[CANCER RESEARCH 64, 5870–5875, August 15, 2004]

Molecular Mechanisms Involved in the Growth Stimulation of Breast Cancer Cells by Leptin

Na Yin, Dan Wang, Hua Zhang, Xia Yi, Xiaojing Sun, Bin Shi, Huijian Wu, Ge Wu, Xinjuan Wang, and Yongfeng Shang

Department of Biochemistry and Molecular Biology, Peking University Health Science Center, Beijing, China

ABSTRACT

Obesity is a risk factor for breast cancer in postmenopausal women. Leptin, an adipocyte-derived cytokine, elicits proliferative effects in some cell types and potentially stimulates the growth of mammary epithelium. Here we show that leptin induced time- and dose-dependent signal transducer and activator of transcription 3 (STAT3) phosphorylation and extracellular signal-regulated kinase (ERK) 1/2 kinase activation in breast carcinoma cells. Blocking STAT3 phosphorylation with a specific inhibitor, AG490, abolished leptin-induced proliferation of MCF-7 cells, whereas blocking ERK1/2 activation by a specific ERK1/2 kinase inhibitor, U0126, did not result in any significant changes in leptin-induced cell proliferation. Our experiments also showed that one member of the p160 family of steroid receptor coactivators, steroid receptor coactivator (SRC)-1, but not glucocorticoid receptor interacting protein 1 (GRIP1) or amplified in breast cancer 1 (AIB1), also functioned in gene transactivation in response to leptin treatment. Glutathione *S*-transferase pull-down experiments showed that SRC-1 physically interacted with the activation domain of STAT3 and that chromatin immunoprecipitation experiments detected the occupancy of SRC-1, but not GRIP1 or AIB1, on the promoter of STAT3 target genes. Our experiments collectively showed that SRC-1 is involved in STAT3 signaling pathway that is implicated in leptin-stimulated cell growth.

INTRODUCTION

It is well known that an excess of fat mass represents a risk factor for breast cancer particularly in postmenopausal women (1, 2). It recently has been shown that leptin receptors are expressed in normal mammary epithelial cells and human breast cancer cell lines (3), and leptin, as a cytokine, can stimulate the proliferation of various cell lines, such as the embryonic cell line C3H10T1/2, the human lung squamous cell line SQ-5, and the mouse pancreatic β cell line MIN6 (4–6). However, the molecular mechanism underlying the cell growth-stimulatory effect of leptin is not totally understood.

Leptin exerts its biological activity through binding to its receptors that belong to the cytokine receptor superfamily (7). Several different leptin receptor isoforms exist, including a long form (ObRb) and a short form (ObRa; Refs. 8, 9). *In vitro* and *in vivo* studies have shown that leptin activates cytokine-like signal transduction via the long form of the leptin receptor. On leptin stimulation, intracellular Janus tyrosine kinases (JAKs) are activated via transphosphorylation and phosphorylate tyrosine residues on the long form leptin receptor and on signal transducers and activators of transcription (STAT) proteins (10). Phosphorylated STAT proteins dimerize and translocate to the nucleus to activate gene transcription.

In this report, we show that the STAT3 signaling pathway is involved in the cell proliferative effect of leptin. We also show that the p160 family of nuclear receptor coactivators, steroid receptor coactivator (SRC)-1, but not GRIP1 and AIB1, interacted with STAT3, was recruited to the STAT3 target gene promoter, and enhanced the target gene transcriptional activation.

MATERIALS AND METHODS

Materials and Reagents. Polyclonal anti-ob-receptor, polyclonal anti-extracellular signal-regulated kinase (ERK) 1/2, and anti-phosphor-ERK1/2 were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Polyclonal anti-STAT3 and anti-phosphor-STAT3 Tyr^{705} were obtained from Upstate Biotechnology (Lake Placid, NY) and New England Biolabs (Beverly, MA), respectively. Monoclonal anti-c-MYC (9E10) was obtained from Santa Cruz Biotechnology. R&D Systems (Minneapolis, MN) provided recombinant human leptin. U0126 and AG490 were obtained from Calbiochem (La Jolla, CA).

Glutathione *S*-Transferase Pull-Down Experiments. Pull-down reactions were performed by incubating *in vitro* transcribed and translated p160 proteins with glutathione *S*-transferase (GST) or GST-STAT3 (700–770) coupled to glutathione beads in binding buffer [20 mM Tris-HCl (pH 7.5) and 137 mM NaCl]. After 1–2 h incubation at 4°C, the beads were washed once with binding buffer, twice with binding buffer containing 0.5 M NaCl, and once with 20 mM Tris-HCl (pH 8).

Luciferase Assays. Cells were transfected with the c-*myc* promoter-driven luciferase construct using Lipofectamine 2000 (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. Forty-eight h after transfection, the cells were harvested, and luciferase and renilla activities were measured using a dual luciferase kit (Promega, Madison, WI). The firefly luciferase data for each sample were normalized based on transfection efficiency measured by renilla luciferase activity. Each experiment was performed in triplicate and repeated at least three times.

Colorimetric MTT Assay. Cells were serum-deprived overnight and exposed to the leptin at different concentrations for 24, 48, or 72 h. The cells then were incubated with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; 0.5 mg/ml) for 4 h at 37°C. After removal of MTT, 150 μl of DMSO were added to the cells, and the absorbance was measured at 540 nm using a microplate reader.

Reverse Transcription-PCR. Two μg total RNA were reverse transcribed using the Superscript first-strand synthesis system for reverse transcription-PCR (Invitrogen). The following primer sets were designed for the amplification of the human ObRa (short and long isoforms) or ObRb (long isoform): ObRa: sense, 5′-CATTTTATCCCCATTGAGAAGTA-3′ and antisense, 5′-CTGAAAATTAAGTCCTTGTGCCCAG-3′; ObRb: sense, 5′-TCACCCAGTGATTACAAGCT-3′ and antisense, 5′-CTGGAGAACTCTGATGTCCG-3′. PCR generated a 273-bp and 1071-bp fragment of the *ObRa* and *ObRb* genes, respectively.

Chromatin Immunoprecipitation Assay. Chromatin immunoprecipitation (ChIP) experiments were performed according to the method described elsewhere (11). For PCR, the following primers were used that cover the region −498 to +20 of the c-*myc* promoter: 5′-CACAGGACAAGGATGCGGTT-3′ (forward primer) and 5′-CCTCTGCCTCTCGCTGGAAT-3′ (reverse primer).

Real-Time Reverse Transcription-PCR. Total RNAs were extracted using TRIzol reagent (Invitrogen), and the expression of c-*myc* mRNA was measured by real-time reverse transcription-PCR using the ABI PRISM 7700 sequence detector and the TaqMan EZ reverse transcription-PCR kit (Applied Biosystems, Foster, CA). The primers and probe for real-time reverse transcription-PCR measurement were forward primer, GCCACGTCTCCACACATCAG; reverse primer, TCTTGGCAGCAGGATAGTCCTT; and probe, 6FAM-ACGCAGCGCCTCCCTCCACTC-TAMRA.

Received 2/23/04; revised 5/20/04; accepted 5/25/04.

Grant support: Grants 30225043 and 30393110 (Y. Shang) from National Natural Science Foundation of China and grant 2002BA711A01–05 (Y. Shang) from the Ministry of Science and Technology of China.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Requests for reprints: Yongfeng Shang, Department of Biochemistry and Molecular Biology, Peking University Health Science Center, 38 Xue Yuan Road, Beijing 100083, China. Phone: 86-10-8280-5118; Fax: 86-10-8280-1355; E-mail: jason@bjmu.edu.cn.

RNA Interference and MTT Assay. pSUPER-RNA interference (RNAi) plasmid was transfected into cells with Lipofectamine 2000 according to the manufacturer's instructions. The cells then were serum-deprived overnight and exposed to 50 nM of leptin for 24, 48, or 72 h before MTT assays. Transfection efficiency was monitored by cotransfection with an *Escherichia coli lacZ* construct (Invitrogen).

RESULTS

Effect of Leptin on the Proliferation of Breast Cancer Cells. The effect of leptin on cell proliferation was examined first in our experimental systems. In these experiments, MCF-7 cells and T47-D cells were serum-starved and then treated with various concentrations of leptin for different time periods. The effect of leptin treatment on cell proliferation then was assessed by MTT methodologies. As shown in Fig. 1, leptin stimulated the growth of MCF-7 cells and T47-D cells in a time- and dose-dependent fashion. Significant stimulatory effect was apparent even at 24 h after treatment of leptin at a concentration of 100 nM, and treatment of leptin at a concentration as low as 10 nM generated a significant growth-stimulatory effect at 48 h of treatment in MCF-7 cells. These experiments agreed well with previous observations (12, 13).

Activation of STAT3 and ERK Signaling Pathways in Growth Stimulation of Breast Carcinoma Cells by Leptin. As stated previously, leptin is believed to exert its biological function through binding to its receptors, which in turn transduce the signal through the activation of STAT3 and ERK pathways. To gain insight into the mechanism underlying the cell proliferative effect of leptin, we first examined the expression of leptin receptors in MCF-7 cells and T47-D cells. These cells were grown in normal growth media for 48–72 h and harvested for total protein or total RNA extraction. The expression of leptin receptor messengers and proteins was examined using reverse transcription-PCR and Western blot analysis, respectively. As shown in Fig. 2*A*, the messengers and the proteins of leptin receptors were detected in MCF-7 cells and T47-D cells.

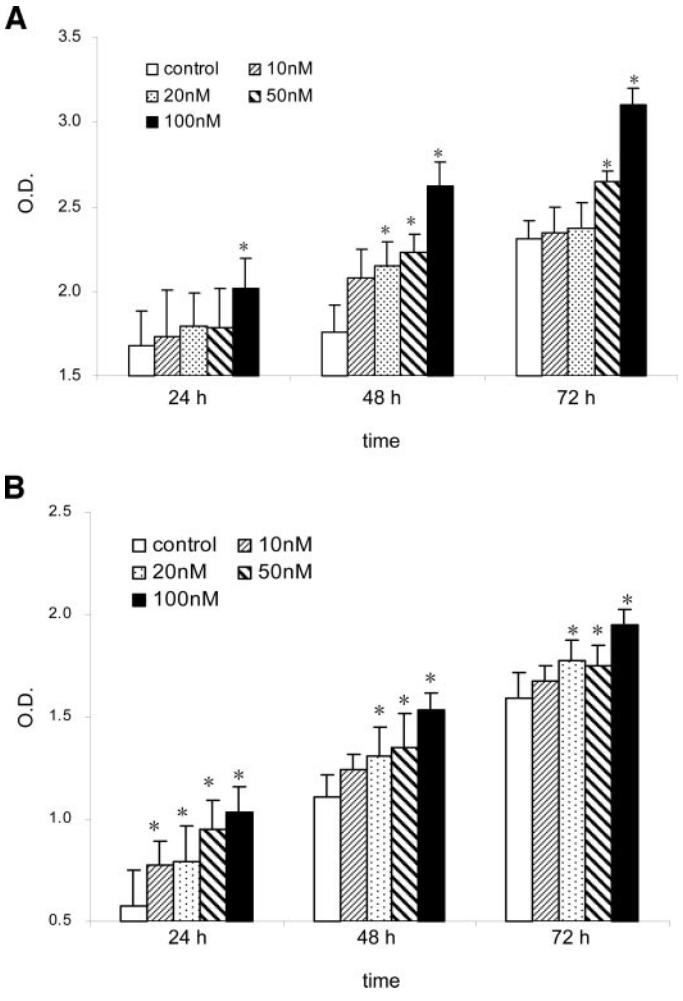

Fig. 1. The effect of leptin on the proliferation of breast cancer cells. *A*, MCF-7 cells and *B*, T47-D cells were incubated with 10–100 nM of leptin for 24, 48, and 72 h, and colorimetric MTT assays then were performed as described in "Materials and Methods." The data represent experiments performed in sextuplet; *significant differences (*versus* the control; $P < 0.05$).

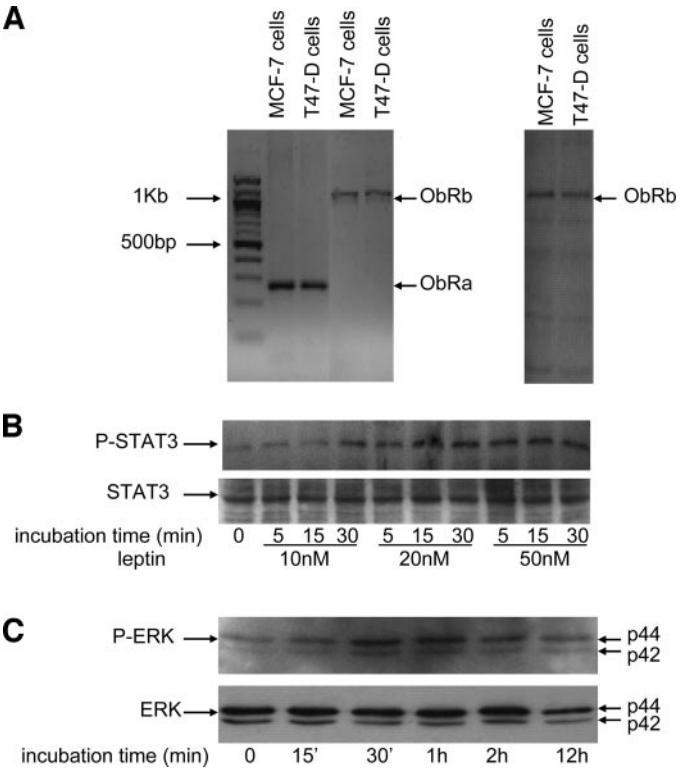

Fig. 2. Activation of STAT3 and ERK signaling pathways in growth stimulation of breast carcinoma cells by leptin. *A*, the expression of ObRa and ObRb mRNAs and ObR protein in MCF-7 and T47-D cells. Total RNA was extracted from MCF-7 and T47-D cells and analyzed by reverse transcription-PCR. Total proteins of MCF-7 and T47-D were examined by immunoblot analysis using a rabbit polyclonal antibody against ObRb. *B*, activation of STAT3 signaling by leptin. MCF-7 cells were treated with different concentrations of leptin for the time indicated, and then lysates (50 μg of protein) were immunoblotted with specific antibodies against total or phosphorylated form of STAT3. *C*, activation of ERK signaling by leptin. MCF-7 cells were treated with 50 nM of leptin for the time indicated, and lysates (50 μg of proteins) then were immunoblotted with specific antibodies against total or phosphorylated form of ERK.

We next examined the changes in STAT3 phosphorylation after the treatment of leptin in MCF-7 cells. Cellular proteins were extracted from MCF-7 cells that were exposed to leptin for various time periods. Lysates then were immunoblotted with a STAT3 phosphor-tyrosine-specific antibody. These experiments showed that STAT3 phosphorylation was stimulated by leptin in a time- and dose-dependent fashion (Fig. 2*B*). As little as 10 nM of leptin increased STAT3 phosphorylation after 30 min of treatment, whereas 20 nM of leptin resulted in an increase of STAT3 phosphorylation after 5 min of treatment. Reprobing the immunoblots with antibodies against STAT3 indicated that the total level of STAT3 was similar in all of the experiments, suggesting that the increased STAT3 phosphorylation was not caused by increased STAT3 expression.

Previous studies have shown that leptin binding to ObRb also leads to the phosphorylation-dependent activation of the ERK pathway (14). Therefore, we examined ERK phosphorylation after leptin stimulation

in the MCF-7 cells. As shown in Fig. 2*C*, the phosphorylation level of ERK1/2 increased after 15 min of 50-nM leptin treatment and peaked at 30 min of the treatment. Reprobing the immunoblots with antibodies against ERK1/2 showed that the total level of ERK1/2 was not changed. These experiments collectively showed that leptin treatment of leptin receptor-expressing MCF-7 cells led to STAT3 phosphorylation and ERK1/2 phosphorylation, suggesting that the cell proliferative effect of leptin in MCF-7 cells could be mediated via STAT3 and/or ERK1/2 signaling pathways.

STAT3 Signaling Pathway, But Not ERK1/2 Signaling Pathway, Is Involved in the Leptin-Induced Cell Proliferation of MCF-7. To investigate whether the STAT3 and ERK1/2 phosphorylation was linked to the cell proliferative effect of leptin and, if so, which signaling pathway, STAT3 or ERK1/2 or both, mediated this effect, we used specific inhibitors to block STAT3 phosphorylation or ERK1/2 phosphorylation, and the cell growth then was measured under the treatment of leptin by MTT assays. As shown in Fig. 3*A*, treatment of MCF-7 cells with JAK2/STAT3 phosphorylation-specific inhibitor AG490 specifically inhibited the phosphorylation of STAT3 protein without affecting the phosphorylation of ERK1/2. Analogously, treatment of MCF-7 cells with ERK1/2 phosphorylation-specific inhibitor U0126 specifically inhibited the ERK1/2 phosphorylation without affecting STAT3 phosphorylation. Examination of the cell proliferation under these conditions indicated that although blocking ERK1/2 phosphorylation had minimal effect on the growth stimulation by leptin, blocking STAT3 phosphorylation totally abrogated the growth stimulation of MCF-7 cells by leptin (Fig. 3*B*), suggesting that the STAT3 signaling pathway is the major growth-stimulating pathway mediating the cell proliferative effect of leptin.

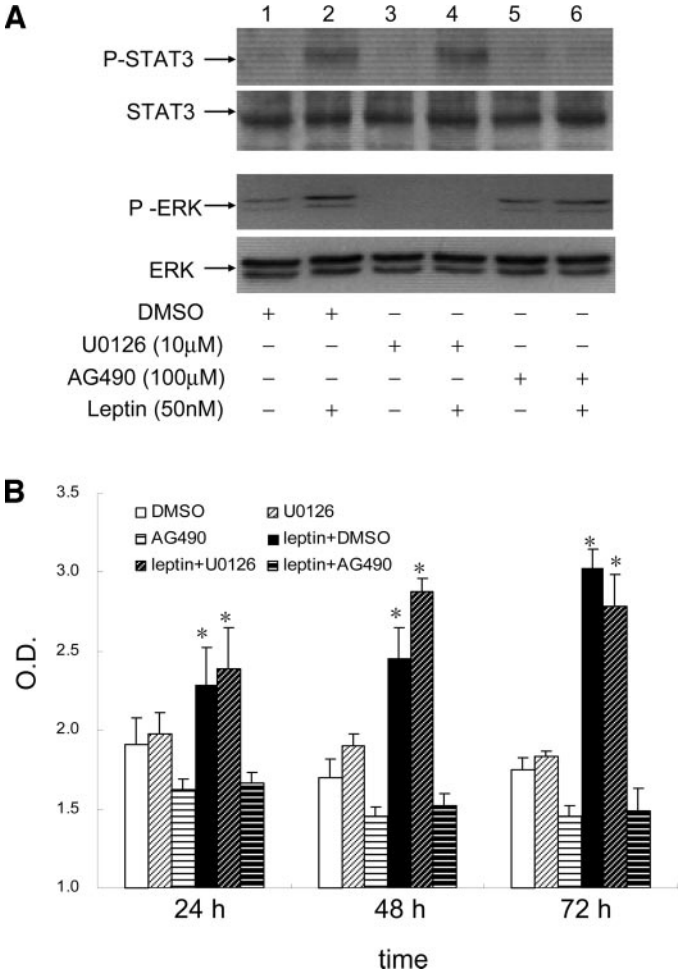

Fig. 3. Effect of ERK phosphorylation inhibitor U0126 and STAT3 phosphorylation inhibitor AG490 on the proliferation of MCF-7 cells induced by leptin. *A*, lysates were prepared from MCF-7 cells that had been serum starved (*Lanes 1, 3,* and *5*), starved and stimulated with 50 nM of leptin (*Lanes 2, 4,* and *6*), or pretreated with U0126 (10 μM; *Lanes 3* and *4*) or AG490 (100 μM; *Lanes 5* and *6*) for 30 min. Total proteins (50 μg) were immunoblotted with specific antibody against total or phosphorylated form of STAT3 or ERK. *B*, serum-starved MCF-7 cells were treated with 50 nM of leptin or left untreated for 24, 48, or 72 h following pretreatment with U0126 or AG490. Colorimetric MTT assays were performed, and the data represent experiments performed in sextuplet; ∗significant differences (*versus* the control; $P < 0.05$).

Leptin Induced the Expression of the STAT3 Target Gene, c-*myc*, and SRC-1, but not GRIP1 or AIB1, Enhanced the Leptin-Induced c-*myc* Transactivation. STAT3 is a cytoplasmic protein, which, once phosphorylated, is translocated into the cell nucleus where it acts as a transcription factor to regulate the rate of gene transcription. To gain further insight into the mechanism involved in STAT3-mediated cell proliferation under leptin treatment in MCF-7 cells, the expression of one of the STAT3 target genes, c-*myc*, was measured first by real-time reverse transcription-PCR under the treatment of leptin in MCF-7 cells. As shown in Fig. 4*A*, treatment of MCF-7 cells with leptin resulted in an increased c-*myc* expression, indicating that leptin treatment could lead to the activation of STAT3 target genes.

STAT3 activates gene transcription through recruitment of coactivators that modify the chromatin architecture. Previous studies have shown that the p160 family coactivators, SRC-1, GRIP1, and AIB1, although originally identified as cofactors involved in nuclear receptor-mediated gene transcription, also participate in gene transcriptional activation mediated by other transcription factors, including STATs. To investigate whether the p160 family coactivators also are involved in leptin-induced activation of STAT3 target genes, transient transfection experiments were performed to determine the enhancement of STAT3 target gene expression by p160 proteins. In these experiments, a c-*myc* promoter-driven luciferase construct was cotransfected with mammalian expression vectors containing SRC-1, GRIP1, or AIB1 cDNAs, and the luciferase activity then was measured in MCF-7 cells under the treatment of leptin. As shown in Fig. 4*B*, SRC-1, but not GRIP1 or AIB1, was able to enhance the expression of the luciferase gene. This discrepancy was not a result of the coactivator protein abundance because the expression levels of SRC-1, GRIP1, and AIB1 in these cells were similar (Fig. 4*D*). The enhanced expression of endogenous c-*myc* gene by SRC-1 also was evident as measured by Western blot analysis (Fig. 4*C*). These observations suggested that there was a differential involvement of p160 coactivators in gene transcription that was consistent with our previous reports (15).

The Interaction between STAT3 and SRC-1 *in Vitro* and *in Vivo* and the Promoter Occupancy of c-*myc* Gene by STAT3 and SRC-1. To further dissect the STAT3 target gene coactivation by SRC-1 coactivator, GST pull-down experiments were performed with *in vitro* transcribed and translated full-length SRC-1 protein and bacteria-expressed GST-fused STAT3 transactivation domain (TAD). GST or GST-STAT3/TAD was incubated with *in vitro* synthesized, ^{35}S-labeled SRC-1 protein for 1 h, and the resultant bead-binding proteins were resolved on 8% SDS-PAGE. *In vitro* transcribed and translated full-length GRIP1 and AIB1 also were included in the experiments. As shown in Fig. 5*A*, only SRC-1, but not GRIP1 or AIB1, was able to specifically interact with STAT3.

To determine whether SRC-1 functions as a transcriptional coactivator of STAT3 under physiologic conditions, we examined the interaction between SRC-1 and STAT3 *in vivo*. MCF-7 cells were stimulated with leptin for 30 min, and coimmunoprecipitations were performed with antibodies against STAT3 (Fig. 5*B*, *Lanes 4* and *6*) and IgG (Fig. 5*B*, *Lanes 3* and *5*). The immunoprecipitates were

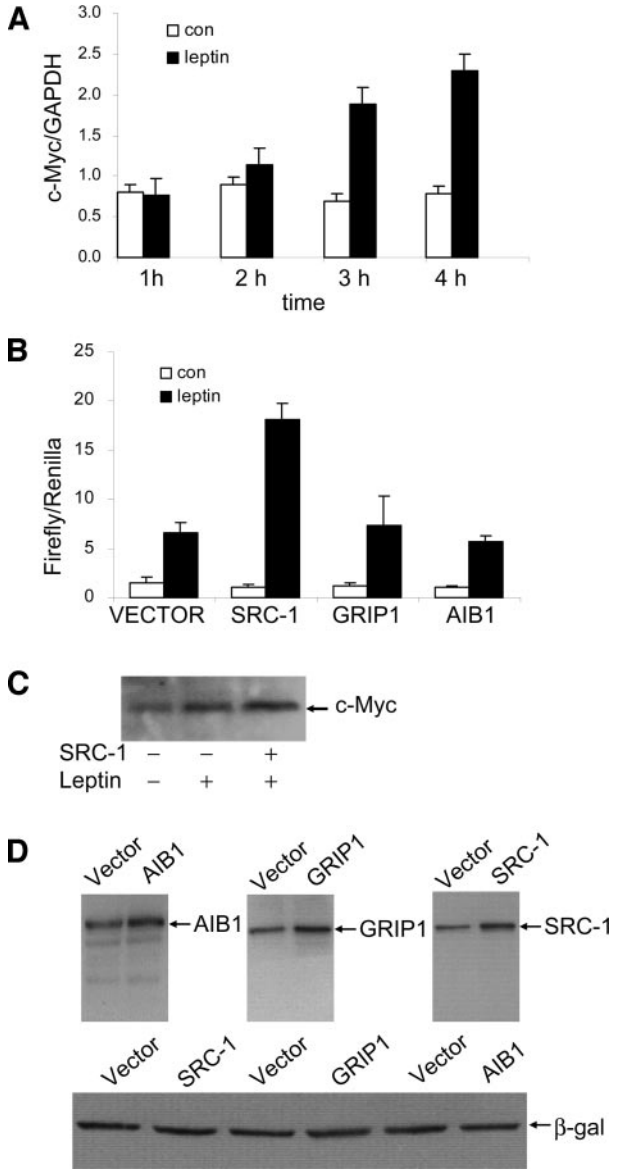

Fig. 4. SRC-1 enhanced leptin-induced c-*myc* transactivation. *A*, stimulation of c-*myc* expression by leptin in MCF-7 cells. MCF-7 cells were grown in phenol-free DMEM supplemented with 10% charcoal-dextran-stripped fetal bovine serum for at least 2 days before experiments. These cells then were serum starved overnight and left untreated or treated with 50 nM of leptin for the time indicated. Total RNAs were extracted, and the expression of c-*myc* gene was measured by real-time reverse transcription-PCR as described in "Materials and Methods." *B*, SRC-1, but not GRIP1 or AIB1, enhanced the leptin-induced c-*myc* transactivation in MCF-7 cells. MCF-7 cells were cotransfected with a c-*myc*-luciferase reporter construct and plasmids for SRC-1, GRIP1, or AIB1 expression. Transfected cells were treated with 50 nM of leptin for 6 h or left untreated (control). Luciferase activities were determined and normalized. The averages of three independent experiments with SD are shown. *C*, overexpression of SRC-1 enhanced leptin-induced c-*myc* expression. MCF-7 cells were transfected with the plasmids for SRC-1 expression, and then left untreated or treated with 50 nM of leptin for 2 h. The protein levels were examined by Western blot analysis using special antibodies against c-*myc*. *D*, MCF-7 cells overexpressing SRC-1, GRIP1, or AIB1. MCF-7 cells were transfected with an expression construct of SRC-1, GRIP1, or AIB1. Forty-eight h after transfection, the total proteins were extracted and examined by immunoblot analysis using the antibodies against SRC-1, GRIP1, or AIB1.

immunoblotted with the SRC-1 antibody, and the results showed that SRC-1 and STAT3 could coimmunoprecipitate (Fig. 5*B*, *Lane 6*), indicating that SRC-1 participates in leptin-induced STAT3 target gene transactivation.

We next examined the presence of SRC-1 protein on the promoter of the STAT3 target gene c-*myc* using ChIP assays (11). In these experiments, soluble chromatins were prepared using a formaldehyde cross-linking protocol, and the occupancy of c-*myc* promoter by SRC-1 and STAT3 was analyzed using specific antibodies against SRC-1 or STAT3 and using a pair of primers spanning the STAT3-responsive region in the c-*myc* promoter (Fig. 5*C*). Detection of GRIP1 and AIB1 also was included in the experiment. As shown in Fig. 5*D*, ChIP experiments detected the occupancy of SRC-1, but not GRIP1 or AIB1, along with STAT3 on the promoter of c-*myc* gene under the treatment of leptin. The increased presence of SRC-1 on

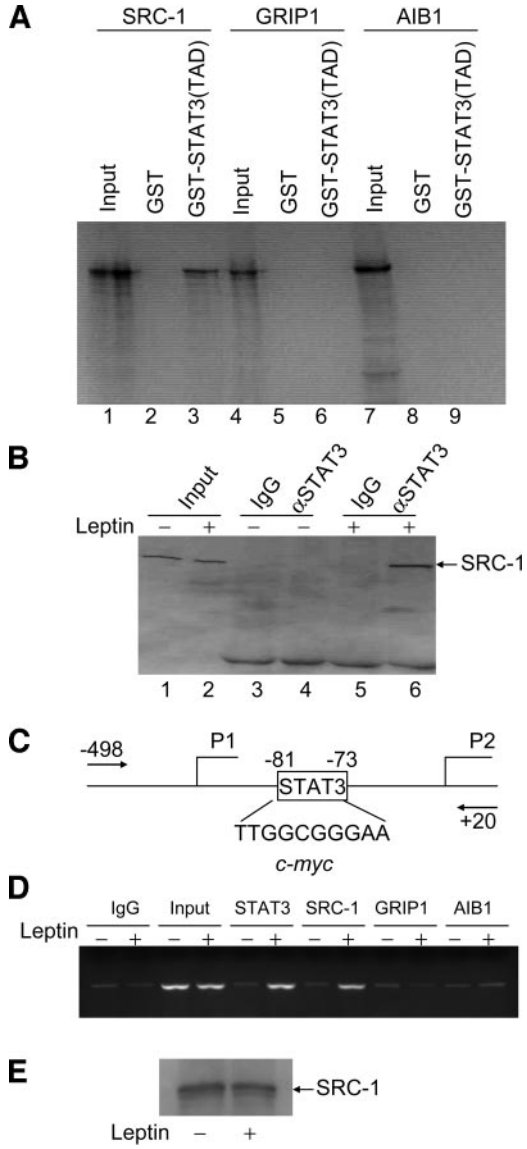

Fig. 5. The interaction between STAT3 and SRC-1 and the promoter occupancy of c-*myc* gene by STAT3 and SRC-1. *A*, SRC-1 interacts with the activation domain of STAT3. GST pull-down experiments were performed as described in "Materials and Methods." Input controls in *Lanes 1*, *4*, and *7* contain 20% of the amount of the ^{35}S-labeled proteins used in the pull-down reactions. *B*, MCF-7 cells were serum starved overnight and then stimulated with 50 nM of leptin or left untreated for 30 min. Cell extracts were directly analyzed by Western blot (*Lanes 1* and *2*) or first immunoprecipitated with antibodies against STAT3 (*Lanes 4* and *6*) or a control serum (*Lanes 3* and *5*), then separated by 10% SDS-PAGE, transferred to a nitrocellulose filter, and immunoblotted with SRC-1 antibody. *C*, schematic representation of the STAT3 consensus binding sites in the c-*myc* promoter and the primers for ChIP assays. *D*, soluble chromatin was prepared from serum-starved MCF-7 cells treated with 50 nM of leptin for 30 min or left untreated, followed by immunoprecipitation with a normal serum or with antibodies against STAT3, SRC-1, GRIP1, or AIB1. The final DNA extractions were amplified using pairs of primers that cover the STAT3 binding sites of the c-*myc* promoter. *E*, the protein level of SRC-1 in the MCF-7 nuclear extracts before and after leptin treatment. MCF-7 cells were serum starved overnight and stimulated with 50 nM leptin for 30 min or left untreated. Nuclear extracts then were analyzed by Western blot analysis with an antibody against SRC-1.

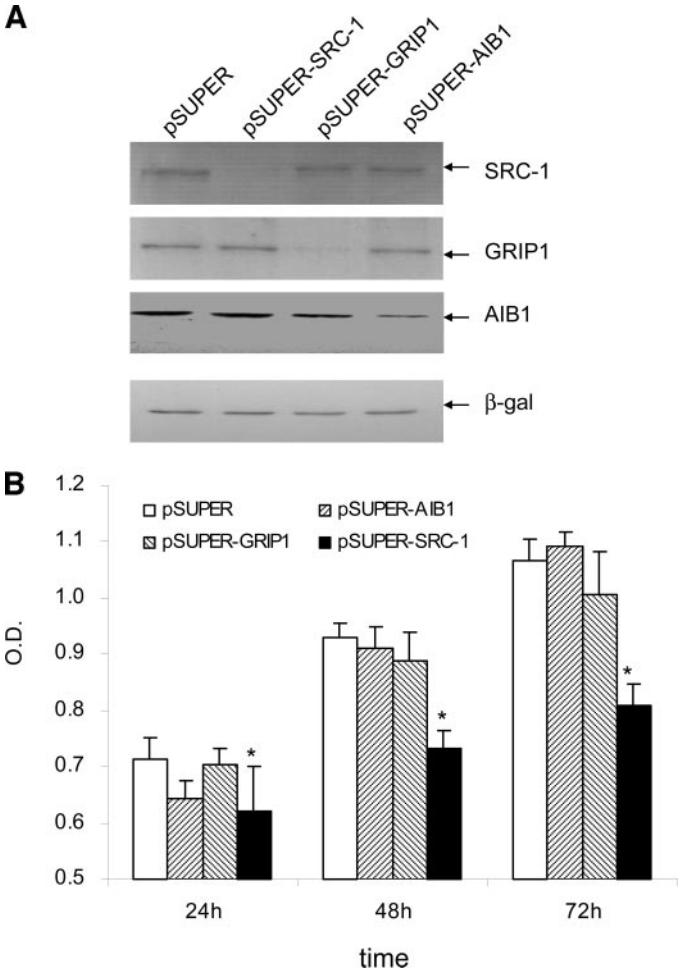

Fig. 6. The effect of SRC-1 silencing on leptin-stimulated MCF-7 cells proliferation. *A,* RNA interference was carried out by introducing pSUPER vectors carrying specific small interfering RNAs against the mRNAs of SRC-1, GRIP1, or AIB1 into MCF-7 cells for 48 h. Total proteins were extracted, and Western blot analysis was performed to monitor the expression of the indicated proteins. Transfection efficiency was monitored by cotransfection with an *Escherichia coli lacZ* construct, and the protein expression of β-galactosidase was detected by Western blot analysis with a β-galactosidase antibody. *B.* Leptin-induced proliferation of MCF-7 cells after RNAi. MCF-7 cells were transfected with pSUPER vectors carrying specific small interfering RNAs against the mRNAs of SRC-1, GRIP1, or AIB1, serum starved overnight, and stimulated with 50 nM of leptin for 24 h, 48 h, and 72 h. Colorimetric MTT assays were performed, and the data represent experiments performed in sextuplet; ∗significant differences (*versus* other groups; $P < 0.05$).

c-*myc* promoter was not caused by leptin-induced accumulation of SRC-1 in the cell nucleus because Western blot measurement did not detect any increase of nuclear SRC-1 with leptin treatment (Fig. 5*E*).

The Effect of SRC-1 Silencing on Leptin-Stimulated MCF-7 Cell Proliferation. Because SRC-1 was the only p160 coactivator involved in the leptin-induced STAT3 target gene activation, we sought to further substantiate the role of SRC-1 in leptin-induced proliferation of MCF-7 cells. We used RNAi approach to silence the expression of SRC-1, GRIP1, or AIB1, and the effect of RNAi on the growth stimulation of MCF-7 cells by leptin was measured by MTT assays. As shown in Fig. 6*A*, the expression of SRC-1, GRIP1, or AIB1 could be effectively silenced by a vector carrying specific sequence against the mRNAs of SRC-1, GRIP1, or AIB1. The growth-stimulatory effect of leptin was severely affected in MCF-7 cells with silenced SRC-1 expression, whereas the growth-stimulatory effect of leptin was not altered in MCF-7 cells with silenced GRIP1 or AIB1 expression. These results further supported the argument that SRC-1, but not GRIP1 and AIB1, plays an important role in leptin signaling pathway that leads to the proliferation of MCF-7 cells.

DISCUSSION

Leptin is a cytokine mainly produced by adipose tissue. Leptin recently has been reported to stimulate the proliferation of various cell types (4–6) and is considered to be a new growth factor. Hyperleptinemia is a common feature of obese women who have a higher risk of breast cancer than women with normal weights, and epidemiologic studies have suggested a correlation between obesity and breast cancer carcinogenesis (1). Therefore, understanding of the molecular mechanism involved in leptin signaling transduction is important in breast cancer prevention and management. We showed in this report that STAT3 signaling pathway is implicated in the cell proliferative effect of leptin.

STAT3 protein has been shown to be an important mediator of cell growth. It plays an essential role during embryonic development, cell survival, and proliferation (16). STAT3 activation often is associated with cell transformation. At the molecular level, STAT3 acts as a transcription activator and regulates a number of genes, such as c-*myc*, *cyclin D1*, *p21^{waf1}*, *Bcl*II, and *Bcl-xL*, which are critically involved in cell growth and proliferation (17–22). We showed that leptin induced the expression of c-*myc* gene and association of STAT3 on the c-*myc* promoter, suggesting the important role of STAT3 in the biological and pathobiological activities of leptin.

The p160 coactivator family is thought to contribute to transcriptional activation by being recruited to the gene promoter and by forming a platform for the binding of other coactivators. Initially identified as coactivators for nuclear receptor-mediated gene transcription, recent studies have shown that the p160 coactivators also are involved in transcriptional activation by various other transcription factors, such as activator protein, serum response factor, nuclear factor κB, and STATs (23–28). In this study, we showed that STAT3 interacted with and recruited one member of the p160 family, SRC-1, but not GRIP1 and AIB1, to its target gene promoter. The differential roles for the three p160 proteins were suggested by our previous reports (11, 15), and our current report further supported the roles of differential gene regulation by p160 proteins.

In conclusion, our current study provides evidence for better understanding of the signaling linking between adipocytes and mammary epithelial cells. These results will be useful for the better understanding of the association between obesity and breast cancer carcinogenesis.

REFERENCES

1. Ballard-Barbash R, Schatzkin A, Carter CL, et al. Body fat distribution and breast cancer in the Framingham Study. J Natl Cancer Inst 1990;82:286–90.
2. Schapira DV, Kumar NB, Lyman GH, Cox CE. Obesity and body fat distribution and breast cancer prognosis. Cancer 1991;67:523–8.
3. Laud K, Gourdou I, Pessemesse L, Peyrat JP, Djiane J. Identification of leptin receptors in human breast cancer: functional activity in the T47-D breast cancer cell line. Mol Cell Endocrinol 2002;188:219–26.
4. Takahashi Y, Okimura Y, Mizuno I, et al. Leptin induces mitogen-activated protein kinase-dependent proliferation of C3H10T1/2 cells. J Biol Chem 1997;272:12897–900.
5. Tsuchiya T, Shimizu H, Horie T, Mori M. Expression of leptin receptor in lung: leptin as a growth factor. Eur Pharmacol 1999;365:273–9.
6. Tanabe K, Okuya S, Tanizawa Y, Matsutani A, Oka Y. Leptin induces proliferation of pancreatic ā cell line MIN6 through activation of mitogen-activated protein kinase. Biochem Biophys Res Commun 1997;241:765–8.
7. White DW, Tartaglia LA. Leptin and OB-R: body weight regulation by a cytokine receptor. Cytokine Growth Factor Rev 1996;7:303–9.
8. Fei H, Okano HJ, Li C, et al. Anatomic localization of alternatively spliced leptin receptors (Ob-R) in mouse brain and other tissues. Proc Natl Acad Sci USA 1997; 94:7001–5.
9. Tartaglia LA, Dembski M, Weng X, et al. Identification and expression cloning of a leptin receptor, OB-R. Cell 1995;83:1263–71.
10. Banks AS, Davis SM, Bates SH, Myers MG Jr. Activation of downstream signals by the long form of the leptin receptor. J Biol Chem 2000;275:14563–72.
11. Shang Y, Hu X, DiRenzo J, Lazar MA, Brown M. Cofactor dynamics and sufficiency in estrogen receptor-regulated transcription. Cell 2000;103:843–52.

12. Hu X, Juneja SC, Maihle NJ, Cleary MP. Leptin—a growth factor in normal and malignant breast cells and for normal mammary gland development. J Natl Cancer Inst 2002;94:1704–11.
13. Dieudonne MN, Machinal-Quelin F, Serazin-Leroy V, et al. Leptin mediates a proliferative response in human MCF7 breast cancer cells. Biochem Biophys Res Commun 2002;293:622–8.
14. Bjorbaek C, Buchholz RM, Davis SM, et al. Divergent roles of SHP-2 in ERK activation by leptin receptors. J Biol Chem 2001;276:4747–55.
15. Shang Y, Brown M. Molecular determinants for the tissue specificity of SERMs. Science 2002;295:2465–8.
16. Takeda K, Noguchi K, Shi W, et al. Targeted disruption of the mouse Stat3 gene leads to early embryonic lethality. Proc Natl Acad Sci USA 1997;94:3801–4.
17. Bromberg JF, Wrzeszczynska MH, Devgan G, et al. Stat3 as an oncogene. Cell 1999;98:295–303.
18. Barre B, Avril S, Coqueret O. Opposite regulation of myc and p21waf1 transcription by STAT3 proteins. J Biol Chem 2003;278:2990–6.
19. Kiuchi N, Nakajima K, Ichiba M, et al. STAT3 is required for the gp130-mediated full activation of the c-myc gene. J Exp Med 1999;189:63–73.
20. Shirogane T, Fukada T, Muller JM, et al. Synergistic roles for Pim-1 and c-Myc in STAT3-mediated cell cycle progression and antiapoptosis. Immunity 1999;11: 709–19.
21. Catlett-Falcone R, Landowski TH, Oshiro MM, et al. Constitutive activation of Stat3 signaling confers resistance to apoptosis in human U266 myeloma cells. Immunity 1999;10:105–15.
22. Giraud S, Bienvenu F, Avril S, et al. Functional interaction of STAT3 transcription factor with the coactivator NcoA/SRC1a. J Biol Chem 2002;277:8004–11.
23. Korzus E, Torchia J, Rose DW, et al. Transcription factor-specific requirements for coactivators and their acetyltransferase functions. Science 1998;279:703–7.
24. Litterst CM, Kliem S, Marilley D, Pfitzner E. NCoA-1/SRC-1 is an essential coactivator of STAT5 that binds to the FDL motif in the á-helical region of the STAT5 transactivation domain. J Biol Chem 2003;278:45340–51.
25. Litterst CM, Pfitzner E. Transcriptional activation by STAT6 requires the direct interaction with NCoA-1. J Biol Chem 2001;276:45713–21.
26. Lee SK, Na SY, Jung SY, et al. Activating protein-1, nuclear factor-κB, and serum response factor as novel target molecules of the cancer-amplified transcription coactivator ASC-2. Mol Endocrinol 2000;14:915–25.
27. Na SY, Lee SK, Han SJ, et al. Steroid receptor coactivator-1 interacts with the p50 subunit and coactivates nuclear factor κB-mediated transactivations. J Biol Chem 1998;273:10831–4.
28. Lee SK, Kim HJ, Na SY, et al. Steroid receptor coactivator-1 coactivates activating protein-1-mediated transactivations through interaction with the c-Jun and c-Fos subunits. J Biol Chem 1998;273:16651–4.