Insulin Antagonizes Interleukin-6 Signaling and Is Anti-inflammatory in 3T3-L1 Adipocytes*

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Adipose tissue secretes different adipokines, including interleukin-6 (IL-6), that have been implicated in the insulin resistance and inflammatory state characterizing obesity. We examined the putative cross-talk between insulin and IL-6 in adipose cells and found that insulin exerts an inhibitory effect on the IL-6 signaling pathway by altering the post-translational modifications of the signal transducer and activator of transcription 3 (STAT3). Insulin reduces the tyrosine phosphorylation and increases the serine phosphorylation of STAT3, thereby reducing its nuclear localization and transcriptional activity. Signaling through the MEK/MAPK pathway plays an important role as treatment with the MEK inhibitor PD98059 reduces the effects of insulin on IL-6 signaling. We also show that the protein tyrosine phosphatase SHP2 is activated upon insulin signaling and is required for the dephosphorylation of STAT3 and that insulin exerts a synergistic effect with IL-6 on suppressor of cytokine signaling 3 expression. As a consequence, the IL-6-induced expression of the inflammatory markers serum amyloid A3 and haptoglobin are significantly decreased in cells incubated with both IL-6 and insulin. Thus, insulin exerts an important anti-inflammatory effect in adipose cells by impairing the IL-6 signal at several levels.

Adipose tissue has long been regarded as important only for the storage and release of lipids. It is now known to be the largest endocrine organ in the body, secreting cytokines, hormones, and growth factors (commonly referred to as adipokines) that are important paracrine/endoctrine regulators (1). Adipose tissue is also thought to play an important role in the low-grade chronic inflammation associated with obesity and insulin resistance (1). Obesity and enlarged fat cells alter the pattern of secreted adipokines, favoring release of the inflammatory cytokine, interleukin-6 (IL-6), and reducing the anti-inflammatory and insulin-sensitizing adipokine, adiponectin (1).

IL-6 is an important activator of inflammation in both liver and adipose tissue. Interestingly, the IL-6 concentration in the interstitial fluid of the adipose tissue is ~50-fold higher than in circulation, which strongly supports its role as an important regulator in this tissue (2). In addition, the adipose tissue accounts for ~30% of the circulating IL-6 concentration (3).

Several studies have shown that IL-6 impairs insulin signaling. For example, IL-6 has been shown to reduce expression of adiponectin in human adipose tissue and the insulin receptor substrate-1 (IRS-1) and glucose transporter 4 in 3T3-L1 cells (2, 4). IL-6 is also a well known inducer of suppressor of cytokine signaling (SOCS) proteins (5), and induction of SOCS1 and SOCS3 has been shown to inhibit insulin signaling by binding to the insulin receptor and IRS-1 (6–8).

Intracellular IL-6 signaling is initiated by the interaction of IL-6 with a plasma membrane receptor complex containing the signal transducer glycoprotein 130 (gp130). This results in the activation of Janus kinases, allowing tyrosine phosphorylation of gp130. In turn, this recruits signal transducers and activators of transcription (STAT) family members, such as STAT3, but also other Src homology 2 domain (SH2)-containing proteins, including the protein tyrosine phosphatase SHP2. STAT3 is phosphorylated by Janus kinase at Tyr-705, which is considered crucial for the homo- or heterodimerization of STAT3 to either STAT3 or STAT1, enabling nuclear translocation (9). Phosphorylation of Ser-727 in STAT3 has been reported to be important for transcriptional activation, but the evidence is contradictory. Some reports have shown that serine phosphorylation increases the transcriptional activation of STAT3 (10–14), whereas others have shown that it reduces or has no effect on transcriptional activity (15–18). There are also reports indicating that the serine and tyrosine phosphorylations may negatively regulate each other (16). The effect of Ser-727 phosphorylation of STAT3 may vary according to cell type, the kinase pathway activated, and the cytokine or growth factor used.

In this study, we have further investigated the cross-talk between insulin and IL-6 and asked whether insulin alters the IL-6 signaling pathway in 3T3-L1 cells. It has previously been implicated that insulin has an anti-inflammatory effect in vivo and in peripheral blood mononuclear cells through an effect on of transcription; SH, Src homology; MAPK, mitogen-activated protein kinase; MEF, mouse embryonic fibroblast; DMEM, Dulbecco’s modified Eagle’s medium; SAA, serum amyloid A; ERK, extracellular signal-regulated kinase; MEK, mitogen-activated protein kinase/extracellular signal-regulated kinase.
Our results show that preincubation of 3T3-L1 cells with insulin significantly reduces the transcriptional activity of STAT3, thus reducing the transcription of the IL-6-induced inflammatory proteins SAA3 and haptoglobin. This is accomplished through a reduced tyrosine phosphorylation of STAT3 and, consequently, nuclear translocation and transcriptional activity in response to IL-6. We also provide evidence that STAT3 tyrosine and serine phosphorylations are regulated by insulin through the protein tyrosine phosphatase SHP2 and the MEK/MAPK signaling pathway, respectively. We conclude that insulin exerts anti-inflammatory effects in 3T3-L1 adipocytes by modulating the intracellular IL-6 signaling pathway.

EXPERIMENTAL PROCEDURES

Cell Cultures—3T3-L1 cells and mouse embryonic fibroblasts (MEFs) were grown in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum, 1% penicillin/streptomycin, and 2 mM L-glutamine. Differentiation was induced in post-confluent 3T3-L1 cells by adding a differentiation mixture (500 μM 3-isobutyl-1-methylxantine, 5 μg/ml insulin, and 10 μM dexamethasone) for 2 days. The cells were then allowed to grow for another 2 days in supplemented DMEM with 5 μg/ml insulin followed by 4 days in normal supplemented DMEM (i.e. no insulin or differentiation mixture). On day 8, medium was changed to DMEM with 1.5% fetal bovine serum for 12 h. The cells were then washed in phosphate-buffered saline and grown for another 12 h in DMEM with 0.5% bovine serum albumin before starting the experiment. The MEFs were starved for 16 h in DMEM with 0.5% bovine serum albumin before the insulin/IL-6 additions. The cells were then incubated with 20 ng/ml IL-6 with or without 100 nM insulin for different times. Insulin was usually added 10 min before IL-6. PD98059 (Calbiochem, San Diego, CA) (40 μM) was added 30 min before IL-6.

Cell Lysates, Immunoprecipitation, and Western Blot—Cells were washed in phosphate-buffered saline and lysed buffer (25 mM Tris-HCl, pH 7.4, 0.5 mM EGTA, 25 mM NaCl, 1% Nonidet P-40, 10 mM NaF, 0.01 mg/ml leupeptin, 1 mM orthovanadate, 100 mM okadaic acid, 1 mM benzamidine, 2 mM AEBSF) was then added to the cells, and the lysate was centrifuged at 12,000 rpm for 10 min. Preparation of nuclear lysate followed the recommendation of the manufacturer of the STAT3 transcription factor assay (Chemicon International, Temecula, CA). Purity of the nuclear fraction was verified by immunoblotting for proteins abundant in the cytoplasm. Protein concentration was measured by the BCA protein assay kit (Pierce). Immunoprecipitations were made by adding 30 μl of Protein G-coupled Dynabeads (Dynal Biotech, Oslo, Norway) that had been preincubated for 1 h with 1 μg of α-gp130 antibody (Santa Cruz Biotechnology, Santa Cruz, CA) to 100 μg of cell lysates and incubated overnight at 4 °C. The lysate (or immunoprecipitated proteins) was boiled in SDS loading buffer and applied on 7.5% SDS-PAGE (Cambrex, Walkersville, MD). Following gel transfer, the nitrocellulose membranes were blocked with 5% fat-free milk powder in phosphate-buffered saline-Tween 20 for 1 h. Membranes were probed with primary antibodies overnight at 4 °C followed by incubation for 1 h with secondary horseradish peroxidase-conjugated antibodies (Amersham Biosciences) diluted 1:1000 in blocking solution. Proteins were visualized by ECL substrate (Amersham Biosciences). The primary antibodies (pY705-STAT3, pY542-SHP2, pY694-STAT5, and pT202/Y204-p44/p42 MAPK) were all from Cell Signaling Technologies (Beverly, MA) and diluted 1:1000 according to the manufacturer’s protocol. STAT3 (BD Biosciences) monoclonal antibody was diluted 1:2000, and pS727-STAT3 (Santa Cruz Biotechnology) was diluted 1:500.

Transcriptional Activity of STAT3—Nuclear lysates from differentiated 3T3-L1 cells treated with IL-6 and insulin for 15, 30, and 60 min were analyzed using a STAT3 transcription factor assay kit (Chemicon International) according to the recommendations of the manufacturer.

Reverse Transcriptase PCR and Quantitation Analysis—RNA was isolated with the RNasey mini kit (Qiagen, Valencia, CA), and the following reverse transcriptase PCR for cDNA preparation was made using the High Capacity cDNA Archive kit (Applied Biosystems, Foster City, CA). Relative quantification was made using the ABI Prism 7700 Sequencing Detection System (Applied Biosystems). Primers and probes were designed using Primer Express software, and sequences are available upon request.

Statistical Analysis—All statistical analyses were performed in Microsoft Excel, and statistical significances were evaluated with the paired Student’s t test when appropriate.

RESULTS

Effect of Insulin on Tyr-705 Phosphorylation of STAT3—To study the effects of insulin on IL-6-induced activation of STAT3, Western blot analysis was used to determine the phosphorylation of Tyr-705 of STAT3 in differentiated 3T3-L1 adipocytes following incubation with IL-6 and/or insulin. The IL-6-induced tyrosine phosphorylation of STAT3 occurred rapidly (data not shown), and insulin did not exert an immediate effect on the phosphorylation of Tyr-705 of STAT3. However, after 30 min a consistent reduction was seen in cells treated with both IL-6 and insulin compared with cells treated with IL-6 alone (Fig. 1A, upper panel). The STAT3 protein content was not altered by any of the additions (lower panel).

Effect of Insulin on Immunoprecipitation of gp130 and STAT3—As IL-6-induced tyrosine phosphorylation occurs when STAT3 is bound to the gp130 receptor, we investigated the effect of insulin on the interaction between STAT3 and gp130 using co-immunoprecipitation experiments. Lysates from differentiated 3T3-L1 cells treated for 120 min with insulin and/or IL-6 were immunoprecipitated with an anti-gp130 antibody followed by immunoblotting for STAT3. The amount of STAT3 associated with gp130 was reduced in samples treated with both IL-6 and insulin compared with samples treated with IL-6 alone (Fig. 1B, upper panel). The blots were probed with α-gp130 antibody to verify that equal amounts of gp130 had been immunoprecipitated (lower panel). These results show that the reduction in IL-6-induced tyrosine phosphorylation of STAT3 in response to insulin is associated with a decreased binding of STAT3 to gp130.

Effect of Insulin on Nuclear Localization of STAT3—We investigated the effect of insulin on IL-6-induced dimerization of STAT3.
and nuclear translocation of STAT3, as these events are dependent on tyrosine phosphorylation (20, 21). Nuclear lysates were prepared and analyzed for STAT3 after 15–60-min incubations with IL-6 and/or insulin. The level of STAT3 in the nucleus was reduced after 15 min in samples treated with both IL-6 and insulin compared with samples treated with IL-6 alone (Fig. 2A). This reduction was seen throughout the study period.

**Effect of Insulin on the Transcriptional Activation of STAT3**

Using a STAT3 transcription factor assay, nuclear lysates from differentiated 3T3-L1 cells that had been incubated with IL-6 and/or insulin were analyzed. A significant reduction of the transcriptional activation was detected after 15 min in samples treated with IL-6 and insulin compared with samples treated with IL-6 alone (Fig. 2B). These results are in agreement with the results of nuclear translocation (Fig. 2A), and the insulin-induced reduction was also observed throughout the studied period.

**IL-6-induced Gene Expression**—The inflammatory proteins serum amyloid A (SAA) and haptoglobin are strongly up-regulated by cytokines such as IL-6 (22, 23). We analyzed the gene expression of SAA3 and haptoglobin in differentiated 3T3-L1 cells that had been incubated with IL-6 and/or insulin. A significant reduction of the transcriptional activation was detected after 15 min in samples treated with IL-6 and insulin compared with samples treated with IL-6 alone (Fig. 2B). These results are in agreement with the results of nuclear translocation (Fig. 2A), and the insulin-induced reduction was also observed throughout the studied period.

**IL-6 also up-regulates expression of the suppressor of cytokine signaling SOCS3** (5). Exposure of differentiated 3T3-L1 cells to IL-6 for 60 min significantly increased the gene expression of SOCS3 ~25-fold (Fig. 3C). In contrast to the gene expression of SAA3 and haptoglobin, addition of insulin increased the IL-6-induced expression of SOCS3 (Fig. 3C). In an attempt to explain this finding, we examined the effect of insulin on STAT5, as previous studies have shown that STAT5B is activated by insulin and enhances SOCS3 expression (7).

Immunoblots showed an increased tyrosine phosphorylation of STAT5 in samples treated with IL-6 or insulin alone for 60 min, but the phosphorylation was greater in the presence of both insulin and IL-6 (Fig. 3D). This indicates that IL-6 and insulin
can synergistically activate STAT5, supporting the suggestion that SOCS3 expression can be induced by both STAT3 and STAT5 activation.

Involvement of SHP2—We examined the effect of insulin on the protein tyrosine phosphatase SHP2, as this enzyme is known to exert a regulating effect on the tyrosine phosphorylation of STAT3 (9). Immunoblotting of lysates from differentiated 3T3-L1 cells that had been incubated with IL-6 and/or insulin showed that insulin slowly increased the tyrosine phosphorylation of SHP2, which in turn is associated with enzyme activation (Fig. 4A). To further investigate the importance of SHP2 in STAT3 phosphorylation, we studied MEFs from mice expressing a mutant variant of SHP2 where the SH2 domains that are necessary for SHP2 activity had been deleted (24). The kinase-dead MEFs showed a high degree of tyrosine phosphorylation of STAT3 in the basal state, and this was not altered by IL-6 and/or insulin (Fig. 4B). These findings support the importance of SHP2 for regulating the tyrosine phosphorylation of STAT3.

Effect of Insulin on Ser-727 Phosphorylation of STAT3—Ser-727 phosphorylation of STAT3 has been shown to either activate or inhibit STAT3, depending on cell type (10–18, 25). Western blot analysis was used to determine the phosphorylation of Ser-727 of STAT3 in differentiated 3T3-L1 adipocytes following incubation with insulin and/or IL-6. Insulin alone caused a rapid (15 min) phosphorylation of Ser-727 (data not shown) that remained throughout the 120-min experiment, whereas the IL-6-induced phosphorylation of Ser-727 declined after 60 min (Fig. 5). Insulin also increased the IL-6-induced phosphorylation of Ser-727 compared with the effect of IL-6 alone (Fig. 5).

Interaction of MAPK Signaling Pathway on IL-6 Signaling—It has previously been reported that the MEK inhibitor PD98059 inhibits serine phosphorylation of STAT3 in a Raf-inducible fibroblast cell line (16). To investigate whether the MAPK signaling pathway affects STAT3 signaling in 3T3-L1 cells, these cells were pretreated with PD98059 30 min before addition of insulin and/or IL-6 for 120 min. Insulin caused an increase in the levels of phosphorylated ERK1 and ERK2, and this was reduced by the addition of PD98059 (Fig. 6A). The results showed that the insulin-induced serine phosphorylation of STAT3 was not only antagonized by the presence of PD98059 but, interestingly, the ability of insulin to antagonize the IL-6-stimulated tyrosine phosphorylation of STAT3 was also prevented in cells pretreated with PD98059 (Fig. 6A).
Anti-inflammatory Effect of Insulin

To further analyze the cross-talk between the MEK/MAPK pathway and the effect of insulin on IL-6 signaling, the nuclear localization and transcriptional activation of STAT3 were studied in 3T3-L1 cells pretreated with PD98059 before addition of insulin and/or IL-6 for 15 min. The ability of insulin to reduce the IL-6-stimulated nuclear localization of STAT3 was almost completely inhibited in the presence of PD98059 (Fig. 6B). Similarly, insulin had no significant inhibitory effect on the IL-6-induced transcriptional activation of STAT3 in cells pretreated with PD98059 (Fig. 6C).

DISCUSSION

In this report, we show that preincubation of 3T3-L1 cells with insulin: 1) reduced the IL-6-induced Tyr-705 phosphorylation of STAT3; 2) reduced the IL-6-induced coprecipitation of gp130 and STAT3; 3) reduced the IL-6-induced nuclear localization and transcriptional activity of STAT3; 4) reduced the IL-6-induced gene expression of SAA3 and haptoglobin; and 5) increased the IL-6-induced Ser-727 phosphorylation of STAT3. In addition, we show the inhibitory effect of insulin was regulated by SHP2 and the MEK/MAPK signaling pathway.

IL-6-induced tyrosine phosphorylation of STAT3 is required for the dimerization of STATs through their SH2 domains (21), and formation of STAT dimers is required for nuclear translocation (9, 20). This is in agreement with our study showing that the insulin-induced reduction of IL-6-stimulated STAT3 tyrosine phosphorylation was accompanied by a reduced nuclear localization of STAT3 as well as a reduced activation of the proinflammatory genes SAA3 and haptoglobin. The reduction in tyrosine phosphorylation of STAT3 seems, in part, to be mediated through SHP2 as this protein tyrosine phosphatase is activated by insulin with a similar time course as the reduced STAT3 tyrosine phosphorylation. SHP2 not only interacts with both STAT1 and STAT3 (27, 31). Our results support this, as we found a reduced coprecipitation of gp130 and STAT3 in the presence of IL-6 and insulin. The same samples also showed an increased coprecipitation between SHP2 and STAT3 (data not shown). The importance of SHP2 was further supported by the finding that MEFs lacking a functional SHP2 activity showed an increased Tyr-705 phosphorylation of STAT3 in both the basal and IL-6-stimulated states. In addition, overexpression of wild-type SHP2 in MEFs completely inhibited IL-6-induced expression of the inflammatory marker genes SAA3 and haptoglobin (data not shown).

Ser-727 phosphorylation of STAT3 has previously been described to either activate or inhibit the transcriptional properties of STAT3 in different cells (10–18, 25). The controversial effect of Ser-727 phosphorylation of STAT3 may depend on cell type, the kinase pathway activated, and/or the cytokine/growth factor used. Our study suggests that serine phosphorylation is inhibitory for the transcriptional activity of STAT3 in 3T3-L1 cells. We found that Ser-727 phosphorylation of STAT3 was rapidly increased by insulin, which is in agreement with a previous study showing that insulin-induced serine phosphorylation of STAT3 occurs within 5 min (32). IL-6-induced Ser-727 phosphorylation of STAT3 declined more rapidly than that seen with insulin, which indicates that Ser-727 phosphorylation by IL-6 and insulin is mediated through different serine/threonine kinases. As several serine/threonine kinases have been described to phosphorylate Ser-727, including ERK, JNK, p38 MAPK, protein kinase C δ, and mTOR kinase (10–12, 14–16, 18, 25, 33), we used specific inhibitors to some of the known pathways involved in order to define the mechanism involved. Specifically, we inhibited the p38, JNK, MEK, phosphatidylinositol 3-kinase, and mTOR pathways. However, the MEK inhibitor PD98059, which blocks signaling upstream of ERK1/2, was found to reduce all the studied effects of insulin on STAT3 signaling (i.e., alterations of Ser and Tyr phosphorylations, nuclear translocation, and transcriptional activity). Also, other studies have found that inhibition of the MEK signaling pathway alters STAT3 phosphorylation and that this can alter the transcriptional activity (16, 34).

Because we were unable to find any insulin-associated changes in the Janus kinase 2-STAT3 interactions (data not shown), we focused on the role of SHP2 for the reduction of STAT3 tyrosine phosphorylation in response to insulin. However, it is unlikely that the rather late effect of insulin on SHP2 activation can solely account for the rapid inhibitory action on STAT3 transcriptional activation as seen in Fig. 2B. To explain this difference, we speculate that the rapid induction of Ser-727 phosphorylation of STAT3 by insulin plays a role in the early reduction of nuclear translocation and transcriptional activation of STAT3 in response to IL-6. Such an effect could be mediated by the impaired binding of STAT3 to gp130 seen in the presence of insulin. The later activation of SHP2 by insulin and the associated gradual reduction in tyrosine phosphorylation of STAT3 would then serve as a mechanism to turn off the IL-6 signal. Interestingly, preliminary experiments indicate that SHP2 can directly interact with STAT3 in an insulin-dependent manner (data not shown), which supports that STAT3 is a substrate for the phosphatase SHP2. This is in accordance with data previously presented by Wang et al. (27).

STAT3 regulates expression of SOCS1–7. This family of proteins contains a central SH2 domain that interacts with Tyr-759 of gp130 to inhibit STAT3 activation, thus acting as negative feedback regulators of STAT signaling (5, 9, 29). SOCS3 is rapidly induced by IL-6 stimulation, and expression of this enzyme peaks within 1 h (5). As we found that insulin reduced the transcriptional activity of STAT3, one would expect a reduced expression of SOCS3. We found, however, that the IL-6-induced expression of SOCS3 was increased by insulin. To address the possible mechanisms for this effect of insulin, we investigated other transcription factors (STATs) that target induction of SOCS3, including STAT5B (7). In contrast to Emanuelli et al. (7), we did not find a clear increase of SOCS3 expression in samples incubated with insulin alone, but we showed that IL-6 and insulin synergistically activated SOCS3, probably through an increased activation of STAT5. These findings provide a mechanism whereby insulin can increase SOCS3 expression while concomitantly reducing STAT3 tyrosine phosphorylation and transcriptional activity. The synergistic effect of IL-6 and insulin on SOCS3 expression is an additional mechanism whereby insulin can turn off the IL-6 signal;
in addition, the insulin signal as SOCS3 is a feedback inhibitor to insulin (7, 35).

The ability of insulin to antagonize IL-6 signaling, and induction of inflammatory proteins like SAA3 and haptoglobin, shows that insulin exerts anti-inflammatory effects in 3T3-L1 adipocytes. This could have important physiological consequences because the adipose tissue plays a major role for the innate inflammatory response (36). Furthermore, the insulin resistance in the adipose tissue can contribute to the increased inflammation seen in obesity and Type 2 diabetes (37, 38).

Acknowledgments—We thank Dr. Tony Pawson and Dr. Reiner Lammers for providing MEF SHP2 cells. We also thank Rosie Perkins for reading and commenting on the manuscript, Stina Mikkelsen for technical assistance, and Dr. Ira Nagaer for designing TaqMan sets.

REFERENCES
1. Hammarstedt, A., Andersson, C. X., Rotter Sopasakis, V., and Smith, U. (2005) Prostaglandins Leukot. Essent. Fatty Acids 73, 65–75
2. Sopasakis, V. R., Sandqvist, M., Gustafson, B., Hammarstedt, A., Schmelz, M., Yang, X., Jansson, P. A., and Smith, U. (2004) J. Biol. Chem. 279, 454–460
3. Mohamed-Ali, V., Goodrick, S., Rawesh, A., Katz, D. R., Miles, J. M., Yudkin, J. S., Klein, S., and Coppack, S. W. (1997) J. Clin. Endocrinol. Metab. 82, 4196–4200
4. Rotter, V., Nagaev, I., and Smith, U. (2003) J. Biol. Chem. 278, 45777–45784
5. Starr, R., Willson, T. A., Murray, L. J., Rayner, J. R., Jenkins, B. J., Gonda, T. J., Alexander, W. S., Metcalf, D., Nicola, N. A., and Hilton, J. S. (1997) Nature 387, 917–921
6. Shi, H., Tzameli, I., Bjorbaek, C., and Flier, J. S. (2004) J. Clin. Endocrinol. Metab. 89, 4171–4175
7. Emanuelli, B., Peraldi, P., Filloux, C., Sawka-Verhelle, D., Hilton, D., and Van Obberghen, E. (2000) J. Biol. Chem. 275, 27439–27449
8. Flier, J. S., Cook, K. S., Usher, P., and Spiegelman, B. M. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 4131–4135
9. Heinrich, P. C., Behrmann, I., Haan, S., Hermanns, H. M., Muller-Newen, G., and Schaper, F. (2003) Biochem. J. 374, 1–20
10. Abe, K., Hirai, M., Mizuno, K., Higashi, N., Sekimoto, T., Miki, T., Hirano, T., and Nakajima, K. (2001) Oncogene 20, 3464–3474
11. Schuringa, J. J., Dekker, L. V., Vellenga, E., and Kruijer, W. (2001) J. Biol. Chem. 276, 27709–27715
12. Turkson, J., Bowman, T., Adnane, J., Zhang, Y., Djeu, J. Y., Sekharan, M., Frank, D. A., Holzman, L. B., Wu, J., Sebti, S., and Jove, R. (1999) Mol. Cell. Biol. 19, 7519–7528
13. Sengupta, T. K., Talbot, E. S., Scherle, P. A., and Ivashkiv, L. B. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 11107–11112
14. Lim, C. P., and Cao, X. (1999) J. Biol. Chem. 274, 31055–31061
15. Jain, N., Zhang, T., Kee, W. H., Li, W., and Cao, X. (1999) J. Biol. Chem. 274, 24392–24400
16. Chung, J., Uchida, E., Grammer, T. C., and Blenis, J. (1997) Mol. Cell. Biol. 17, 6508–6516
17. Wen, Z., and Darnell, J. E., Jr. (1997) Nucleic Acids Res. 25, 2062–2067
18. Ng, J., and Cantrell, D. (1997) J. Biol. Chem. 272, 24542–24549
19. Dandona, P., Aljada, A., Mohanthy, P., Ghanim, H., Hamouda, W., Assian, E., and Ahmad, S. (2001) J. Clin. Endocrinol. Metab. 86, 3257–3265
20. Milocco, L. H., Haslam, J. A., Rosen, J., and Seidel, H. M. (1999) Mol. Cell. Biol. 19, 2913–2920
21. Shuai, K., Horvath, C. M., Huang, L. H., Qureshi, S. A., Cowburn, D., and Darnell, J. E., Jr. (1994) Cell 76, 821–828
22. Ramadori, G., Van Damme, J., Rieder, H., and Meyer zum Buschenfelde, K. H. (1988) Eur. J. Immunol. 18, 1259–1264
23. do Nascimento, C. O., Hunter, L., and Trayhurn, P. (2004) Biochem. Biophys. Res. Commun. 313, 702–708
24. Saxton, T. M., Henkemeyer, M., Gasca, S., Shen, R., Rossi, D. J., Shalaby, F., Feng, G. S., and Pawson, T. (1997) EMBO J. 16, 2352–2364
25. Yokogami, K., Wakisaka, S., Avruch, J., and Reeves, S. A. (2000) Curr. Biol. 10, 47–50
26. Gunaje, J. J., and Bhat, G. J. (2001) Biochem. Biophys. Res. Commun. 288, 252–257
27. Wang, C. Z., Su, H. W., Hsu, Y. C., Shen, M. R., and Tang, M. J. (2006) Mol. Biol. Cell 17, 2839–2852
28. Stahl, N., Farruggella, T. J., Boulton, T. G., Zhong, Z., Darnell, J. E., Jr., and Yancopoulos, G. D. (1995) Science 267, 1349–1353
29. Lehmann, U., Schmitz, J., Weissenbach, M., Sobota, R. M., Hörtner, M., Friederichs, K., Behrmann, I., Tsiaris, W., Sasaki, A., Schneider-Mergener, J., Yoshimura, A., Neel, B. G., Heinrich, P. C., and Schaper, F. (2003) J. Biol. Chem. 278, 661–671
30. Terstegen, L., Gatsios, P., Bode, J. G., Schaper, F., Heinrich, P. C., and Graeve, L. (2000) J. Biol. Chem. 275, 18810–18817
31. Wu, T. R., Hong, Y. K., Wang, X. D., Ling, M. Y., Dragoi, A. M., Chung, A. S., Campbell, A. G., Han, Z. Y., Feng, G. S., and Chiu, Y. E. (2002) J. Biol. Chem. 277, 47572–47580
32. Ceresa, B. P., and Pessin, J. E. (1996) J. Biol. Chem. 271, 12121–12124
33. Ceresa, B. P., Horvath, C. M., and Pessin, J. E. (1997) Endocrinology 138, 4131–4137
34. Krasilnikov, M., Ivanov, V. N., Dong, J., and Ronai, Z. (2003) Oncogene 22, 4092–4101
35. Ueki, K., Kondo, T., and Kahn, C. R. (2004) Mol. Cell. Biol. 24, 5434–5446
36. Pajvani, U. B., Trujillo, M. E., Combs, T. P., Iyengar, P., Jelicks, L., Jeckels, L., Roth, K. A., Kittis, R. N., and Scherer, P. E. (2005) Nat. Med. 11, 797–803
37. Smith, U., Axelsen, M., Carvalho, E., Eliasson, B., Jansson, P. A., and Wesslau, C. (1999) Annu. N. Y. Acad. Sci. 892, 119–126
38. Rondinone, C. M., Wang, L. M., Lonnroth, P., Wesslau, C., Pierce, J. H., and Smith, U. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 4171–4175