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Chronic Leptin Treatment Sensitizes MCF-7 Breast Cancer Cells to Estrogen

Adamo Valle, Jorge Sastre-Serra, Jordi Oliver and Pilar Roca

Grupo Multidisciplinar de Oncología Traslacional, Institut Universitari d'Investigació en Ciències de la Salut, Universitat de les Illes Balears, Ciber Fisiopatología Obesidad y Nutrición (CB06/03) Instituto Salud Carlos III, Palma de Mallorca

Key Words

Leptin • Estrogen • Breast cancer • Obesity • MCF-7 • Estrogen receptor

Abstract

Background/Aims: Obesity is associated with an increased risk of estrogen-dependent breast cancer. The adipokine leptin, whose levels are chronically increased in obese people, has been shown to stimulate ER positive cancer cell growth. Considering previous evidence of a crosstalk between leptin and estrogen signaling, the objective of this study was to establish the influence of chronic leptin treatment on estrogen-dependent cell growth. Methods: To this aim, we use the estrogen receptor (ER) positive MCF-7 breast cancer cell line treated chronically with leptin and analyzed estrogen-dependent cell growth, ERs (ERα and ERβ) expression, ER-dependent transcriptional activity as well as cell survival to the antiestrogenic agents tamoxifen and ICI 182,780. Results: Leptin signaling pathway kept activated after chronic stimulation (7 days) with leptin showing significant phosphorylation of JAK2 and STAT3 and higher cell proliferation rate. Chronic leptin at 100 ng/mL dose increased ERα to ERβ ratio and consistently enhanced estrogen-dependent transcriptional activity, increasing E2-dependent cell growth and resistance to antiestrogen agents. Conclusion: This study supports the existence of a crosstalk between leptin and estrogen, in which leptin might play an important role potentiating the mitogenic action of estrogen, probably by alteration of ERα to ERβ ratio.

Introduction

For many years, obesity has been recognized as a significant risk factor for postmenopausal breast cancer but the specific mechanisms underlying this relationship are not fully understood [1-3]. It has been suggested that aromatization of androgens in the expanded adipose tissue of obese women leads to elevated estrogen levels contributing to the pathogenesis of hormone-responsive breast cancer [4, 5]. However, with the recognition of adipose tissue as an active endocrine organ, other adipokines are also being subject of investigation providing

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Dr. Adamo Valle
Dept. Biologia Fonamental I Ciències de la Salut, Universitat de les Illes Balears
Cra. Valldemossa km 7.5 E-07122- Palma de Mallorca (Spain)
Tel. +34 971 259 644, Fax +34 971 173 184, E-Mail adamo.valle@uib.es

complementary explanation for the increased risk of breast cancer in obese postmenopausal women. In fact, cytokines such as TNF-α, IL-6, adiponectin and leptin have been shown to be directly or indirectly implicated in breast cancer pathogenesis [6-8].

Leptin, the product of Ob gene, is a 16 κDa protein mainly synthesized by adipose tissue and, at a lower extent, by other organs such as stomach, placenta, muscle, immune cells and mammary gland [9, 10]. Leptin concentration in the blood increases as body weight and fat mass increase and regulates energy homeostasis by suppressing food intake and increasing energy expenditure acting directly on hypothalamic nuclei [11-13]. In addition to its central nervous system activities, however, leptin has been shown to influence multiple functions in peripheral tissues expressing the leptin receptor (ObR) [14-16]. In mammary gland, leptin has been shown to be a mitogenic factor necessary for normal mammary development but also for tumor formation. In fact, mice deficient in leptin (ob/ob) or leptin receptor (db/db) exhibit a significant impairment of postnatal mammary development and a decreased incidence of both spontaneous and oncogene-induced mammary tumors [17, 18]. In vitro studies have shown that leptin stimulates proliferation of normal and cancerous mammary epithelial cells [7, 19]. Furthermore, leptin has been shown to promote tumor growth by other mechanisms including inhibition of apoptosis, enhancement of cell invasion and expression of matrix degrading enzymes [20-22].

Part of the leptin action has been proposed to be also mediated by the crosstalk of this pathway with that of the estrogen receptor pathway in breast cells [23-26]. In fact, leptin has been shown to increase aromatase expression, which evidences that leptin could enhance estrogen production in adipose tissue and thus influence mammary cell proliferation [27, 28]. Studies in breast cancer cell lines have shown that leptin increases estrogen receptor α (ERα) expression, and enhances ERα-dependent transcription [23, 25, 26]. Consistently, leptin interferes with the action of antiestrogen ICI 182,780 via post-transcriptional modulation of ERα [25]. Despite all this growing evidence of a molecular crosstalk between ObR and ER axis, few studies have demonstrated a real functional interaction between these hormones on cell growth. Several studies have failed to find summative or synergic stimulation of cell proliferation when cells were simultaneously treated with leptin and estrogen [25, 29]. To our knowledge, only Chen and colleagues reported cooperation between leptin and estrogen in sustaining cell growth of ZR-75-1 breast cancer cells after 6 days of treatment [20]. The lack of a functional crosstalk may be caused by the use of short-term leptin treatments in these studies (24-72 h). In fact, plasma leptin levels are proportional to body fat content, implicating that obese people are under chronic exposure to high circulating concentrations of this cytokine. Therefore, in order to investigate the long-term impact of leptin on estrogen sensitivity in estrogen-dependent breast cancer cells, we studied cell growth of MCF-7 breast cancer cells previously cultured in media supplemented with physiological doses of leptin (10-100 ng/mL) for several (1-7) days. To better characterize the influence of chronic leptin on ER signaling in breast cancer we also studied ER status (ERα and ERβ), ER transcriptional activity and resistance to antiestrogen therapy. Our results increase understanding of the crosstalk between leptin and estrogen signaling in breast cancer, especially when the levels of circulating leptin are continuously high as in hyperleptinemia, which is commonly observed in obese patients.

Materials and Methods

Reagents

Dulbecco's modified Eagle's (DMEM) and Opti-MEM medium was from GIBCO (Paisley, UK). Leptin, 17β-estradiol (E2), tamoxifen and ICI 182,780 were from Sigma Aldrich (St. Louis, MO, USA). Routine chemicals were supplied by Panreac (Barcelona, SPAIN), Bio-rad Laboratories (Hercules, CA, USA) and Sigma Aldrich.

Cell culture and proliferation assay

MCF-7 breast cancer cells were purchased from ATCC and routinely grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% antibiotics (penicillin and streptomycin) at 37°C in 5% CO_2. For leptin treatments, cells were cultured with FBS-DMEM supplemented with 0, 10, 50 and 100 ng/mL leptin for 1, 2, or 7 days. For estrogen treatments, cells were seeded in 96-well culture dishes with phenol-red free DMEM supplemented with 10% charcoal-stripped FBS and the same amount of leptin. Twenty-four hours after E2 deprivation, cells were treated with E2 concentrations ranging from 0.01 to 10 nM and cell density measured at 0 and 48 h by MTT assay. For anti-estrogen treatments, cells were cultured in complete media (FBS-DMEM) supplemented with leptin for 6 days and then seeded in 96 well plates with the same amount of leptin for further 24 hours. Tamoxifen (5, 10, 15 and 20 μM), ICI 182,780 (5, 10, 20, 40 μM) or vehicle alone (0.025% DMSO) was added to wells and cell viability measured at 0 and 48 h by MTT reduction assay. Briefly, 10 μL/well of a MTT solution (5 mg/mL) were added and incubated for two hours to let metabolically active cells to

yield an insoluble purple formazan product. Supernatants were discarded and 100 µL/well of DMSO were added to dissolve the formazan crystals. Optical density (OD) was measured on a microplate autoreader (BIO-TEK Instruments, Winooski, Vermont, USA) at 570 nm against reference wavelength (630 nm). Eight wells were used per treatment and each experiment was performed in triplicate.

Flow cytometry

Cells were cultured in 12-well plates with FBS-DMEM supplemented with leptin for 1, 2 or 7 days. For E2 treatment, medium was replaced by phenol-red free DMEM supplemented with Charcoal-stripped FBS 24 h prior E2 addition. Cells were tripsinized, washed with phosphate buffer saline (PBS) and fixed in methanol overnight at 4°C. After being washed twice with PBS, DNAs were stained in the dark with 50 µg/ml propidium iodide in the presence of 50 µg/ml of RNAse A. Samples were analyzed for DNA ploidy using a Coulter Epics XL-MCL Flow Cytometer (Beckman Coulter, Miami, FL, USA).

Luciferase assay

Dual-Luciferase assay (SABioscience, Frederick, MD, USA) was used to monitor the activity of ER-induced signal transduction in response to E2 in leptin-treated cells. MCF-7 cell culture was cotransfected with ER-responsive Firefly luciferase construct and a constitutively expressing Renilla construct as an internal control. Briefly, MCF-7 cells were cultured in the presence or absence of 100 ng/mL leptin for 7 days. 24 hours prior transfection cells were plated in 96-well plates (70-80% confluence) in Opti-MEM medium without antibiotics. Cells were transfected with 100 ng of ERE-responsive construct for 6 hours using Lipofectamine™ 2000 according to the manufacturer's procedure. Upon transfection, the cells were shifted to E2 free medium (phenol red free DMEM supplemented with 10 % charcoal-dextran stripped FBS) for 16 hours and then treated with 1 or 10 nM E2 or vehicle alone (DMSO 0.025%) for another 18 hours. Luciferase activities were measured using Dual-Glo™ Luciferase assay system (Promega Corporation, Madison, WI, USA). The luciferase (firefly) activity was determined and the Renilla activity was used for normalizing transfection efficiencies and monitoring cell viability. The results were summarized from at least three sets of transfections and presented as the mean ± SEM.

Western blot

For Western blot analysis, 50 µg of protein from cell lysate were fractioned by SDS-PAGE (12% polyacrylamide gel) and electrotansferred onto nitrocellulose filters. Membranes were incubated in a blocking solution of 5% nonfat powdered milk in 20 mM Tris-HCl, 0.13 mM NaCl and 0.1% Tween 20. Antisera against ObR, ERα, ERβ (Santa Cruz Biotechnologies, CA, USA), pSTAT3, STAT3, p-JAK and JAK (Cell signaling technology, Danvers, MA, USA), were used as primary antibodies. Protein bands were visualized by the Immun-Star Western C kit reagent (Bio-Rad) Western blotting detection system. The chemiluminiscence signal was captured with a Chemidoc XRS densitometer (Bio-Rad) and analyzed with Quantity One software (Bio-Rad).

Statistics

The Statistical Program for the Social Sciences software for Windows (SPSS, version 18.0; SPSS Inc, Chicago, IL) was used for all statistical analyses. Data are presented as means ± standard error of the mean (SEM). Statistical differences between control and E2 or leptin treated cells were assessed by Student's t-test. Statistical significance was set at $P < 0.05$.

Results

Effect of chronic leptin on MCF-7 cell proliferation

We examined MCF-7 cell proliferation at leptin concentrations deemed to represent physiologic levels from lean to obese humans (10-100 ng/ml) for 1, 2 and 7 days periods. As shown in Fig. 1A, leptin increased cell proliferation in a dose-dependent manner with maximal proliferation observed at 100 ng/ml leptin. Cells showed higher proliferative response at 2 days of leptin treatment, with chronic (7 days) leptin treatment showing attenuated growth rate. Cell cycle state was evaluated by flow cytometry to confirm that leptin stimulation resulted in cell cycle progression. One and two days of leptin treatment appear to speed up the cell cycle progression increasing cells in S and G2/M phases. Cells exposed chronically to leptin (7 days) showed slight but significant increases in S and G2/M phases at all leptin concentrations assayed.

Activation of leptin signaling pathway in MCF-7 cells after chronic leptin treatment

High sustained concentration of leptin may result in leptin desensitization by expression of the suppressor of cytokine signaling 3 (SOCS-3) which negatively regulates leptin-induced signaling [30]. Leptin binding to its receptor leads to canonical phosphorylation of JAK2, which, in turn, phosphorylates STAT3 resulting in STAT3 dimmerization and nuclear translocation. SOCS3 induced by leptin inhibits JAK2 and STAT3 phosphorylation, acting as a negative feed-back loop [31, 32]. To check whether ObR axis is repressed in chronically leptin stimulated MCF-7 cells, we analyzed the ObR levels and phosphorylation state of JAK2 and STAT3. As shown in Fig. 2, ObR levels were decreased in cells chronically exposed to leptin. No differences were found, however, in cells treated for shorter periods (1 or 2 days) or different leptin doses (data not shown). This desensitization at receptor level after chronic exposure to leptin is consistent

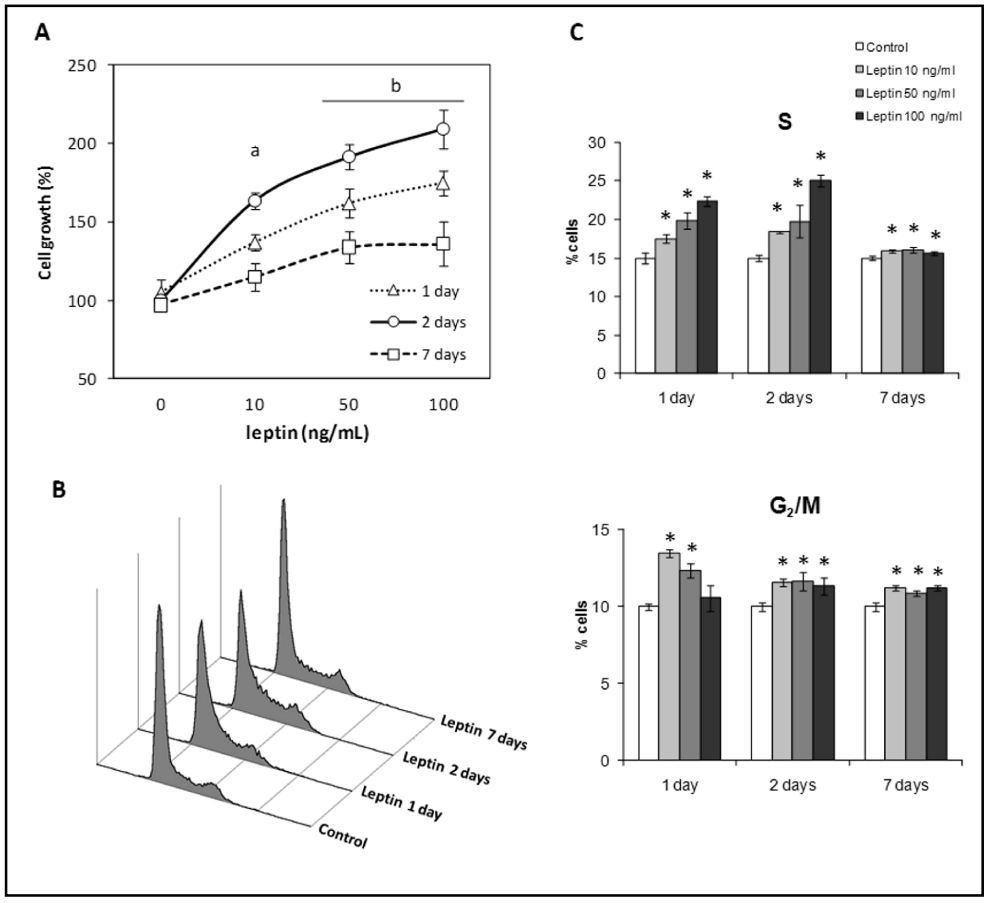

Fig. 1. Effects of leptin on cell proliferation and cell cycle in MCF-7 cells. A) Dose-response curves for leptin on MCF-7 cell proliferation. Cells were maintained in the media for 1, 2 or 7 days in various concentrations of leptin. Cell proliferation was analyzed by MTT assay as described in Materials and Methods. Data are mean ± sem of three experiments and are normalized as percentage of the control value (0 ng/mL leptin). [a]$p<0.05$ 10 nM leptin vs. control for 1 and 2 days treatment; [b]$p<0.05$ 50 and 100 nM leptin vs. control for all time treatments B) Representative histograms of propidium iodide-stained MCF-7 cells treated with or without 100 ng/mL leptin for 1, 2 or 7 days. C) Flow cytometry analysis of MCF-7 cells treated with leptin for 1 or 7 days. Bars represent the mean ± sem of cells distributed in S and G2/M phases expressed as percentage of total viable cells. *$p<0.05$ leptin vs. control.

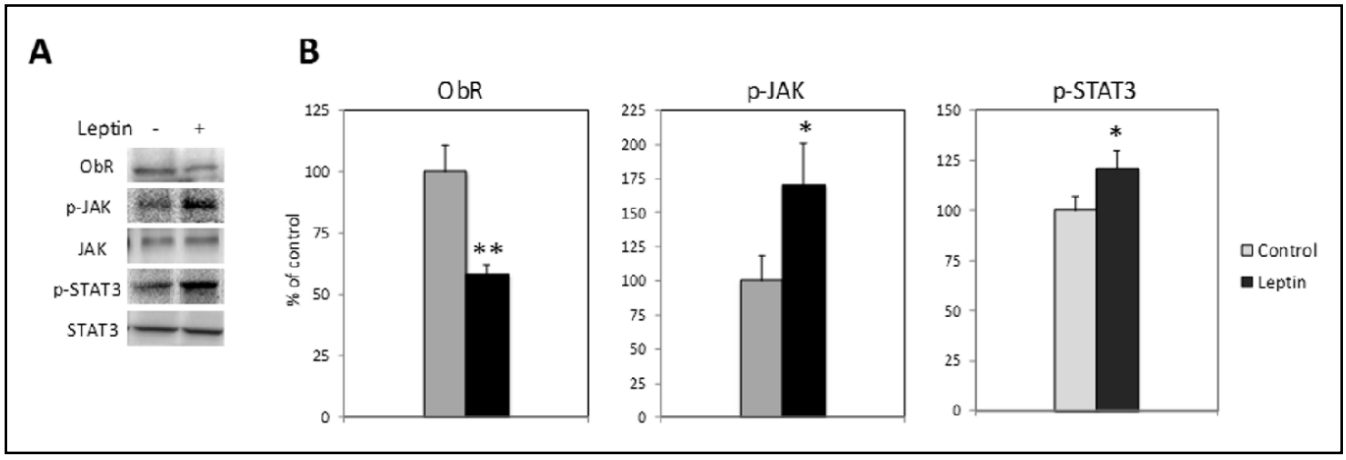

Fig. 2. Activation of leptin signaling pathway in MCF-7 cells chronically exposed to leptin. A) Representative western blot showing relative levels of leptin receptor (ObR) and phosphorylation state of JAK and STAT3 in MCF-7 cells treated with or without 100 ng/mL leptin for 7 days. Antibodies against ObR, p-STAT3, total STAT3, p-JAK and total JAK were used. B) Histograms representing the mean ± sem of band intensity expressed as percentage of control cells. Similar results were observed from three repeated experiments. *$p<0.05$, **$p<0.01$ leptin vs. control.

with the drop in mitogenic activity shown in Fig. 1. Despite decreased ObR levels, both JAK and STAT3 were found to maintain a higher phosphorylation state than untreated MCF-7 cells, suggesting that leptin signaling, although attenuated, was not fully repressed after 7 days of leptin treatment.

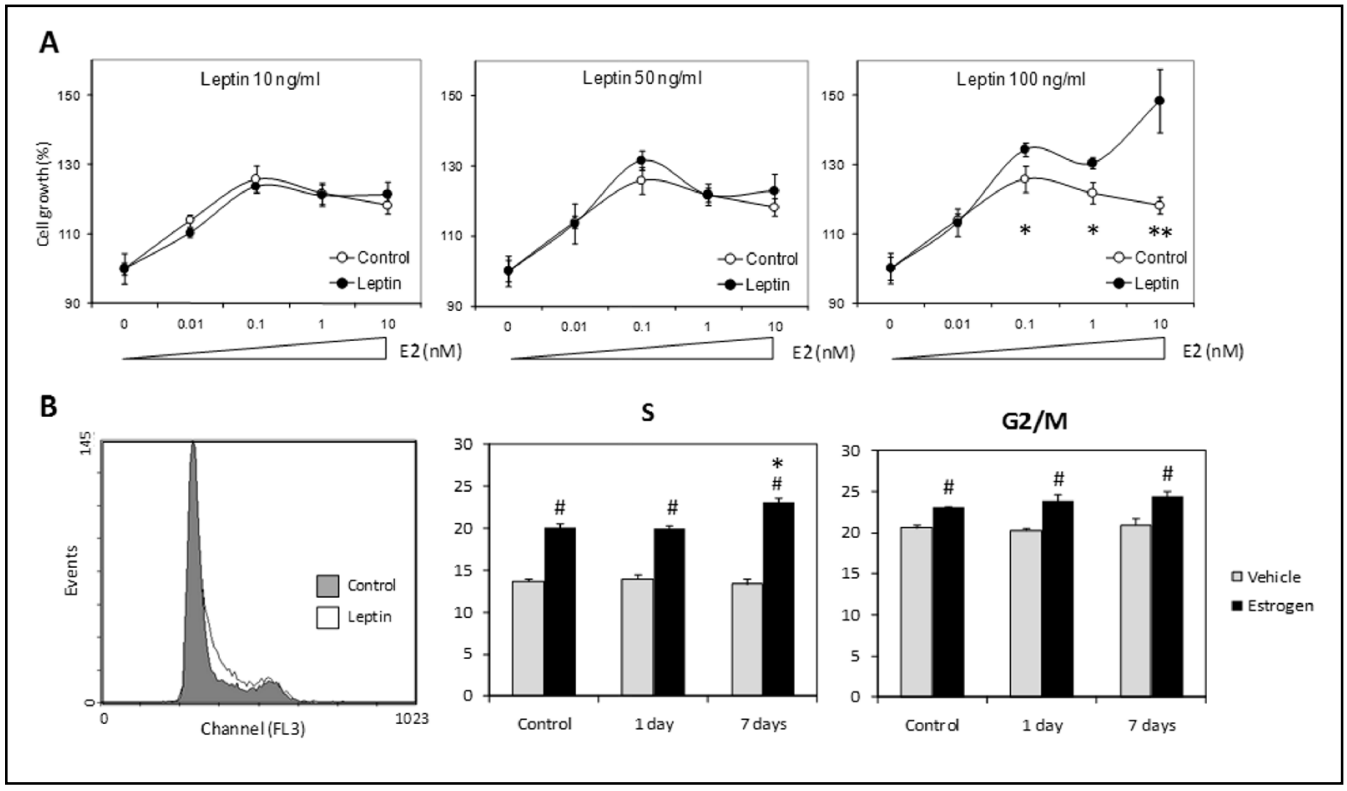

Fig. 3. Effects of estrogen on cell proliferation and cell cycle in MCF-7 cells chronically exposed to leptin. A) Graphs showing dose-response curves for E2 on MCF-7 cells maintained in media for 7 days with various concentrations of leptin. Cell proliferation was analyzed by MTT assay as described in Materials and Methods. At 4th day of leptin treatment, cells were E2-deprived for 24 h (5th day) shifting media to charcoal-stripped-FBS-DMEM supplemented with the same leptin dose. Cell density was measured at point 0 and 48 h after E2 treatment (6th and 7th day leptin treatment). Data are mean ± sem of three experiments and are normalized as percentage of the control value (0 ng/mL leptin). B) Effect of leptin exposure in E2-induced cell cycle progression. Left panel show representative histogram of cells incubated with or without 100 ng/ml leptin for 7 days and treated with 10 nM E2 for 48 hours. Right panels summarize the effect of leptin exposure in S and G2/M phases after 48 h of E2 exposure (10 nM). Bars represent the mean ± sem of cells distributed in S and G2/M phases expressed as percentage of total viable cells. *p<0.05, **p<0.01 leptin vs. control; #p<0.05 estrogen vs. vehicle.

Effects of estrogen on cells proliferation of chronically leptin-treated MCF-7

Estrogen is a well-known factor risk for breast cancer and promotes cell growth in estrogen receptor positive cells. We examined the ability of estrogen to induce proliferation when cells are chronically exposed to leptin. MCF-7 cells were treated with different amounts of leptin for 7 days and shifted to estrogen-free media 24 h prior to incubate cells with vehicle or physiological doses of estrogen (0.01-10 nM) for further 48 h. As shown in Fig. 3A, estrogen induced a dose-response curve with a maximum growth at 0.1 nM E2. Similar dose-response curves were obtained for cells treated chronically with 10 or 50 ng/mL leptin. Nevertheless, cells treated chronically with the highest leptin dose (100 ng/mL) were more responsive to estrogen at concentrations ranging from 0.1 nM to 10 nM. Cell cycle analysis showed a significant higher percentage of cells entering the S phase in response to estrogen in cells chronically t reated with 100 ng/ml leptin (Fig. 3B). No differences were found in the E2-induced increase of cells entering the G2/M or S phase at lower leptin doses (data not shown).

Chronic leptin increases ERα/ERβ ratio and ERE-dependent transcriptional activity

To delve deeper into the molecular mechanisms underlying the sensitization to E2 in cells chronically

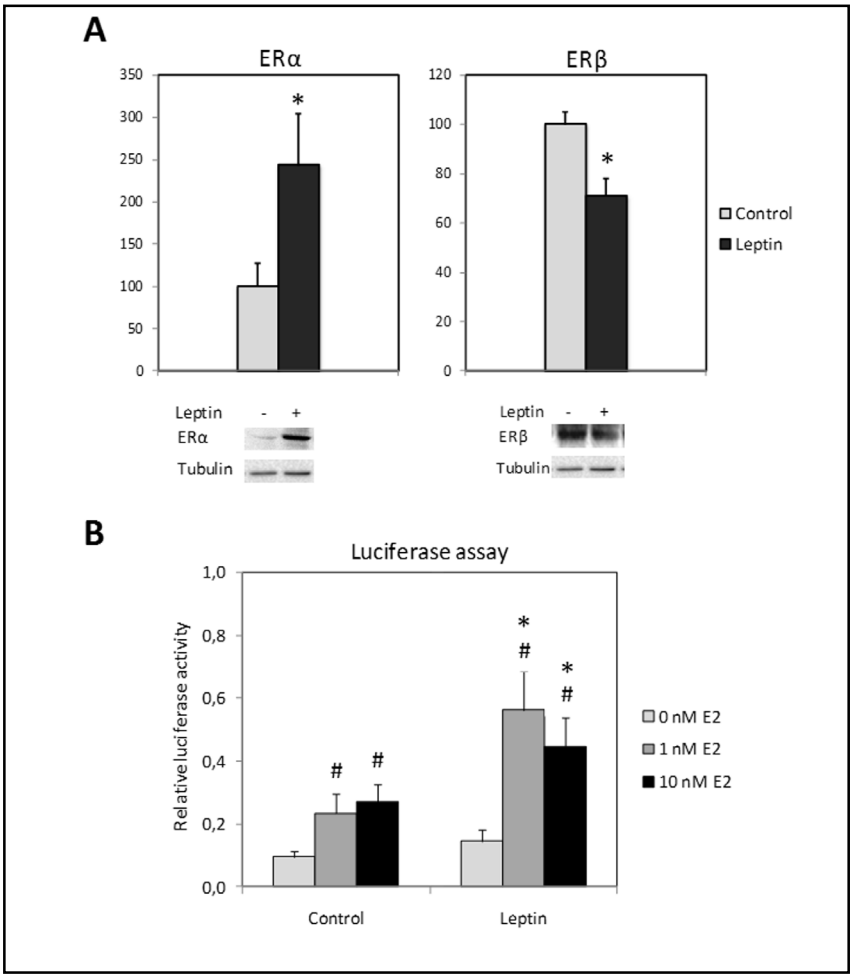

Fig. 4. A) Leptin regulated expression of ERα and ERβ. Protein expression was measured in MCF-7 cells treated with or without 100 ng/mL leptin for 7 days by Western blotting using specific antibodies against ERα and ERβ. Tubulin was used as internal control for loaded amount of total protein. Bars represent the mean ± sem of band intensity expressed as percentage of control cells. Similar results were observed from three repeated experiments. B) Effect of chronic leptin on ERE-dependent transcriptional activity. MCF-7 cells incubated with or without 100 ng/mL leptin for 7 days were seeded in 96 well plates and transfected for 6 hours with 100 ng of DNA per well using Lipofectamine 2000™. All transfection mixtures contained an ER-responsive Firefly luciferase construct and a constitutively expressing Renilla construct (40:1). Upon transfection, cells were estrogen-deprived for 16 h and treated for further 18 h with E2 (1 o 10 nM) or vehicle alone (DMSO 0.025%). Luciferase activity was measured in cell lysates using a luminometer. Relative luciferase activity was calculated for each sample using firefly luciferase activity upon normalization with renilla luciferase values. *p<0.05 leptin vs. control; #p<0.05 estrogen vs. vehicle.

exposed to leptin, we examined the protein levels of estrogen receptors subtypes ERα and ERβ. ERα protein expression was significantly increased in MCF7 cells treated for 7 days with 100 ng/mL leptin whereas ERβ protein levels were decreased (Fig. 4A). Thus, leptin treatment induced a 3-fold increase in the ERα to ERβ ratio of MCF7 cells (3.00 ± 0.54 vs. 1.00 ± 0.24).

It is well known that the classical estrogen receptor (ERα) mediates the proliferative actions of E2 in ER positive cells, whilst the proposed inhibitory role of ERβ on cell growth is yet subject of debate [33-36]. To check whether the alteration induced by leptin in the specific ERs abundance might alter E2-dependent transcriptional activity, we used a ERE reporter assay in MCF-7 cells treated with leptin. As shown in Fig. 4B, estrogen significantly stimulated ERE-dependent transcription above the basal level. MCF-7 cells cultured with leptin for 7 days showed a 2-fold higher E2-induction of ERE-dependent transcription than control cells. These data suggest chronic leptin treatment alters the expression profile of ER subtypes and enhances E2-dependent transcriptional activity. Curiously, no differences were observed in transactivation intensity between 1 nM and 10 nM E2. This lack of dose-dependent response, in contrast with the observed proliferation curve of leptin-treated cells, suggests that other factors rather than intensity of transactivation (e.g. cooperation with other transcription factors or duration of transactivation) may be mediating the E2 dose-dependent proliferation observed in cells chronically treated with 100 ng/mL leptin (Fig 3A).

Chronic leptin exposure increases the resistance to antiestrogen therapy

ERα expression is closely related to the progression of breast cancer and the sensitivity to endocrine treatments of breast tumor. Hormonal therapy treats ER positive breast cancers in two ways: i) by lowering the amount of estrogens in the body or ii) by blocking the action of estrogens on breast cancer cells. Tamoxifen and ICI 182,780 are antiestrogen agents widely used

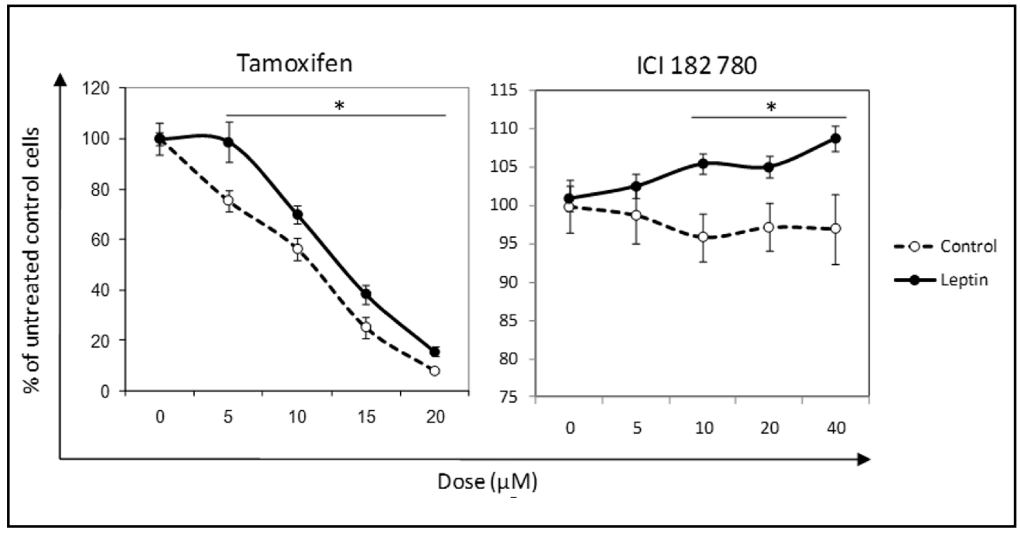

Fig. 5. Leptin attenuates the effects of tamoxifen and ICI 182,780. MCF-7 cells were maintained in media supplemented with or without 100 ng/mL leptin for 7 days and treated with tamoxifen or ICI 182,780 for further 48 h. Cell viability was analyzed by MTT assay as described in Materials and Methods. Data are mean ± sem of three experiments and are normalized as percentage of the control value (0 ng/mL leptin). *p<0.05 leptin vs. control.

to block estrogenic activity in breast tumors. To test whether chronic exposure to leptin may compromise the effectiveness of these drugs, viability of leptin-exposed MCF-7 cells was analyzed after 48 hours treatment with different doses of tamoxifen or ICI182,780. As shown in Fig. 5, MCF-7 cells treated with leptin for 7 days showed a higher resistance to tamoxifen in comparison to non-treated cells. Tamoxifen treatment of MCF-7 cells achieved significant citotoxic effects at 5 μM concentration, whereas leptin treated cells did not exhibit significant death at the same dose. Tamoxifen IC50 values calculated from our MCF-7 cells survival curves was 11.7 μM, whereas chronic supplementation with leptin increased IC50 values for tamoxifen up to 14.5 μM. We also examined the cytostatic effect of ICI 182,780. Doses ranging from 5 to 40 μM inhibited cell growth in control cells, whereas leptin chronically treated were able to proliferate (Fig. 5).

Discussion

A connection between postmenopausal breast cancer and obesity has been known for many years, even though the underlying mechanism remains to be fully elucidated [37]. In postmenopausal obese women, adipose tissue is an important source of estrogen production by aromatization of androgens, which leads to increased estrogenic stimulation and higher breast cancer risk in comparison to normal weight women [38, 39]. Growing evidence suggests that leptin may be also involved in the pathogenesis and progression of estrogen-dependent breast carcinoma. Despite the growing molecular evidence of a functional crosstalk between ObR and ER axis, few studies have focused on the interaction at cell growth level and most failed to find any synergistic or additive effect [24, 25, 29]. Plasma leptin levels are proportional to body fat content, which involves that obese people, though circadian rhythm, are under chronic exposure to high circulating concentrations of leptin [40]. In fact, Chen et al. found that leptin and E2 cooperate on proliferation of ZR-75-1 breast cancer cells after 6 days of simultaneous treatment with both hormones [20]. Given that most studies have focused on short-time effects of leptin (24-72 hours), our aim was to explore whether long-term leptin treatment might influence estrogen sensitivity in MCF-7 cells.

Our results confirmed that leptin is a potent mitogen for MCF-7 breast cancer cells. We found a great significant increase in cell proliferation measured at 24 and 48 h of leptin treatment. In comparison, long-term leptin treatment (7 days) resulted in an attenuated but still significant stimulation of growth rate. Such attenuation may be related to ObR desensitization by negative feed-back mechanisms [31, 32]. In fact, we observed a down-regulation of ObR levels after chronic exposure to leptin (Fig 2). Despite down-regulation of leptin receptor, we found that both JAK2 and STAT3 still maintain a higher phosphorylation state than untreated cells, which suggests that chronic leptin treatment sets a new baseline of activation of the signaling pathway.

The ability to respond to estrogen signaling was next examined by analyzing dose-response curves to estrogen in MCF-7 cells chronically treated with different

leptin concentrations. We found that the cells exposed to 100 ng/mL leptin showed significant higher response to E2. Since this dose is above the concentration found in most obese women (50 ng/mL), the physiological relevance of the present data could be questionable. However, it has been shown that both normal mammary gland and breast tumor cells produce leptin, which is supposed to act in an autocrine manner [41]. In fact, *in vivo* studies have reported much higher leptin levels in human fat interstitial fluid than in blood [42]. This mechanism of leptin production can lead to local leptin levels in breast tumors that are as much as several fold higher compared with the circulation, although this is something that cannot routinely be measured. Altogether, these observations suggest that leptin concentration tested in the present study range within the physiological concentrations in the interstitial fluid surrounding breast tumors of obese women.

Estrogens exert their physiological effects on target tissues by interacting with the estrogen receptors, ERα and ERβ. The classic estrogen receptor, ERα, is closely related to the progression of breast cancer and the sensitivity to endocrine treatments of breast tumor. In contrast, recent studies have indicated a protective role for ERβ against breast cancer development [35, 43, 44]. Forced expression of ERβ in MCF-7 cells counteracts the mitogenic effects of ERα probably working as a competitive inhibitor [35]. Therefore, the relative ratio of ERα to ERβ is thought to be a significant factor underlying the biological activity of estrogen [45]. In the present study we showed that chronic leptin induces a robust increase in the ERα to ERβ ratio in MCF-7 breast cancer cells. In agreement, *in vivo* experiments using a nude mouse xenograft model showed that injection of leptin in the tumor for 15 days produced a similar alteration in the ERα to ERβ ratio [46]. Consistently, positive correlation between the expression of ObR and ERα has been reported in human breast cancer biopsies [24], although other authors have not observed such association [47]. In contrast, the association between ERβ and leptin in breast cancer has rarely been reported, with a study showing a positive correlation between leptin/ObR and ERβ expression [47].

Increased ERα to ERβ ratio may be a mechanism by which leptin could influence estrogenic signaling. Consistently, chronic leptin-treated cells showed a 2-fold higher induction of the ERE-reporter gene than control cells when treated with the same E2 doses. Alteration in the ER profile and E2-dependent transcriptional activity by leptin may have clinical implications with regard to the use of hormone-therapy in obese women. In fact, a previous work by Garofalo et al. demonstrated that leptin is able to attenuate the effects of the pure antiestrogen ICI 182, 780 (faslodex) in MCF-7 cells [25]. In agreement, here we found that MCF-7 cells chronically supplemented with leptin were able to continue growing even in the presence of ICI 182,780. Considering that the cytostatic activity of ICI 182,780 is based on ERα degradation, it seems that a common mechanism, involving ERα stabilization, may explain the higher E2 sensitivity induced by leptin and the resistance to this antiestrogenic drug. Nevertheless, to our knowledge, no epidemiological studies have investigated the effectiveness of Faslodex in postmenopausal obese women. On the other hand, in the present study we have also shown that leptin attenuates the cytotoxic effect of tamoxifen in MCF-7 cells. However, epidemiological studies suggest that obesity does not decrease the effectiveness of tamoxifen for breast cancer recurrence and mortality [48]. These epidemiological data are intriguing considering the multiple molecular mechanisms by which leptin seems to promote estrogen signaling, which leads to reformulate the question, namely whether the inhibition of leptin signaling may improve the effectiveness of anti-estrogenic agents.

In summary, our study is the first to explore the effects of chronic leptin on estrogen signaling and breast cancer proliferation. Chronic leptin increased ERα to ERβ ratio and consistently enhanced estrogen-dependent transcriptional activity, increasing estrogen-dependent cell growth and resistance to antiestrogens in the ER positive MCF-7 breast cancer cell line. These findings give supportive evidence of the existence of a crosstalk between leptin and estrogen and contribute to the understanding of the association between obesity and breast cancer progression.

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