Leptin Induces, via ERK1/ERK2 Signal, Functional Activation of Estrogen Receptor α in MCF-7 Cells*

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Leptin is a hormone with multiple biological actions, produced predominantly by adipose tissue. In humans, plasma levels correlate with total body fat, and high concentrations occur in obese women. Among its functions, leptin is able to stimulate normal and tumor cell growth. We demonstrated that leptin induces aromatase activity in MCF-7 cells evidencing its important role in enhancing in situ estradiol production and promoting estrogen-dependent breast cancer progression. Estrogen receptor α (ERα), which plays an essential role in breast cancer development, can be transcriptionally activated in a ligand-independent manner. Taking into account that unliganded ERα is an effector of mitogen-activated protein kinase (MAPK) signal and that leptin is able, via Janus kinase, to activate the Ras-dependent MAPK pathway, in the present study we investigate the ability of leptin to transactivate ERα. We provided evidence that leptin is able to reproduce the classic features of ERα transactivation in a breast cancer cell line: nuclear localization, down-regulation of its mRNA and protein levels, and up-regulation of a classic estrogen-dependent gene such as pS2. Transactivation experiments with a transfected reporter gene for nuclear ER shown an activation of ERα either in MCF-7 or in HeLa cells. Using a dominant negative ERK2 or the MAPK inhibitor PD 98059, we showed that leptin activates the ERα through the MAPK pathway. The N-terminal transcriptional activation function 1 appears essential for the leptin response. Finally, it is worth noting that leptin exposure potentiates also the estradiol-induced activation of ERα. Thus, we are able to demonstrate that the amplification of estrogen signal induced by leptin occurs through an enhancing in situ E2 production as well as a direct functional activation of ERα.

Leptin, the product of the ob gene, mainly secreted by adipocytes, is involved in the control of body weight and results strongly correlated to the body fat mass (1–4). Recently, leptin was reported to stimulate the proliferation of various cell types (5–11) leading to consider leptin as a novel growth factor. Although leptin is mainly synthesized by breast adipose tissue, its expression has also been detected in normal and tumoral human mammary epithelial cells (12, 13). In addition, it has been shown that leptin receptors (short and long isoforms) are expressed in normal mammary epithelial cells (14) as well as in human breast cancer cell lines (11, 15). These data suggest an important role of leptin on mammary gland development and tumorigenesis, giving more emphasis to the epidemiological studies that evidence a relationship between obesity and breast carcinogenesis.

Obesity is an important health concern, because it is associated with a variety of metabolic disorders and an increased risk of developing cancer (16). It is now well established that postmenopausal women with upper body fat predominance experience a higher risk of breast cancer (17, 18). The association between obesity and breast carcinoma is usually ascribed to estrogen excess, derived from androgen aromatization in peripheral fat deposits (19, 20). In our recent work we have demonstrated that leptin is able to stimulate, through mitogen-activated protein kinase (MAPK) and signal transducers and activators of transcription (STAT) signals, aromatase expression in the MCF-7 cell line evidencing its important role in enhancing in situ estradiol production and promoting cell proliferation (21). In addition, a potential relationship between leptin and estrogens stems also from the evidence that estrogens appear to modulate leptin gene expression in adipose tissue (22, 23). Although estrogen receptor-positive breast tumors are usually more responsive to therapy than estrogen receptor-negative tumors, there is a report demonstrating that estrogen receptor-positive breast tumor status in obese women is actually associated with a poorer prognosis than estrogen receptor-negative status (24). Also, the T-47D cells, an estrogen receptor-positive cell line, evidenced a dramatic increase in anchorage-independent growth after treatment with leptin (25).

Estrogen receptors (ERα and ERβ) are members of the superfamily of nuclear steroid hormone receptors, which are able to regulate the transcriptional activity of target genes by interacting with different DNA response elements (26). The estrogen receptor α (ERα) signaling plays an essential role in pro-

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1 The abbreviations used are: MAPK, mitogen-activated protein kinase; STAT, signal transducers and activators of transcription; ER, estrogen receptor; DMEM, Dulbecco’s modified Eagle’s medium; CS, calf serum; BSA, bovine serum albumin; PMSF, phenylmethylsulfonyl fluoride; HEGO, human ERα expression vector; NLS, nuclear localization signal; RT, reverse transcription; E2, estradiol; JAR2, Janus kinase 2; ERK, extracellular signal-regulated kinase; EMSA, electrophoretic mobility shift assay; ERE, estrogen-responsive element.
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Leptin was shown to induce the functional transactivation of ERα in a dose-dependent manner, resulting in increased luciferase activity. The effect of leptin on ERα transactivation was blocked by the ERα antagonist ICI 182,780, indicating that the activation involves the functional transactivation pathway of ERα.

To investigate the mechanism of leptin-induced ERα transactivation, the authors performed a series of experiments using MCF-7 cells. They found that leptin treatment resulted in a significant increase in the expression of the ligand-responsive luciferase reporter plasmid (pS2) and in the phosphorylation of ERα at Ser118, indicating that leptin activates the MAPK signaling pathway.

Western blot analysis showed that leptin treatment induced a time-dependent increase in the expression of ERα and its phosphorylated form. The effect of leptin on ERα transactivation was also blocked by the MAPK inhibitor PD98059, suggesting that MAPK signaling is involved in leptin-induced ERα transactivation.

The authors also investigated the role of MAPK pathways in leptin-induced ERα transactivation using specific inhibitors. The results showed that the MEK1/2 inhibitor U0126 blocked the increase in luciferase activity induced by leptin, while the MEK1/2 activator MEK1/2 did not affect the activity.

In conclusion, the study provides evidence for a novel mechanism by which leptin can induce the functional transactivation of ERα in breast cancer cells, involving the activation of the MAPK signaling pathway. This finding has important implications for the development of targeted therapy strategies for breast cancer.

**Experimental Procedures**

Materials— Dulbecco’s modified Eagle’s medium/nutrient mixture F-12 Ham (DMEM/F-12), t-glutamine, Eagle’s non-essential amino acids, penicillin, streptomycin, calf serum, bovine serum albumin, and penicillin-streptomycin. Cells were maintained in DMEM/F-12 containing 5% CS, 1% L-glutamine, 1% Eagle’s minimum essential medium (EMEM), and 1 mg/ml penicillin-streptomycin.

**Immunocytochemistry Staining**—Paraffin-embedded MCF-7 and HeLa cells (2% paraformaldehyde for 30 min) were used for immunocytochemical staining. Endogenous peroxidase activity was inhibited by hydrogen peroxide (3% in absolute methanol for 30 min), and non-specific sites were blocked by normal horse serum (10% for 30 min).

**RT-PCR Assay**—The evaluation of ERα and pS2 mRNA expression was performed by semi-quantitative RT-PCR (39). For ERα, pS2, and the internal control gene 36B4, the primers were: 5’-GGTGGTACAC-TACCCGAGG-3’ (ERα forward) and 5’-CAGATTGTTCATGCGAGG-3’ (ERα reverse), 5’TCTATATGTAATACCGACGACG-3’ (pS2 forward) and 5’TGGTAGTGTCAGCAAGCAGC-3’ (pS2 reverse), and 5’TCAACATCTCCCCCTTCTC-3’ (36B4 forward) and 5’-CAATATCCATATCTCGT-3’ (36B4 reverse) to yield products of 1172, 210, and 408 bp, with 20, 15, and 15 PCR cycles, respectively.

**Western Blot Analysis**—MCF-7 cells were grown in 100-mm dishes up to 70–80% confluence and then lysed. Protein lysates were obtained by homogenization containing 50 mM HEPES (pH 7.5), 150 mM NaCl, 1 mM EGTA, 10% glycerol, 1% Triton X-100, a mixture of protease inhibitors (aprotinin, PMSF, and sodium orthovanadate). Equal amounts of total protein were resolved on an 11% SDS-polyacrylamide gel. Proteins were transferred to a nitrocellulose membrane, probed with the antibody F-10 against ERα, and then subjected to immunostaining with a goat anti-mouse IgG and reagents.

**Transfection Assay**—MCF-7 cells were transfected into 24-well plates with 500 μl of regular growth medium/well the day before transfection. The medium was replaced with DMEM lacking phenol red as a carbon source on the day of transfection, which was performed with FuGENE 6 reagent as recommended by the manufacturer with the mixture containing 0.5 μg of reporter plasmid pXETL. A set of experiments was performed cotransfecting XETL and pCMV5myc vector containing the cDNA encoding dominant-negative ERK2 K52R (ERK2–, 0.5 μg/well). HeLa cells were cotransfected with XETL, HEGO, and pSG5/HE15, pSG5/HE19, S104/106/118A-ER plasmid was mutated in serine residues 104, 106, and 118 to Ala (a gift from Dr. A. Lannigan, University of Virginia, Charlottesville, VA); HE241G ERα plasmid mutant that lacks a nuclear translocation signal (NLS) (32–500) (kindly provided by Dr. P. Chambon, CNRS-INSERM, University of Louis Pasteur, Strasbourg, France). pcMV5myc vector containing the cDNA encoding dominant-negative ERK2 K52R (ERK2–; gift from Dr. M. Cobb, Department of Pharmacology, Southwestern Medical Center, Dallas, TX).

**Cell Cultures**—Wild-type human breast cancer (MCF-7) cells were a gift from E. Surmacz (Philadelphia, PA). Human uterine cervix adenocarcinoma (HeLa) cells were obtained from the American Type Culture Collection (Manassas, VA). MCF-7 cells were maintained in 100-mm dishes containing 5% CS, 1% t-glutamine, 1% Eagle’s non-essential amino acids, and 1% mouse penicillin-streptomycin. Cells were cultured in phenol red-free DMEM containing 5% charcoal-stripped fetal calf serum (CS-fetal calf serum), 0.5% BSA, and 2 mM t-glutamine, for 24 h before each experiment.

**Immunocytochemical Staining**—Paraffin-embedded MCF-7 and HeLa cells (2% paraformaldehyde for 30 min) were used for immunocytochemical staining. Endogenous peroxidase activity was inhibited by hydrogen peroxide (3% in absolute methanol for 30 min), and non-specific sites were blocked by normal horse serum (10% for 30 min).
HEPES, pH 8, 1 mM EDTA, 50 mM KCl, 10 mM dithiothreitol, 10% glycerol, 1 mg/ml BSA, 50000 cpm of labeled probe, 20 μg of MCF-7 nuclear protein, and 5 μg of poly(dI-dC)). The above-mentioned mixture was incubated at room temperature for 20 min in the presence or absence of unlabeled competitor oligonucleotide. The entire reaction mixture was electrophoresed through a 6% polyacrylamide gel in 0.25 Tris borate-EDTA for 3 h at 150 V. Gel was dried and subjected to autoradiography at −70 °C.

**Statistical Analysis**—Each datum point represents the mean ± S.E. of three different experiments. Data were analyzed by analysis of variance testing the STATPAC computer program.

**RESULTS**

**Leptin Modulates ERα Nuclear Immunoreactivity in MCF-7 Cells**—It is well documented that ERα is predominantly localized in the nucleus (42–44) and, upon ligand activation, undergoes conformational changes leading to homodimerization and target gene regulation (45).

To provide evidence that leptin is able to modulate ERα nuclear localization in MCF-7 cells, we performed two sets of immunostaining experiments using different culture conditions. Fig. 1 shows that, in MCF-7 cells maintained in medium without serum for 24 h (A–C) and then treated with vehicle (A) or 100 nM E2 (B) or 1000 ng/ml leptin (C) for 24 h. No immunodetection was observed replacing the anti-ERα antibody with an irrelevant mouse IgG (insets). Each experiment is representative of at least 10 tests. Scale bars: 5 μm.

**Leptin down-regulates ERα expression in MCF-7 cells.** MCF-7 cells were incubated in serum-free medium for 24 h (A–C) and then treated with vehicle (A) or 100 nM E2 (B) or 1000 ng/ml leptin (C) for 24 h. No immunodetection was observed replacing the anti-ERα antibody with an irrelevant mouse IgG (insets). Each experiment is representative of at least 10 tests. Scale bars: 5 μm.

**Fig. 2. ERα nuclear signaling induced by leptin in long term deprived MCF-7 cell line.** The MCF-7 cell line was incubated in serum-free medium for 96 h (A–C) and then treated with vehicle (A) or 100 nM E2 (B) or 1000 ng/ml leptin (C) for 24 h. No immunodetection was observed replacing the anti-ERα antibody with an irrelevant mouse IgG (insets). Each experiment is representative of at least 10 tests. Scale bars: 5 μm.

**Fig. 3. Effect of leptin on ERα mRNA and protein levels.** A, semiquantitative RT-PCR of ERα mRNA. MCF-7 cells were stimulated for 24 h with E2 (100 nM) or leptin (1000 ng/ml); 36B4 mRNA levels were determined as a control. C, immunoblot of ERα from MCF-7 cells treated with E2 (100 nM) or leptin (1000 ng/ml) for 24 h; β-actin serves as loading control. B and D, the histograms represent the mean ± S.E. of three separate experiments in which the band intensities were evaluated in terms of optical density arbitrary units and expressed as the percentage of the control assumed as 100%. *, p < 0.05 compared with control; **, p < 0.01 compared with control.
treatment with either E₂ (Fig. 2B) or leptin (Fig. 2C) for 24 h induced a strong ERα immunoreactivity in the nuclear compartment. No immunoreactivity was observed either by replacing the anti-ERα antibody by irrelevant mouse IgG (insets in Figs. 1 and 2) or by using the primary antibody pre-absorbed with an excess of receptor protein (data not shown).

**Leptin Down-regulates ERα Expression**—E₂ is known to down-regulate the levels of ERα in breast cancer cell line through an increased turnover of the E₂-activated ERα protein and a reduced transcription rate of its own gene (46). This down-regulation represents an additional hallmark of ERα activation by an agonist. To evaluate if leptin may exhibit a like-estrogen action, we investigated the down-regulatory effects of ERα mRNA and total protein levels in MCF-7 cells. A treatment of 24 h with either 1000 ng/ml leptin or E₂ 100 nM displayed in both circumstances a similar pattern of response consistent with a down-regulation of both ERα mRNA (Fig. 3, A and B) and protein content (Fig. 3, C and D). ERα mRNA levels were compared by semiquantitative RT-PCR and standardized on the mRNA levels of the housekeeping gene 36B4 (Fig. 3, A and B).

**Leptin Up-regulates pS2 mRNA**—To provide further evidence for the ability of leptin to activate per se ERα, we investigated upon leptin exposure the expression of a classic estrogen-dependent gene, pS2. We observed, by RT-PCR, in MCF-7 cells treated with 1000 ng/ml leptin for 24 h, a strong increase of pS2 mRNA that was inhibited by the addition of the pure anti-estrogen ICI 182,780 (Fig. 4, A and B).

**Leptin Induces Functional Activation of ERα in MCF-7 and HeLa Cells**—To corroborate the specificity of leptin to transactivate the endogenous ERα, we transiently transfected MCF-7 cells with the gene reporter XETL, which carries firefly luciferase sequences under the control of an estrogen response element upstream of the thymidine kinase promoter. A significant enhancement of XETL expression was observed in the transfected cells exposed to 1000 ng/ml leptin for 48 h (p < 0.01) (Fig. 5). Similar results were obtained in estrogen receptor-negative HeLa cells cotransfected with HEGO and XETL plasmids tested in the same experimental conditions (Fig. 5). Remarkably the anti-estrogen ICI 182,780 was shown to effi-

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**Fig. 4. Leptin up-regulates pS2 mRNA.** A, semiquantitative RT-PCR of pS2 mRNA. MCF-7 cells were treated in the absence (control) or in the presence of E₂ (100 nm) or leptin (1000 ng/ml) for 24 h. The pure anti-estrogen ICI 182,780 (10 μM) was used. 36B4 mRNA levels were determined as a control. B, the histograms represent the mean ± S.E. of three separate experiments in which band intensities were evaluated in terms of optical density arbitrary units and expressed as the percentage of the control assumed as 100%. *, p < 0.01 compared with control; ●, p < 0.01 compared with E₂-treated samples; ○, p < 0.01 compared with leptin-treated samples.

**Fig. 5. Leptin activates ERα in MCF-7 and HeLa cells.** MCF-7 cells were transfected with the luciferase reporter plasmid XETL. HeLa cells were cotransfected with XETL and HEGO plasmids. The cells were treated in the absence (control) or in the presence of 100 and 1000 ng/ml of leptin (Lep) for 48 h. 10 μM of the pure anti-estrogen ICI 182,780 was used. The values represent the means ± S.E. of three different experiments. In each experiment, the activities of the transfected plasmids were assayed in triplicate transfections. *, p < 0.01 compared with control.
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Fig. 6. MAPK signal is involved in the activation of ERα. MCF-7 (A) and HeLa (B) cells were transiently transfected with XETL or cotransfected with XETL and HEGO, respectively. The cells were serum-starved overnight with or without PD 98059 (PD) and were untransfected or transiently transfected with dominant negative ERK2 plasmid and then were treated for 48 h in the presence or absence of leptin (1000 ng/ml). The values represent the means ± S.E. of three different experiments. In each experiment, the activities of the transfected plasmids were assayed in triplicate transfections. *p < 0.01 compared with control.

Effect of in Vitro Leptin Treatment on ERE DNA-binding Activity in MCF-7 Cells—The results obtained with the functional studies were corroborated by electrophoresis mobility shift (EMSA). Nuclear extracts from MCF-7 cells were analyzed by EMSA using a 32-bp DNA probe containing an estrogen-responsive element (ERE) from the human pS2 gene. In the EMSA shown in Fig. 9 we obtained a specific protein-DNA complex using nuclear extracts prepared from MCF-7 cells (lane 1). The formation of this complex was abolished by the addition of a 200-fold molar excess of non-radiolabeled probe (lane 2). A treatment of 48 h with 1000 ng/ml leptin or 100 nM E2 induced a strong increase in ERE DNA-binding activity (lanes 3 and 4), which was reversed in the presence of the pure anti-estrogen ICI 182,780 when compared with basal level (lane 5).

Using nuclear extracts from MCF-7 cells either treated with MAPK inhibitor PD 98059 or transiently transfected with dominant negative ERK2, the ERE DNA-binding activity induced by leptin treatment was drastically reduced (lanes 6 and 7).

DISCUSSION
An association between breast cancer and obesity has been recognized for at least 40 years, even though the mechanism underlying such relationship remains to be fully elucidated (16). Although in situ estrogen production by adipocytes has been considered an important risk factor for breast cancer progression (16–18), an additional candidate that may play a major role in the same scenario is leptin. Leptin is a hormone with multiple biological actions that is produced predominantly by adipose tissue and is present at high concentrations in obese women who are exposed to a higher risk in developing breast cancer (1–3). In the same vein several actions of leptin, including the stimulation of normal and tumor cell growth, migration and invasion, and enhancement of angiogenesis, suggest that this hormone is involved in breast cancer progression (5–11, 48). This assumption is sustained by recent findings that show how normal mammary gland morphogenesis is impaired in both non-transgenic genetically obese leptin-deficient and genetically obese leptin receptor-deficient mice (25). Similar results were obtained for transgenic transforming growth factor-α/lep<sup>ob</sup> mice, which did not develop mammary tumors, in contrast to transgenic transforming growth factor-α mice that were either homozygous lep<sup>ob</sup> or heterozygous lep<sup>ob</sup> lean for the leptin gene, which exhibited mammary tumor incidence rates of 50% and 60%, respectively, by 24 months of age (25). All these findings seem to underscore an important role for leptin on mammary gland development and mammary gland tumorigenesis giving new interpretative clues to under-
stand the epidemiological relationship between obesity and breast carcinogenesis.

The two isoforms of leptin receptor are both present in different breast cancer cell lines (11, 14, 15). The short and long isoforms induce the activation of one or more members of the Janus (or JAK) family of tyrosine kinases, which form a complex with the cytokine receptor subunits, thereby inducing autophosphorylation as well as phosphorylation of the receptor. These phosphorylated tyrosines form binding sites for various signaling molecules, which are themselves thought to be phosphorylated by JAK kinases like STAT proteins. The same phosphorylated tyrosine sites bind SHP2 protein-containing phosphatase. SHP2 is proposed as a positive regulator of leptin signaling through MAPK activation by the recruitment of the adapter protein growth receptor bound 2 and the activation of the Ras/Raf pathway. A secondary pathway for leptin-induced MAPK signaling is mediated directly via JAK2 (49).

Stemming from the evidence that MAPK signal induces the functional activation of unliganded ERα (34, 37), it was reasonable to investigate the potential role of leptin in stimulating ERα. In the present study we have demonstrated for the first time that leptin amplifies ERα activation by estradiol. HeLa cells were transiently cotransfected with XETL and either HEGO or Ser-104/106/118A-ER plasmids. The cells were treated for 48 h in the absence (Control) or in the presence of leptin (1000 ng/ml). The values represent the means ± S.E. of three different experiments. In each experiment, the activities of the transfected plasmids were assayed in triplicate transfections. *, p < 0.01 compared with control.
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Fig. 9. Effects of in vitro leptin treatment on ERE DNA-binding activity in MCF-7 cells. Nuclear extracts from MCF-7 cells were incubated with a double-stranded ERE-specific consensus sequence probe labeled with [γ-32P]ATP and subjected to electrophoresis in a 6% polyacrylamide gel (lane 1). Competition experiments were performed by adding as competitor a 200-fold molar excess of unlabeled probe (lane 2). MCF-7 nuclear extracts treated with 100 nM of E2 or 1000 ng/ml of leptin (Lep) for 48 h incubated with probe is shown in lanes 3 and 4. The pure anti-estrogen ICI 182,780 (10 μM) (lane 5) was added in leptin-treated MCF-7 nuclear extracts. MCF-7 cells were serum-starved overnight with PD 98059 (lane 6) or transiently transfected with dominant negative ERK2 (lane 7) and then treated for 48 h with leptin.

time that leptin was able to induce functional transactivation of ERα that was abrogated in the presence of either MAPK inhibitor or dominant negative ERK2. This addresses a crucial role of MAPK signal to stimulate ERα upon leptin exposure. In different breast cancer cell lines, it has been demonstrated how the interaction between insulin/insulin-like growth factor-1 and estradiol signaling occurs also through the direct transcription activation of ERα via MAPK (34). In this concern, it is well documented that the human ERα is phosphorylated by ERK on Ser-118 (34, 37, 50). The phosphorylation of this serine is required for full activity of the ERα AF-1 domain. Overexpression of active ERK kinase or the active p21ras, resulting in the ERK1/ERK2 activation, enhances estrogen-induced transcriptional activity of the wild type ERα but not of a mutant ERα with an alanine in place of Ser-118 (34).

All these observations fit with our data demonstrating that: 1) only the construct bearing the N-terminal domain (AF-1) was able to activate ERα reporter gene; 2) ERα mutated in serine residues 104/106/118 is no longer stimulated by leptin, and it is still transactivated by E2 even though to a lesser extent with respect to wild type. This finding recalls a recent report (51) evidencing how the full activation of liganded receptor requires the integrity of phosphorylated serine residues 104/106/118. In addition, it has been demonstrated, by the same authors, how the mutation of serine 104/106/118 affects the physical and functional interaction of full-length ERα with p160/SRC and CBP in the absence of ligand. Thus, it is reasonable to assume that the ERα, mutated in Ser-104/106/118 and being unable to recruit cofactor, fails to activate cell transcription machinery upon leptin stimulation.

It emerges from recent findings that in human vascular smooth muscle cells MAPK activation per se results in a nuclear translocation of ERα, which supports the MAPK-mediated phosphorylation of ERα as a prerequisite for its nuclear localization (50). Thus the sustained MAPK activation induced by leptin may explain why even after prolonged incubation of MCF-7 cells in serum-free medium, leptin treatment for 24 h is able to induce ERα nuclear localization as revealed by our immunostaining data, whereas ERα immunoactivity was just scantily detectable in untreated cells. This finding appears to be unrelated to the cell type specificity, which we reproduced in HeLa cells, where leptin was able to induce ERα wild type nuclear localization but failed to do so in the presence of ERα lacking nuclear localization signal (NLS) (data not show).

Therefore, in MCF-7 cells, concomitantly with leptin treatment, the classic biological features of ERα wild type functional transactivation were observed: 1) a nuclear compartmentalization of ERα; 2) the down-regulation of its mRNA and total protein content; and 3) the up-regulation of a classic estrogen-dependent gene such as pS2, which was inhibited by the pure anti-estrogen ICI 182,780.

These results fit well with the EMSA findings. In this assay using 32P-ERE sequence of pS2 promoter in the presence of nuclear extracts from MCF-7 cells, we observed that, upon leptin treatment, a strong increase in DNA binding occurred in the same extent of that induced by estradiol and was markedly reduced in the presence of either MAPK inhibitor or dominant negative ERK2.

These data broaden further the potential relationship existing between leptin and estrogens. Indeed it has been reported that estrogens appear to modulate leptin gene expression in adipose tissue (22, 23). On the other hand, we recently reported for the first time that leptin is able to induce the aromatase gene expression in MCF-7 cells via AF-1 (21), thus addressing clearly how leptin may be involved in the pathophysiology of breast, modulating in situ estrogen production also in epithelial cells.

Now we are able to demonstrate that the potential amplification of estrogen signals induced by leptin has two active components: the enhanced aromatase activity and a direct activation of ERα in the absence of the natural ligand. In addition, the potentiating effects of leptin on E2-induced activation of ERα address how different functional domains as effectors of two distinct signals may cooperate in a synergistic way. This gives a great emphasis to the role of leptin in promoting breast cancer in obese women through the potential use of readily aromatizable androgens in breast tissue, by enhancing in situ estradiol production together with its ability to directly activate ERα in epithelial breast cancer.

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