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Leptin prevents apoptosis of trophoblastic cells by activation of MAPK pathway

Antonio Pérez-Pérez [a], Julieta Maymó [b], José L. Dueñas [c], Raimundo Goberna [a], Juan Carlos Calvo [b], Cecilia Varone [b], Víctor Sánchez-Margalet [a,*]

[a] Departamento de Bioquímica Médica y Biología Molecular, Facultad de Medicina Hospital Universitario Virgen Macarena, Universidad de Sevilla, España, Spain
[b] Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina
[c] Servicio de Ginecología y Obstetricia, Hospital Universitario Virgen Macarena, Universidad de Sevilla, España, Spain

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ABSTRACT

Leptin (Ob), the peripheral signal produced by the adipocyte to regulate energy metabolism, can also be produced by placenta, where it may work as an autocrine hormone. Recently, we have demonstrated that leptin promotes proliferation and survival of trophoblastic cells. In the present work we aimed to study the signal transduction pathways that mediate the trophic effect of leptin in placenta, by using the human placenta choriocarcinoma JEG-3 cell line, as well as trophoblastic cells from human placenta.

We have assayed the early phase of apoptosis, triggered by serum deprivation, by using Annexin V–propidium iodide (PI) labeling and flow cytometric analysis, as well as the late phase of apoptosis by studying the activation of caspase-3. We have studied the major signalling pathways known to be triggered by the leptin receptor, and we have investigated the relative importance of these pathways in the effect of leptin by using pharmacological inhibitors. We have found that leptin stimulates Janus kinase (JAK)-signal transducers and activators of transcription (STAT) pathway by promoting JAK-2 and STAT-3 tyrosine phosphorylation. We have also demonstrated the activation of mitogen-activated protein kinase (MAPK) pathway by studying phosphorylation of extracellular-signal regulated kinase (Erk) kinase (MEK) and Erk1/2. PI3K pathway is also triggered by leptin stimulation as assessed by the study of protein kinase B (PKB) phosphorylation. These signaling pathways were confirmed in trophoblastic cells obtained from placenta of healthy donors. The effect of leptin on JEG-3 survival was completely reversed by blocking Erk1/2 activation employing the MEK inhibitor PD98059, whereas it was not affected by PI3K inhibition using wortmannin. These data suggest that the leptin antiapoptotic effect in placenta is mediated by the MAPK pathway.

Leptin (Ob), the protein product of the *LEP* gene [1], is a hormone synthesized mainly in adipose cells [2] to regulate weight control in a central manner [3]. However, leptin can also be expressed in other tissues, such as placenta and stomach [4,5]. Leptin is released into the circulation, and plasma levels correlate with total body fat mass [6]. On the other hand, there is now increasing evidence that leptin has systemic effects apart from those related to energy homeostasis, including regulation of neuroendocrine, reproductive, hematopoietic, and immune functions [7].

Recently, pleiotropic effects of leptin have been identified in reproduction and pregnancy [8]. Compelling evidence in the last years implicated leptin in reproductive processes such as the regulation of ovarian function, oocyte maturation, embryo development, and implantation [9–11]. Molecular interactions at the embryo-maternal interface during the time of adhesion and subsequent invasion are crucial to the process of embryonic implantation [11]. This process takes place during the first weeks of pregnancy, when the well-differentiated primary cells of the placenta known as trophoblastic cells grow in an invasive fashion. There is evidence suggesting that cytokines produced by the maternal endometrium and the developing embryo play an important role in this signaling process. Although numerous cytokine-receptor pairs are expressed by the maternal endometrium and the embryo during implantation, the knowledge of the cytokine functions is limited [12]. The primary amino acid sequence of leptin indicated that it could belong to the long-chain helical cytokine family [13]. In fact, leptin receptor (Ob-R) shows sequence homology to members of the class I cytokine-receptor (gp130) superfamily [14]. In this line, we have found that leptin may function as a proinflammatory cytokine on human monocytes [15] and lymphocytes [16], promoting the production of Th1-type cytokines. Leptin directly stimulates proliferation and activation of human monocytes [15], and human circulating T lymphocytes when they are co-stimulated by phytohemagglutinin PHA or concanavalin A (Con A)[1] [16]. Moreover, we have previously

* Corresponding author. Address: Department of Biochemistry and Molecular Biology, School of Medicine, Virgen, Macarena University Hospital, Av. Dr. Fedriani 3, 41071 Seville, Spain. Fax: +34 955008105.
E-mail address: margalet@us.es (V. Sánchez-Margalet).

[1] *Abbreviations used:* Con A, concanavalin A; MAPK, mitogen-activated protein kinase; JAK, Janus kinase; STAT, signal transducers and activators of transcription; DMEM, Dulbecco's modified Eagle's medium; FCS, fetal calf serum; DTT, dithiothreitol; PBS, phosphate-buffered saline; FITC, fluorescein isothiocyanate.

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demonstrated that leptin promotes proliferation and cell survival of human peripheral blood mononuclear cells via mitogen-activated protein kinase (MAPK) activation [15,17]. We have also studied the signaling pathways activated by leptin receptor in peripheral blood mononuclear cells [18], where the long isoform of leptin receptor stimulates the Janus kinase (JAK)-signal transducers and activators of transcription (STAT) [19], PI3K, and MAPK pathways [20].

Recently, we have demonstrated that leptin is a trophic and mitogenic factor for trophoblastic cells by virtue of inhibiting apoptosis and promoting proliferation [21]. Therefore, we have provided some evidence for the possible role of the leptin produced by trophoblastic cells in the physiology of the placenta. Thus, leptin may be an important autocrine trophic factor for the growth and maintenance of the placenta during pregnancy, when circulating leptin levels are increased [22] and mainly due to the increased leptin production by trophoblastic cells [4]. In fact leptin levels return to normal levels after delivery [10]. However, little is known about the molecular mechanisms underlying these effects of leptin on trophoblastic cells. On the other hand, intracellular signaling pathways activated by leptin have been studied in many different systems [23]. The aim of the present work was to investigate the signal transduction pathways activated by leptin in placenta and to elucidate the relative importance of the different signaling pathways to mediate the antiapoptotic effect of leptin.

Materials and methods

Reagents

Human recombinant leptin was from R&D Systems (Minneapolis, MN). Antibodies against leptin receptor (C-terminal) and JAK-2 were from Santa Cruz (Santa Cruz, CA). Antibodies against PKB, MEK 1-2, STAT-3, and caspase-3 were from BD Biosciences Pharmingen™. Monoclonal antibodies to phosphotyrosine (α-PY) were purchased from Transduction Laboratories (Lexington, KY). Pharmacological inhibitors: PD980059 and wortmannin were from Sigma–Aldrich (Saint Louis, Missouri). Annexin V-FITC Apoptosis Detection Kit I was from BD Biosciences Pharmingen™.

Cell culture and treatments

The human choriocarcinoma cell line JEG-3 was generously provided by Susana Genti-Raimondi (Universidad Nacional de Córdoba, Córdoba, Argentina). Cells were grown in Dulbecco's modified Eagle's medium (DMEM) with 4.5 g/l glucose, supplemented with 10% fetal calf serum (FCS), 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM glutamine (Invitrogen), and 1 mM sodium pyruvate (all from Biological Industries, Beit Haemek, Israel) at 37 °C in 5% CO_2 [21]. Stimulation experiments were performed as follows: JEG-3 cells were treated for 10 min in the absence or presence of different concentrations of leptin, then washed with cold PBS and solubilized for 30 min at 4 °C in lysis buffer containing 20 mM Tris, pH 8, 1% Nonidet P-40, 137 mM NaCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 1 mM dithiothreitol (DTT), 10% glycerol, 1 mM phenylmethylsulfonyl fluoride, and 0.4 mM sodium orthovanadate [19]. After centrifugation, the soluble cell lysates were used for immunoprecipitation or Western blot analysis. Protein concentration was determined by a kit from Bio-Rad (Richmond, CA), using bovine serum albumin as standard.

Placental explants processing and treatments

Human placentas ($n = 9$) were obtained after cesarean section or vaginal delivery following normal term pregnancies in the Virgen Macarena University Hospital. The Institutional Ethical Review Board approval was obtained for the use of the human placenta. Every placenta was immediately suspended in ice-cold phosphate-buffered saline (PBS) and transported to the laboratory, where they were washed 2–3 times in sterile PBS to remove excess blood. Placentas were dissected to remove fetal membranes and endometrial tissue. Villous tissue free of visible infarct, calcification, or hematoma was sampled from at least five cotyledons at a distance midway between the chorionic and basal plates. These core parts of cotyledons were cut into multiple cubic segments (10–15 mg wet weight) and thoroughly rinsed with cold (4 °C) Hanks' medium pH 7.4 (137 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 1 mM $MgSO_4$, 0.3 mM Na_2HPO_4, 0.4 mM KH_2PO_4, and 4 mM $NaHCO_3$), containing 95% O_2 and 5%CO_2, 10 mM glucose, and 0.1% BSA. Placental explants (50 mg) were randomly distributed in the tubes containing 1 ml of a Hanks' medium (four replicates per treatment). Incubation of placental explants started in a shaking water bath at 37 °C for an equilibration period of 5 min. Then, explants were incubated during 10 min in the same incubation medium supplemented with or without recombinant leptin (0.1–10 nM). The experiments were ended by removing the explants from the bath. They were washed twice in PBS by centrifugation for 2 min at 2000*g* and 4 °C and resuspended in 500 μl of lysis buffer. The explants were incubated in lysis buffer at 4 °C for 30 min on an orbital shaker and then centrifuged at 10000 *g* for 20 min. The supernatants were transferred to new tubes and treated as cellular lysates for Western blot assays.

Immunoprecipitation and Western blot analysis

The soluble cellular lysates (0.5 mg of protein) were precleared with 50 μl of protein A–Sepharose (Pharmacia, Uppsala, Sweden) for 2 h at 4 °C by end-over-end rotation.

The precleared cellular lysates were incubated with appropriate antibodies for 3 h at 4 °C [19]. Next, 50 μl of protein A–Sepharose was added to immune complexes and incubation was continued for 2 h at 4 °C. The immunoprecipitates were washed three times with lysis buffer. We added 40 μl of SDS–stop buffer containing 100 mM of DTT to the immunoprecipitates followed by boiling for 5 min. The soluble supernatants were then resolved by 8–16% SDS–PAGE and electrophoretically transferred onto nitrocellulose membranes [19,20]. The membranes were blocked with Tris-buffered saline–0.05% Tween-20 (TBST) containing 5% nonfat dried milk for 1 h at 23 °C. The blots were then incubated with primary antibody for 1 h, washed in TBST, and further incubated with secondary antibodies linked to horseradish peroxidase. Bound horseradish peroxidase was visualized by a highly sensitive chemiluminescence system (SuperSignal from Pierce, Rockfold, IL) [24]. The bands obtained in the blots were scanned and analyzed by the PCBAS2.0 program. Values are expressed as means ± SEM. Student's *t*-test was used for comparisons, with differences being considered statistically significant at $p < 0.05$.

FITC–Annexin V/PI double staining

JEG-3 cells were treated with or without leptin for 5 h in the presence or absence of the pharmacological inhibitors wortmannin or PD980059. The cells were washed with PBS, detached with a rubber policemen, and resuspended in binding buffer (10 mM Hepes, pH 7.4, 140 mM NaCl, and 2.5 mM $CaCl_2$). Fluorescein isothiocyanate (FITC)–Annexin V and PI were added to a final concentration of 1 μg/ml [23]. The mixture was incubated for 10 min and then analyzed by flow cytometry. A total of 20,000 cells were routinely acquired in a FACScalibur flow cytometer. Data were analyzed using CELLQuest software (BDIS). Trophoblastic cell

population was gated in side-forward scattering to analyze FL-1 (FITC–Annexin) and FL-2 (PI). This test discriminates intact cells (Annexin V^-/PI^-), early apoptotic cells (Annexin V^+/PI^-), and late apoptotic necrotic cells (Annexin V^+/PI^+). Data were also analyzed using the computer program WinMDI version 2.8.

Results

Leptin activates JAK-STAT signaling pathway in JEG-3 trophoblastic cells

To study the activation of JAK kinases by the leptin receptor in JEG-3 trophoblastic cells, we stimulated them with human leptin and analyzed the phosphorylation of JAK-2 by Western blot using antibodies that specifically recognize the tyrosine phosphorylated form of JAK-2 (Fig. 1A). The amount of JAK-2 in every sample was controlled by anti-JAK-2 immunoblot (Fig. 1A). Maximal activation of JAK-2 was observed at 10 nM leptin (data not shown), but 0.1 nM was enough to partially activate JAK-2. The long isoform of the leptin receptor has been shown to bring about ligand-dependent increase in tyrosine phosphorylation of the leptin receptor by recruiting receptor-associated kinases of the Janus family [20,25]. In JEG-3 cells, we have found that human leptin stimulates tyrosine phosphorylation of the long form of the leptin receptor, as assessed by immunoprecipitation with an antibody against the C-terminus of the protein and immunoblotting with antibodies against phosphotyrosine (Fig. 1B). As shown in Fig. 1B, this effect was dependent on the dose at 10 min of treatment. Maximal phosphorylation was observed at 10 nM leptin. The phosphorylated band corresponded with an apparent molecular mass of about 190 kDa, consistent with previously reported data in different systems [20,26].

We next sought to study the possible activation of STAT-3 by human leptin in JEG-3 cells [19]. Solubilized lysed samples were analyzed by Western blot using antibodies that specifically recognize the tyrosine phosphorylated form of STAT-3 antibody. The amount of total STAT-3 protein was checked by anti-STAT-3 immunoblotting (Fig. 1C). As shown in Fig. 1C, tyrosine phosphorylation of STAT-3 was observed in response to human leptin treatment. The leptin effect on STAT-3 phosphorylation was dependent on the dose (Fig. 1C), and maximal effect was achieved at 1 nM leptin, but the effect was also observed at 0.1 nM leptin.

Leptin activates PI3K pathway

Previous studies have shown that leptin activates PI3K in myotubes, β-cells, hepatocytes and PBMC [20,27–30]. To measure activation of PI3K pathway in JEG-3 cells in response to human leptin, we measured the activation of the central kinase of this pathway, i.e. PKB, by immunoblot using antibodies that specifically recognize the phosphorylated form of PKB. As shown in Fig. 2A, leptin dose-dependently stimulated PKB phosphorylation. Maximal effect was observed at 10 nM leptin, but the effect was also observed at 0.1 nM leptin. GSK3 is the target of PKB, which is inhibited by the phosphorylation on Ser-21 (the α-isoform) and Ser-9 (the β-isoform) [31]. We therefore employed antibodies that recognize the Ser-phosphorylation of GSK3 and controlled the amount of GSK3 protein by immunoblot using anti-GSK3 antibodies. As shown in Fig. 2B, leptin dose-dependently increased the phosphorylation of GSK3. Maximal effect was observed at 1 nM leptin, but an increase in phosphorylation was observed at 0.1 nM.

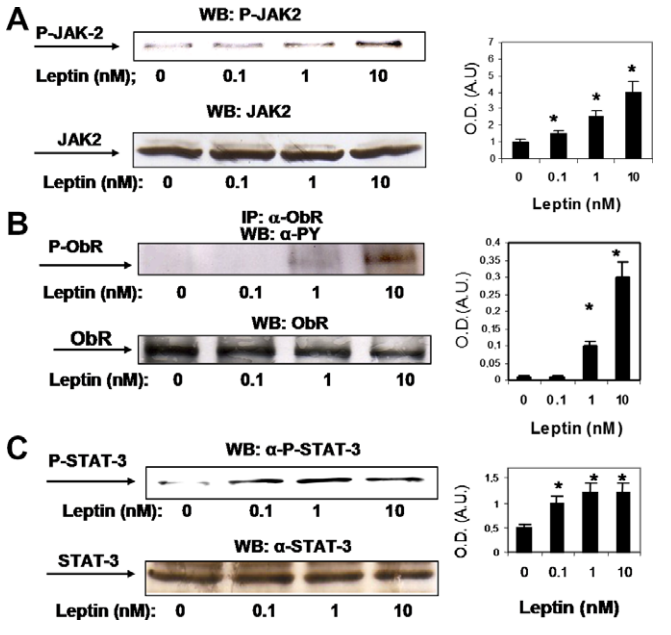

Fig. 1. Leptin activates JAK-STAT signaling pathway in JEG-3 trophobastic cells. JEG-3 cells were incubated in the absence or presence of increasing concentrations of leptin for 10 min in DMEM 0% FCS. Cells were lysed and soluble clarified cell lysates were subjected to immunoprecipitation with anti-Ob-R antibodies. Immunoprecipitates were then resolved by SDS–PAGE and Western blot with anti-phosphotyrosine antibodies (B). For the activation of JAK-2 and STAT-3, cell lysates were analyzed by immunoblotting using the specific antibody against the phosphorylated form of JAK-2 (A) and STAT-3 (C). Data shown are representative of three independent experiments. Densitograms with standard error are shown. $^*p < 0.05$ versus control.

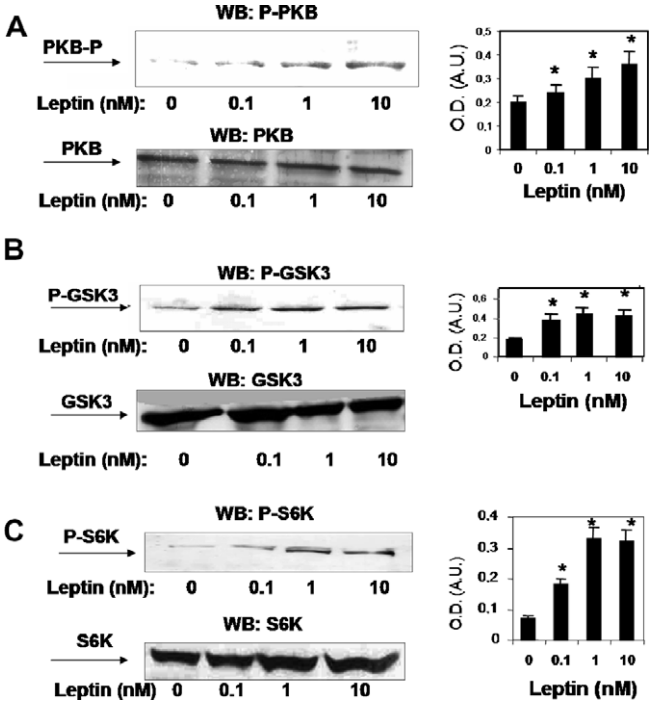

Fig. 2. Leptin activates PI3K pathway. JEG-3 cells were incubated in the presence of increasing concentrations of leptin for 10 min. Cells were lysed and soluble lysates were analyzed by immunoblotting using the specific antibody against the phosphorylated form of PKB (A), GSK3 (B), and p70S6K (C). The same lysates were analyzed by immunoblot using the antibodies that recognizes the non-phosphorylated kinases to control the amount of every kinase in the samples. Data shown are representative of four independent experiments. Densitograms with standard error are shown. $^*p < 0.05$ versus control.

Another kinase downstream of PKB is p70S6 kinase. Since p70S6 kinase activity is correlated with its phosphorylation state, specifically at Thr421 and Ser424 [32], we employed polyclonal antibodies that detect p70S6 kinase only when phosphorylated at Thr421/Ser424. The amount of p70S6 kinase in every sample was controlled using anti-p70S6K antibodies. As shown in Fig. 2C, leptin dose-dependently stimulated the phosphorylation of p70S6K, with the maximal effect on the phosphorylation observed at 1 nM leptin and the minimal effect at 0.1 nM.

Leptin activates MAPK pathway

On the basis of the previously described effect of leptin on MAPK pathways in different systems such as PBMC [20,26,33,34], we checked whether MEK and its target Erk1–2 are activated by leptin in trophoblastic cells by studying its phosphorylation level, which reflects their activation state. Thus, we employed antibodies that specifically recognize the phosphorylated forms of the kinases, and we used anti-MEK and anti-MAPK antibodies for the control of the immunoblot. As shown in Fig. 3A, leptin dose-dependently stimulated the phosphorylation of MEK. Maximal effect was achieved at 10 nM leptin and minimal effect was observed at 0.1 nM leptin. Similar leptin dose–response effect was observed for the leptin activation of MAPK (Fig. 3B). As shown in Fig. 3B, leptin stimulated tyrosine/threonine phosphorylation of MAPK as assessed by specific immunoblot with the anti-doubly phosphorylated MAPK antibody. Both Erk1 and Erk2 were phosphorylated in JEG-3 cells after 10 min of incubation with human leptin.

Leptin activation of JAK-STAT, PI3K and MAPK are confirmed in physiological trophoblastic cells

In order to check whether leptin may activate the same signaling pathways in normal trophoblast cells as those that we have found in the JEG-3 cells, we performed dose–response experiments using normal placenta trophoblast explants. As shown in Fig. 4, leptin dose-dependently stimulated the phosphorylation of JAK-2, STAT-3, MEK, PKB and p70S6K. Maximal effect of leptin for the activation of every kinase was obtained at 10 nM leptin, but a significant effect was observed at 0.1 nM leptin, similar to the observed results obtained from JEG-3 cells.

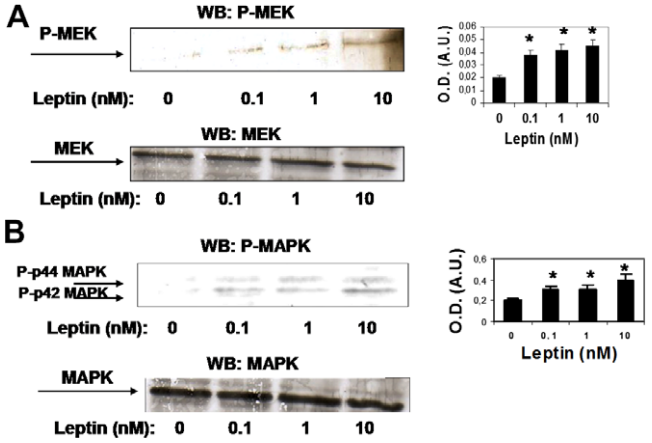

Fig. 3. Leptin activates MAPK pathway. JEG-3 cells were incubated in the presence of increasing concentrations of leptin for 10 min. Cells were lysed and soluble lysates were analyzed by immunoblotting using the specific antibody against the phosphorylated form of MEK (A) and doubly phosphorylated MAPK (B). The same lysates were analyzed by immunoblot using the antibodies that recognizes the non-phosphorylated kinases to control the amount of each kinase in the samples. Data shown are representative of four independent experiments. Densitograms with standard error are shown. $^{*}p < 0.05$ versus control.

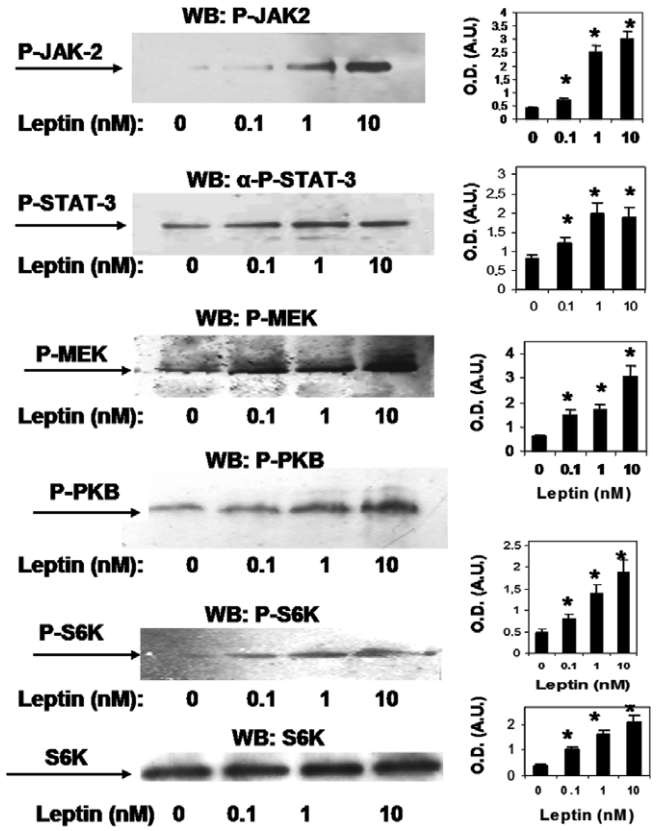

Fig. 4. Leptin activates JAK-STAT, PI3K, and MAPK pathways in physiological trophoblastic cells. Placental explants (50 mg) were incubated for 10 min in the presence of different concentrations of leptin, washed and lysed. Soluble lysates were analyzed by immunoblot using antibodies that specifically recogize the acivated form of the kinases: JAK-2 (A), STAT-3 (B), MEK (C), PKB (D), and p70S6K (E). The samples were controlled by immunoblot against total S6K (F). Data shown are representative of four independent experiments. Densitograms with standard error are shown. $^{*}p < 0.05$ versus control.

Leptin prevents apoptosis of JEG-3 trophoblastic cells by activating MAPK pathway

We have previously found that leptin has a trophic effect on JEG-3 cells, preventing the apoptosis promoted by serum deprivation [17]. We cultured JEG-3 cells in the absence of serum with and without leptin and in the presence of the PI3K inhibitor wortmannin or the MEK inhibitor PD98059. We measured the early apoptotic events by simultaneous detection of Annexin-V–FITC/PI staining using flow cytometry. As shown in Fig. 5, leptin diminished apoptotic cell population even in the presence of 50 nM wortmannin. However, the presence of 100 μM PD98059 completely reverted the antiapoptotic effect of leptin, suggesting that MAPK pathway rather than PI3K is mediating the antiapoptotic effect of leptin on trophoblastic JEG-3 cells.

The signaling pathway involved in the antiapoptotic effect of leptin in JEG-3 cells was confirmed by studying the activation of caspase-3, which was assessed by immunoblot using antibodies against the activated (cleaved) form of caspase-3. As shown in Fig. 6, leptin prevented the activation of caspase-3 induced by serum deprivation during 16 h, and this effect was reverted by the MEK inhibitor PD98059, whereas the PI3K inhibitor wortmannin did not revert the antiapoptotic effect of leptin.

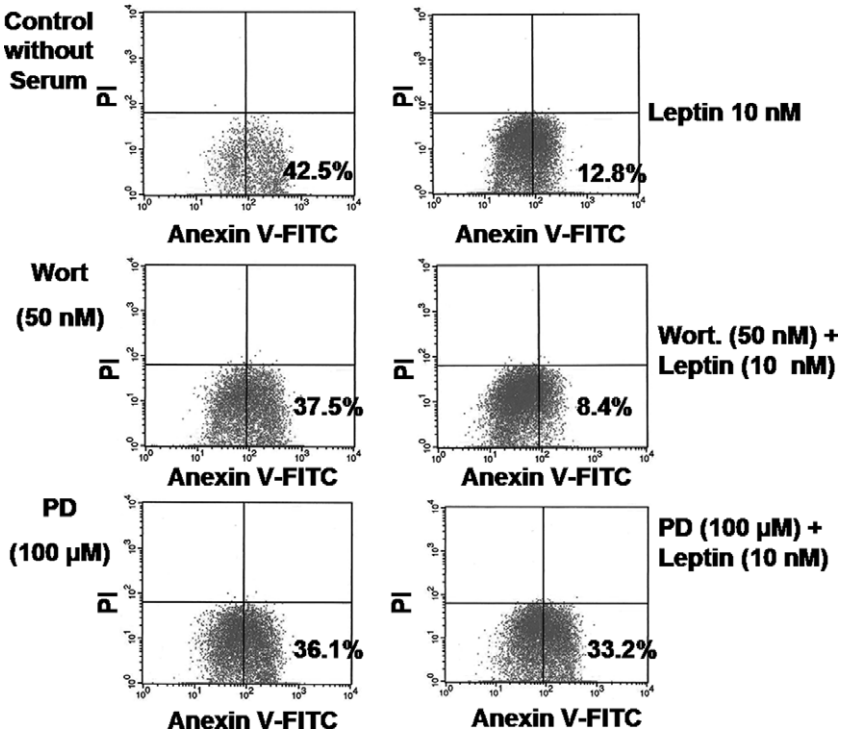

Fig. 5. Leptin prevents early apoptotic events in JEG-3 trophoblastic cells by activating MAPK pathway Cells were incubated with or without 10 nM leptin for 5 h, in the absence or presence of 50 nM wortmanin, or 100 µM PD98059. Data are dot–plot diagrams of FITC–Annexin V/PI flow cytometry of JEG-3 cells. The lower left quadrants show the viable cells, which exclude PI and are negative for FITC–Annexin V binding. The upper right quadrants contain the non-viable, necrotic, and late apoptotic cells, positive for FITC–Annexin V binding and for PI uptake. The lower right quadrants represent the apoptotic cells, FITC–Annexin V-positive, and PI-negative. A representative experiment of four independent experiments is shown.

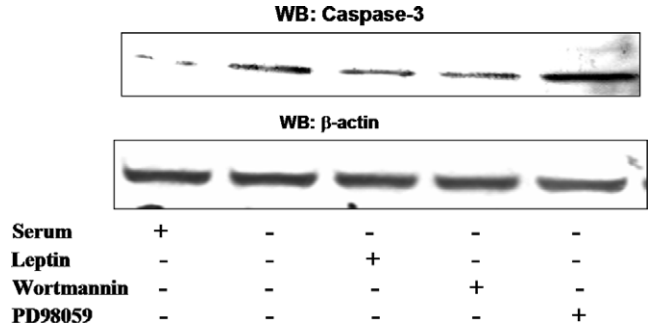

Fig. 6. Leptin prevents caspase-3 activation in JEG-3 trophoblastic cells by activating MAPK pathway. Cells were incubated in the absence of serum with or without 10 nM leptin for 16 h, and in the absence or presence of 50 nM wortmannin, or 100 µM PD98059. Cells were lysed and soluble lysates were analyzed by immunoblotting using the specific antibody against the phosphorylated form of caspase-3. The experiment was controlled using cells cultured in the presence of serum. The amount of protein in every sample was checked by immunoblot using anti-β-actin antibody. A representative experiment of three independent experiments is shown.

Discussion

Leptin is considered not only as an adipocyte-derived hormone to signal in the central nervous system but an important local factor in placenta that may function as a trophic factor, promoting growth and preventing the apoptotic process [21]. These effects may be of physiological relevance since trophoblastic cells are an important source of leptin production [4], so that circulating leptin levels are increased in pregnancy [22].

Moreover, leptin levels are increased under stressful condition for placenta cells that are present in some pathophysiological pregnancy disorders, such as preeclampsia or gestational diabetes [22,35,36]. The leptin overproduced by placenta under these stressful circumstances may be helpful to prevent the stress-mediated apoptosis of the trophoblastic cells. However, little is known about the molecular mechanisms underlying these effects.

In this study, we employed JEG-3 human choriocarcinoma cells, but normal trophoblastic explants from healthy donors were also studied to confirm the physiological relevance of the pathways activated by leptin. These cells maintain many characteristics of human trophoblast cells and have been widely used to study placental cellular signaling [37,38]. Moreover, these cells express both leptin and its receptor [21]. Both leptin and its receptor share structural and functional similarities with the IL6 family of cytokines [13,14]. The leptin receptor also has signaling capabilities comparable with IL6-type cytokine receptors [39]. Thus, to further understand the signal transduction of leptin in trophoblastic cells, we wanted to assess the major signaling pathways known to be activated by leptin receptor in other systems [40], such as peripheral blood mononuclear cells [18], these are JAK-STAT, PI3K, and MAPK pathways.

We have found that leptin treatment in JEG-3 trophoblastic cells triggers the activation of JAK-2 and STAT-3, which is in contrast to data obtained in BeWo trophoblastic cells, whereas another group has reported that leptin neither enhanced JAK-2 phosphorylation nor activated STAT-3 and STAT-1 proteins, but they found that JAK-2 is constitutively activated [41].This discrepancy may be due to differences in the cell lines. That is why we aimed to investigate this signaling pathway in physiological trophoblastic cells, by using human placental explants. We found that human placental JAK-2 and STAT-3 were tyrosine phosphorylated in response to leptin in a dose-dependent manner. This is the first time that the JAK-STAT pathway is studied in normal placenta, and data clearly demonstrate that this is a signaling pathway recruited by leptin receptor. JAK-2 is the most important JAK isoform to medi-

ate physiological effects of leptin [25,42]. We have not tried other STAT forms, and therefore, the possible role of STATs other than STAT-3 in leptin receptor signaling in placental trophoblastic cells cannot be ruled out. On the other hand, STAT-3 activity has been correlated with trophoblast invasiveness [43].

PI3K, PKB, and mTOR have also been found to be signaling pathways regulating the invasive differentiation of human trophoblasts [44], however this is the first time, is shown the activation of PI3K by leptin in trophoblastic cells, by studying the phosphorylation of downstream kinases PKB and S6K. On the other hand the activation of the PI3K pathway by leptin has been demonstrated in many other systems, including immune cells [18,23,45]. Therefore the activation of PI3K pathway in placenta should not be striking. Thus, we have found that leptin dose-dependently stimulates the phosphorylation of PKB and S6K in both JEG-3 and placental trophoblast explants. In summary, PI3K pathway can also be recruited by leptin in placental trophoblastic cells.

Leptin has previously been found to activate MAPK in different systems, mediating a proliferative response [20,26,33,34]. We have found that leptin activates MAPK pathway in placenta as assessed by studying MEK and MAPK phosphorylation in both JEG-3 and placental trophoblast explants. Leptin activation of MAPK pathway has been previously found to be the mechanism whereby leptin promotes cell survival preventing apoptosis [46,47], for instance we have found that the antiapoptotic effect of leptin on blood monocytes is mediated by the activation of MAPK [17]. Now, in trophoblastic JEG-3 cells we have found that leptin prevents the apoptotic process triggered by the deprivation of serum by means of the activation of MAPK pathway, since the pharmacological inhibition of MAPK can block the antiapoptotic effect of leptin. In fact, we demonstrated that leptin prevents both the early (phosphatidylserine exposure) and late events (caspase-3 activation) of apoptosis via MAPK pathway. However, PI3K inhibition did not prevent the antiapoptotic effect of leptin, suggesting that PI3K activation may mediate other functions of leptin in placenta, and that MAPK pathway is the major signaling pathway to mediate the antiapoptotic effect of leptin in placenta.

In conclusion, major leptin receptor signaling pathways are triggered by leptin in human placenta and the antiapoptotic effect of leptin is mainly mediated by the activation of the MAPK pathway.

Acknowledgments

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