosphorylation [9]; or that SOCS1 and SOCS3 block insulin signaling by ubiquitin-mediated degradation of IRS-1 and IRS-2 [11]. Since insulin and IGF-I receptors share IRS-1 as an intracellular signal transducer [8], it can be speculated that SOCS1 plays a role in IGF-I receptor-mediated signal transduction. It has been shown that SOCS2 and SOCS3 can bind to the activated IGF-I receptor [12,13], but the effect of SOCS1 on IGF-I receptor-mediated signaling has yet to be clarified.

The prominent biological function of IGF-I receptor-mediated signaling is documented in the program of skeletal-muscle differentiation. Skeletal-muscle development involves a multi-step process that begins with the determination of the myogenic precursor from pluripotent mesodermal stem cells and concludes with the terminal differentiation of committed myoblasts, and is characterized by cell-cycle withdrawal, expression of muscle-specific proteins, and formation and maturation of myofibers. Expression of myogenin, one of the muscle-restricted basic helix–loop–helix transcription factors, provokes resting myoblasts to terminal differentiation [14]. The transcriptional activation of the myogenin promoter is mainly regulated by two transcription factors, MyoD and myocyte enhancer factor 2 (MEF2).

IGFs are a crucial regulator of muscle differentiation, which act through the IGF-I receptor to activate two separate signal transduction pathways: the mitogen-activated protein (MAP) kinase pathway and the IRS–phosphoinositide 3-kinase (PI3K)–Akt pathway. These two pathways have opposing role in muscle differentiation. Activation of the former pathway yields phosphorylated MEK1, which directly associates with MyoD to prevent MyoD from binding to the myogenin promoter, resulting in inhibition of muscle differentiation [15]; activation of the latter pathway induces myogenin expression by increasing the transcription activity of MEF2, resulting in the progression of muscle differentiation [16].

To elucidate the role of SOCS1 in intracellular signaling via the IGF-I receptor, we analyzed the effects of SOCS1 on the skeletal-muscle differentiation program. Enhanced skeletal-muscle differentiation was observed in a primary culture of myoblasts obtained from SOCS1-deficient mice. Forced expression of SOCS1 inhibited the differentiation of myoblasts into myotubes. In addition, analysis of the profile of the intracellular proteins involved in IGF receptor-mediated signaling revealed that SOCS1 modified the balance between the MAP kinase pathway and the IRS–PI3K–Akt pathway by enhancement of MEK and suppression of the IRS-1/Akt pathway. These results indicate that SOCS1 might play an important role in muscle differentiation through the modulation of the IGF-I receptor-mediated signaling pathway.

Materials and methods

Primary mouse myoblast culture. Limb skeletal muscle was dissected out and placed in sterile phosphate-buffered saline (Nacalai Tesque, Kyoto, Japan). The tissue was minced finely with sterile razor blade and digested with tissue of dissociation medium at 37 °C for 30 min. The resulting slurry was passed through a 100 μm cell strainer to remove large pieces of tissue and was suspended with DMEM (Sigma–Aldrich, St. Louis, USA). The filtrate was spun at 1500 rpm for 4 min, the pellet was resuspended in DMEM and the cells were plated on 6 cm collagen-coated tissue culture dishes (Iwaki, Chiba, Japan). The cells were cultured at 37 °C for 10 min. The supernatants were removed to a 35 mm laminin plate (Becton–Dickinson Labware) and cultured at 37 °C for 20 min. Then, the medium was replaced by growth medium (Ham's F-10 medium supplemented with 20% fetal bovine serum and 2.5 mg/ml BFGF) and re-plated to culture plate. After 3 or 4 days, the cells were induced to differentiation by replacing the growth medium with differentiation medium (DMEM with 2% horse serum).

Cell culture. C2C12 mouse myoblasts (American Type Culture Collection, Manassas, VA) were maintained in DMEM supplemented with 10% fetal bovine serum. We used a subclone of C2C12 mouse myoblasts which produce marginal amounts of IGF-II in examination of induction of SOCS1. C2C12 mouse myoblasts stably expressing SOCS1 were maintained in DMEM supplemented with 10% fetal bovine serum and 500 μg/ml G418 (Nacalai Tesque). To induce differentiation, cells at confluency were washed once with serum-free DMEM and replaced by differentiation medium (DMEM with 2% horse serum).

Transient transfection assays. C2C12 cells were plated on 48-well dishes at a density of 1.5×10^4 cells/well. On the following day, 0.75 mg of reporter plasmid, 0.1 mg pRLtk control plasmid, 4.5 ml Super Fect transfection reagent (Qiagen, Valencia, CA), and 50 ml DMEM were mixed and incubated for 10 min at 25 °C, and then combined with 250 ml DMEM supplemented with 2% FBS. The resultant 300 ml of the mixture was added to each well. And cells were incubated for 3 h at 37 °C. Thereafter, the medium was replaced with serum-free DMEM with or without factors. After 20 h, cells were lysed and subjected to measurement of luciferase activity using Dual-Luciferase Reporter Assay System (Promega).

Adenovirus infection. C2C12 cells were plated on 6-well plates (2×10^5 cells/well) and cultured for 1 day in DMEM with 10% FBS, then those cells were infected with 200 moi each of adenovirus bearing either mouse SOCS1 or LacZ gene. SOCS1 stable transformants were also subjected to the same treatment and infected with 20 moi each of adenovirus bearing either constitutively active Akt or LacZ gene. After incubation for 12 h, differentiation of those cells was initiated by the shift of serum concentration from 10% to 1%. In another experiments adenovirus was infected to C2C12 cells after 2 day culture in DMEM with 2% horse serum. At the indicated time points, cells were fixed with 2% formaldehyde and subjected to immunostaining.

Immunofluorescence. C2C12 cells plated on glass coverslips were fixed with 2% formaldehyde for 15 min at room temperature, followed by incubation with 100 mM glycine/PBS for 15 min to quench excess formaldehyde. Then the cells were permeabilized with 0.1% Triton X-100/PBS for 5 min at room temperature. After blocking non-specific binding sites in 5% goat serum/PBS for 1 h at room temperature, the cells were incubated with MHC (cloneMF20) antibody at 1:100 dilution in buffer A (PBS containing 0.05% Tween 20 and 1% BSA) for

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