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Review: Leptin gene expression in the placenta — Regulation of a key hormone in trophoblast proliferation and survival

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ABSTRACT

Leptin is a 16000 MW protein originally described as an adipocyte-derived signaling molecule for the central control of metabolism. However, pleiotropic effects of leptin have been identified in reproduction and pregnancy. The leptin gene is expressed in placenta, where leptin promotes proliferation and survival of trophoblast cells. Study of the major signaling pathways known to be triggered by leptin receptor has revealed that leptin stimulates JAK/STAT, MAPK and PI3K pathways in placental cells. Leptin also exerts an antiapoptotic action in placenta and this effect is mediated by the MAPK pathway. Moreover, leptin stimulates protein synthesis by activating the translational machinery via both PI3K and MAPK pathways. Expression of leptin in placenta is highly regulated, suggesting that certain key pregnancy molecules participate in such regulation. An important hormone in reproduction, hCG, induces leptin expression in trophoblast cells and this effect involves the MAPK signal transduction pathway. Moreover, the cyclic nucleotide cAMP, which has profound actions upon human trophoblast function, also stimulates leptin expression and this effect seems to be mediated by crosstalk between the PKA and MAPK signaling pathways. Estrogens play a central role in reproduction. 17β-estradiol upregulates leptin expression in placental cells through genomic and non-genomic actions, probably via crosstalk between estrogen receptor-α and the MAPK and PI3K signal transduction pathways. Taken together these findings give a better understanding of the function of leptin and the regulatory mechanisms of leptin expression in human placental trophoblast and further support the importance of leptin in the biology of reproduction.

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1. Introduction

Leptin, the product of the *LEP* gene, is a 16,000 molecular weight non-glycosylated polypeptide of 146 amino acids discovered in 1994 by Zhang et al. [1]. Its crystal structure reveals a four-helix bundle similar to the long-chain helical cytokine family that includes IL-6, IL-11, IL-12, LIF, G-CSF, CNTF, and oncostatin M. This cytokine-type hormone is able to exert multiple functions; the best characterized is the regulation of food intake and energy expenditure. In this regard, leptin is produced by white adipose tissue and secreted in response to increased energy store and its levels in blood correlate with total body fat stores [2]. Leptin has potent weight reducing effects *in vivo*. In *Lep/Lep* mice, the gene encoding leptin is mutated, resulting in obesity [1]. Pleiotropic effects of leptin have been identified including the inhibition of insulin secretion from

pancreatic β-cells, the stimulation of glucose utilization and lipolysis in adipocytes, and sugar transport across the small intestine. Leptin has also been implicated in the modulation of thermogenesis, homeostasis, angiogenesis, hematopoiesis, osteogenesis, chondrogenesis, neuroendocrine and immune functions. The current status of leptin is consistent with its production by various tissues and organs such as the stomach, skeletal muscle, pituitary cells and the placenta [2]. Compelling evidence also has implicated leptin in reproductive functions such as the regulation of ovarian function, oocyte maturation, embryo development and implantation [3].

The aim of this paper is to review the actions of leptin in placental cells and the signal transduction pathways activated by the hormone. Moreover, the regulation of leptin expression by different placental hormones and the mechanisms involved will be discussed.

2. Leptin is a modulator of embryo implantation

Embryo implantation represents the most critical step of the reproductive process. The blastocyst becomes intimately connected

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to the maternal endometrial surface to form the placenta [4]. Implantation involves a complex sequence of signaling events that are crucial to the establishment of pregnancy. A large number of identified molecular mediators have been postulated to be involved in this early feto−maternal interaction, including hormones, adhesion molecules, cytokines, growth factors, lipids and others [5].

In this context, a significant role of leptin in implantation has been proposed. Secretory endometrium is a target tissue for leptin action, and oocytes and preimplantation embryos express leptin receptor (LEP-R) mRNA, indicating that leptin may be necessary for embryonic development. Furthermore, leptin mRNA is specifically expressed at the blastocyst stage, suggesting a function in the blastocyst-endometrial dialog [3]. In line with this, leptin has concentration and stage-dependent effects on embryonic development *in vitro*. In a human *in vitro* model, it was observed that leptin was present in conditioned media from human blastocysts whether or not they were cocultured with endometrial epithelial cells. The higher leptin secretion found in competent human blastocyst cultures, compared with arrested blastocysts, suggests that this molecule may be a marker of cell viability. Differences between arrested and competent blastocysts suggest autocrine/paracrine regulation of leptin between endometrial epithelial cells and preimplantation embryos [6].

In humans, mutations of both leptin and leptin receptor have been identified. As in rodents, these mutations are recessive. Human subjects with leptin or leptin receptor mutation are obese and sterile, and exhibit significant growth retardation and central hypothyroidism, with reduced secretion of growth hormone and thyrotropin. Leptin-deficient mice are sterile; however, fertility can be restored by exogenous administration of leptin probably due to a corrected interaction at the endometrium−embryo interface [7].

The obligatory nature of leptin signaling in mammalian implantation was illustrated by experiments in the mouse demonstrating that endometrial leptin receptor expression was pregnancy-dependent and that intrauterine injection of a leptin peptide antagonist or a leptin antibody impaired implantation. Leptin levels in women suffering spontaneous first trimester abortions were abnormally low, implying a direct role in pregnancy maintenance [8]. Little is known about leptin deficiencies in humans. Leptin is present in the human oocyte and preimplantation embryo. Since the endocrinology of pregnancy in general, and leptin in particular, are not well conserved between species, the extrapolation of data from rodent to human physiology is not feasible [9].

Further evidence for the importance of leptin in implantation is the fact that the hormone increases the expression in cytotrophoblasts of matrix metalloproteinase, which is needed for the invasion process [10].

Deregulation of leptin metabolism and/or leptin function in the placenta may be implicated in the pathogenesis of various disorders during pregnancy, such as recurrent miscarriage, gestational diabetes, intrauterine growth retardation, and preeclampsia [8].

3. Leptin modulates placental proliferation and survival

Several studies have shown that leptin induces trophoblast cell proliferation, stimulates hormone and cytokine production [11], and regulates fetal growth and development [8].

We have demonstrated that leptin enhances BeWo and JEG-3 cell proliferation in a dose and time-dependent fashion. Moreover, cell proliferation was diminished by inhibiting endogenous leptin expression with an antisense oligonucleotide (AS). Cell population distribution during the different stages of the cell cycle showed a displacement of cells toward a G2/M phase after leptin treatment. Leptin treatment also upregulated expression of cyclin D1, one of

the key cell cycle-signaling proteins [12]. Proliferation and death processes are intimately related. In this regard, we have demonstrated that leptin diminished apoptosis as well [12,13]. These results raise the question of the signal transduction pathways involved in leptin action.

Both leptin and its receptor share structural and functional similarities with the IL6 family of cytokines. The leptin receptor also has signaling capabilities comparable with IL6-type cytokine receptors [14]. Leptin receptors are located primarily on syncytiotrophoblast, accessible to maternal rather than to fetal circulating leptin. The human leptin receptor gene encodes four transmembrane proteins with different C-terminal lengths and sequences and one soluble isoform lacking the transmembrane region. All transmembrane isoforms are identical in their extracellular region and transmembrane domain and also share the first 29 amino acids of the intracellular tail [15]. Short and long leptin receptor isoforms are expressed in the placenta. The soluble receptor has also been characterized in human placenta [16]. We have demonstrated that multiple signal transduction pathways are activated in response to leptin both in JEG-3 cell culture and in human term placenta [13]. Leptin is able to stimulate Janus kinase (JAK)-signal transducers and activators of transcription (STAT) pathway by promoting JAK-2 and STAT-3 tyrosine phosphorylation in the human placenta choriocarcinoma JEG-3 cell line, as well as in trophoblast cells from human term placenta. STAT-3 activity has been correlated with trophoblast invasiveness [17]. The signal transduction pathways involving mitogen-activated protein kinase (MAPK) are also activated. Leptin induced the phosphorylation of the extracellular-signal regulated kinase kinase (MEK) and the extracellular-signal regulated kinase 1/2 (ERK 1/2) [13]. It was demonstrated that leptin activation of the MAPK pathway mediates a proliferative response in different cell types such as human peripheral blood mononuclear cells [18]. The PI3K pathway is also triggered by leptin stimulation as assessed by the study of protein kinase B (PKB) phosphorylation [13]. PI3K, PKB, and mTOR have also been found to be signaling pathways regulating the invasive differentiation of human trophoblasts [19].

The relative importance of MAPK and PI3K pathways in leptin action was studied by using pharmacological inhibitors. In this context, it was found that the effect of leptin on JEG-3 survival is completely reversed by blocking ERK 1/2 activation, employing the MEK inhibitor PD98059, whereas it was not affected by PI3K inhibition using wortmannin. In fact, it was demonstrated that leptin prevents both the early and late events of apoptosis via the MAPK pathway. These data suggest that PI3K activation may mediate other functions of leptin in placenta, and that the MAPK pathway is the major signaling pathway to mediate the antiapoptotic effect of leptin in placenta [13].

To further investigate the mechanism by which leptin stimulates cell proliferation, we analyzed protein synthesis in JEG-3 cells and trophoblast cells. We observed that leptin induces not only protein synthesis but also the phosphorylation state of EIF4EBP1 (PHAS-I) and EIF4E. The initiation-factor EIF4E binds to the cap structure at the 50-end of the mRNA and mediates the assembly of the initiation-factor complex EIF4E. The assembly of this complex is inhibited by EIF4E-binding proteins (EIF4EBPs) such as EIF4EBP1 (PHAS-I). Phosphorylation of these EIF4EBPs releases EIF4E from inactive EIF4EBP-EIF4E complex, allowing EIF4E to bind to EIF4G, and EIF4A to form the active EIF4F complex [20]. The activity of this complex is also regulated by phosphorylation of EIF4E. We also showed that leptin stimulates protein synthesis by activating the translational machinery via both PI3K and MAPK pathways [20,21]. Leptin activation of the translational machinery in placenta may be relevant both physiologically and pathophysiologically since a decrease in EIF4EBP1 phosphorylation has been recently found in

fetuses with intrauterine growth restriction resulting from impaired placental development [20].

4. Leptin expression in placenta

The synthesis and secretion of leptin as well as the expression of its functional receptors by trophoblast cells has been widely demonstrated suggesting that leptin may act through a paracrine or autocrine mechanism. Human leptin mRNA and protein are co-localized in the syncytiotrophoblast and in fetal vascular endothelial cells, tissues in direct contact with maternal and fetal blood respectively. First trimester cytotrophoblasts also express leptin mRNA and protein [15]. Circulating leptin levels are elevated during pregnancy, reaching a peak during the second trimester and at the end of pregnancy, whereas maternal plasma leptin levels decline to normal values 24 h after delivery. During the third trimester of pregnancy, leptin receptor levels show a marked expression [8]. Previous studies have demonstrated that leptin is a modulator of placental endocrine function. The placenta is an important source of maternal circulating leptin. Plasma leptin concentrations are significantly elevated in pregnant women as compared with those in age and body mass index-matched nonpregnant women [22]. Although increased adiposity during pregnancy might (as in obesity) be expected to underlie the hyperleptinaemia of pregnancy, leptin levels are elevated to an extent that cannot be attributed to the increased body mass index [22]. As trophoblast cells produce leptin locally, the effective concentration of this hormone may be even higher in the placenta.

Once bound to placental receptors, leptin triggers local and peripheral effects. Placental leptin induces hCG production in trophoblast cells, enhances mitogenesis, stimulates amino acid uptake, and increases the synthesis of extracellular matrix proteins and metalloproteinases. Leptin may also have a local autocrine immunomodulatory or anti-inflammatory role [15]. Concentrations of leptin in human cord blood correlate with placental size. This is in agreement with the role of leptin in regulating placental growth, which potentially leads to placental hypertrophy under conditions of leptin overproduction. Leptin also stimulates angiogenesis in primary cultures of human endothelial cells. These observations support the possibility that leptin might provide a link between the immune and the endocrine system responses of placental cells [15]. Leptin has also a potential role in fetal growth. Maternal placental leptin may be produced in response to fetal growth and relative to the placental delivery of nutrients. In addition, it was suggested that leptin stimulates pancreatic development, resulting in early insulin production and increased fetal growth [23].

First trimester placental villi secrete 50-fold higher levels of leptin compared to term villi and this secretion is potentiated by IL1A, 17β-estradiol and IL6 [24]. It was also demonstrated that the human leptin gene is actively engaged by hypoxia through mechanisms that are common to other hypoxia-inducible genes [25].

Various regulatory elements have been identified within the leptin promoter, e.g., cAMP and glucocorticoid response elements, and CCATT/enhancer and SP1 binding sites, suggesting a direct regulation of leptin expression through different transcriptional pathways [1]. A placental specific enhancer located 1.9 kb upstream of the human leptin gene was identified. It works in choriocarcinoma lines but not in adipose cells [26]. Because of the functional significance of leptin in pregnancy, it would be expected that certain key molecules for this process participate in the regulation of leptin expression in placenta. Fig. 1 shows a schematic representation of in silico analysis of leptin promoter. It shows both confirmed and putative elements to different transcription factors.

4.1. Regulation of leptin expression in placental cells by hCG

HCG is secreted by villous trophoblasts and is likely to act on other receptor-bearing cells in the fetoplacental environment [27]. It was previously demonstrated that leptin has a stimulatory effect on hCG secretion when added to primary cultures of human term placental trophoblast cells and in first-trimester trophoblast cells [11]. Leptin action on hCG secretion apparently depends on the time of pregnancy, not affecting hCG secretion at term while enhancing hCG secretion by first trimester placental explants. On the other hand leptin secretion is significantly stimulated in cytotrophoblast cells by treatment during 4 h with GnRH-II [28]. In this regard, it has been reported that leptin secretion in human cytotrophoblast cells was significantly inhibited when incubated during 4 h with increasing concentrations of hCG [29]. It is probably that among the different hormones secreted by the placenta, GnRH, hCG and leptin are involved in an autocrine/paracrine loop regulating placental function principally during the first trimester of pregnancy. We have investigated the effect of hCG on leptin expression in BeWo cells and placental explants. We observed a significant upregulation and this effect was dose and time-dependent [30].

We also found that hCG increased leptin expression by acting at the transcriptional level and that a minimal promoter region spanning up to −218 bp may be responsible for this induction. In silico analysis of this DNA fragment revealed potential consensus elements for different transcription factors such as C/EBP and Sp1 with a core similarity of 1. Previous reports have demonstrated the involvement of both C/EBP and Sp1 in the regulation of leptin gene in adipocytes [31]. Furthermore, C/EBPs transcriptional factors were reported to be regulated by hCG during follicular development [32]. Other investigators also described an hCG upregulation of C/EBPα expression in rat primary cultures of Leydig cells and in human adipose cells [33,34].

HCG mediates its action through the LH/hCG receptor, and its major function is to maintain the progesterone production of corpus luteum during early pregnancy. HCG has probably many other functions, being one of the earliest embryonic signals; it is already expressed in eight-cell embryos and is secreted in high local concentrations by the blastocyst entering the uterine cavity. Therefore, it is probably one of the embryonic signals involved in the embryo-maternal dialog regulating implantation [35]. Binding of hCG to its receptor generates signal transduction through the activation of the associated heterotrimeric G-proteins.

It was found that (Bu)$_2$cAMP not only did not enhance hCG effect but even inhibited hCG-dependent leptin expression in placental cells. It was recently described that the downregulation of the LH/CG receptor was obtained by treating rats with hCG or by the chronic elevation of cAMP production [36]. It is possible that the downregulation of the hCG receptor would explain the lack of cAMP stimulation on hCG effect when the level of the cyclic nucleotide is raised. Although the physiological role of hCG in promoting cAMP production is well documented, the LH/hCG receptor has also been shown to mediate activation of the MAPK [37], JAK and PI3K pathways [38]. For example, it is reported that in a human endometrial epithelial cell line (HES) and baboon epithelial endometrial cells, CG does not activate the AC-cAMP-PKA pathway but it can rapidly induce phosphorylation of ERK 1/2 in a PKA-independent manner [37]. We have shown that hCG treatment specifically activates MEK and ERK 1/2 phosphorylation in placental cells. The involvement of MAPK signaling pathway activation in leptin upregulation was demonstrated both by using MEK inhibitor, PD98059 and by the transfection of cells with a plasmid encoding a dominant negative mutant of ERK2. Data obtained by using both methods demonstrated that MAPK pathway plays a major role in transducing gonadotropin signaling toward leptin upregulation. The PI3K
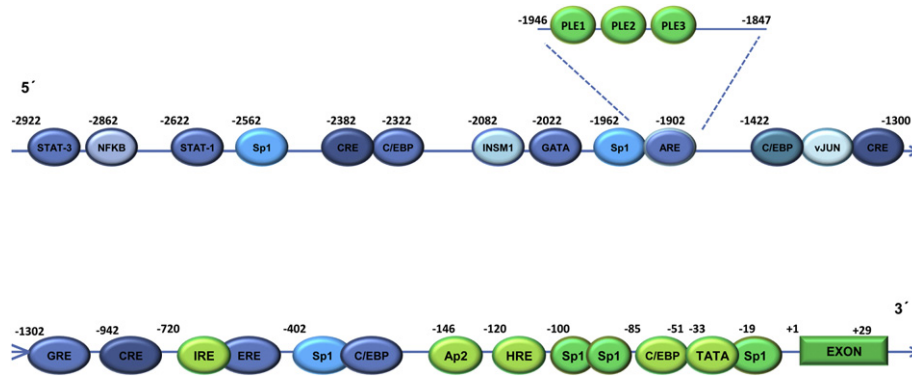
Fig. 1. *Leptin promoter elements analysis.* The schematic representation shows reported (green circles) and putative (blue circles) regulatory elements for different transcription factors. The enlargement of the promoter region shows the positions of the placental leptin enhancer (PLE 1, 2 y 3). *In silico* analysis to identify putative elements was performed with the MatInspector Release Professional 8.0.3 program. *C/EBP*, CCAAT/enhancer-binding protein; *GRE*, glucocorticoid response element; *CRE*, cAMP response element-binding protein; *ERE*, estrogens response element; *IRE*, insulin response element; *ARE*, androgens response element; *HRE*, hypoxia response element; *Sp1*, stimulant protein 1; *INSM1*, Insulinoma-associated protein 1.

pathway did not seem to be involved in leptin regulation by hCG [30]. Fig. 2 shows a schematic representation of the mechanisms involved in the hCG upregulation of leptin.

4.2. Regulation of leptin expression in placental cells by cAMP

The cAMP is one of the oldest known signaling molecules. Increases in intracellular cAMP lead to the activation of cAMP-dependent protein kinase (PKA), which phosphorylates intracellular substrates, including the cAMP response element-binding protein (CREB). CREB is a cAMP-regulated transcriptional regulatory protein that binds to consensus cAMP-responsive DNA elements (CREs) within target genes [39]. It has been shown that increases in intracellular cAMP result in decreased expression of leptin mRNA and leptin secretion in rat adipocytes [40]. It was also shown in these cells that the inhibitory effect of cAMP on leptin release is PKA-dependent [41]. In contrast, it has been demonstrated that leptin secretion in BeWo cells, and in term human placental tissue cultured in monolayer, is augmented by forskolin, an activator of PKA [42]. However, several experiments have provided evidence that cAMP affects some cellular processes independently of PKA [43]. It is well documented that cAMP has profound effects upon human trophoblast function as demonstrated in numerous *in vitro* studies of normal and transformed trophoblast cells [44].

Little is known about leptin regulation by cAMP in human placenta. When the effect of cAMP on leptin expression in BeWo, JEG-3 cells and placental explants was investigated, a significant dose-dependent upregulation was observed. Moreover, cAMP was able to increase leptin promoter activity and leptin mRNA transcription [45]. The stimulatory effect of cAMP on leptin expression has also been reported in placental chorionic tissue [42]. The fact that human leptin gene has a placenta-specific enhancer indicates that the regulation of leptin production in placental trophoblasts is different from that in adipocytes.

4.3. Crosstalk between PKA and MAPK in leptin expression

It is known that the PKA pathway plays a central role in biological signaling of various hormones in the placenta, such as epinephrine, prostanoids, and hCG [44]. The induction effect of cAMP on leptin expression might result from the activation of PKA or from PKA-independent events induced by this nucleotide. Among PKA intracellular substrates, it is CREB that is important for the expression of many cAMP-responsive genes in different cell types and in response to diverse signals [46]. cAMP is able to induce CREB phosphorylation not only in choriocarcinoma cells but also in placental explants. Moreover, the overexpression of the catalytic subunit of PKA or the transcription factor CREB caused a significant increase in leptin promoter activity in a dose-dependent manner. The participation of the PKA pathway in cAMP induction of leptin expression was further demonstrated when the overexpression of a dominant negative regulatory subunit of PKA (PKI) produced a decrease in leptin expression. Specific inhibition of PKA with H89, a selective inhibitor, suppressed cAMP induction of leptin expression both in BeWo cells and in placental explants. Furthermore, the PKA pathway acts at a transcriptional level since H89 and SQ22,536, an adenylyl cyclase inhibitor, were able to inhibit leptin promoter activity induced by cAMP, demonstrating that a decrease in endogenous cAMP level also affects basal leptin expression [45]. All these data strongly suggest that an increase in cAMP level stimulates leptin expression through the activation of the PKA signaling pathway.

On the other hand, the MAPK pathway is essential for reproduction in general. It has been involved in oocyte maturation and in the control of trophoblast penetration and invasion [47]. It is well established that the ERK pathway is involved in placental development [48]. The involvement of this signaling pathway in cAMP upregulation of leptin gene in placental cells was demonstrated using PD98059 that partially blocked leptin induction caused by the overexpression of the catalytic subunit of the PKA. In this context, it has been reported that the MAPK cascade can modulate PKA activity by several mechanisms [49].

The cAMP/PKA pathway and the MAPK cascades modulate common processes in the cell and multiple levels of crosstalk between these signaling pathways have been described [49]. In some cell types, and under certain circumstances, activation of PKA results in activation of the ERK 1/2 pathway, whereas in other cell types and under other culture conditions, PKA blocks ERK 1/2 signaling [50]. Possible crosstalk between these pathways in trophoblast cells was assessed to investigate the molecular mechanisms underlying cAMP effect on leptin upregulation. In this way, cAMP treatment specifically activated ERK 1/2 phosphorylation in placental cells [45]. Furthermore the inhibition of MAPK pathway partially prevented CREB phosphorylation by cAMP. Phosphorylation of CREB at serine 133 was initially attributed to PKA. However, studies have established that several kinases can phosphorylate CREB at the same residue. ERK 1/2 cannot directly phosphorylate CREB but it can activate by phosphorylation of members of the pp90rsk family of protein kinases (RSK1–3), which in turn translocate to the nucleus and directly phosphorylate CREB [51]. In
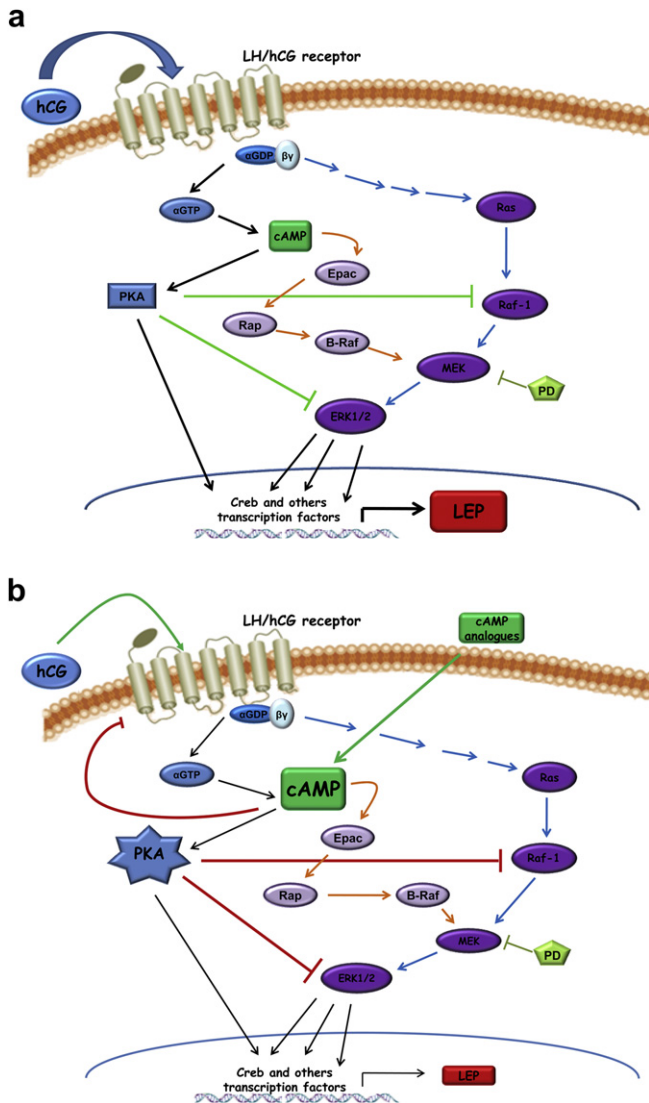
Fig. 2. *HCG upregulates leptin expression in trophoblastics cells*. The proposed model shows hCG mechanisms involved in leptin-induced expression based on current data. hCG stimulates leptin expression in placenta mainly by the MAPK pathway (a). However, when hCG is combined with exogenous cAMP (b), intracellular cAMP rises. This leads to overactivation of PKA, hCG receptor desensitization and MAPK pathway inhibition. All these together could account for the inhibition of leptin expression. αGDP, alpha subunit of G-protein associated to guanosine diphosphate; αGTP, alfa subunit of G-protein associated to guanosine triphosphate; βγ, beta and gamma subunits of G-protein; B-RAF, proto-oncogene serine/threonine-protein kinase; Epac, exchange protein activated by cAMP; RAP, Small Ras-like GTPase Rap; PD, PD98059. Open arrows indicate stimulation; flat arrows, inhibition.

addition, the inhibition of PKA resulted in a significant increase in ERK phosphorylation in placenta, suggesting that PKA activation might cause ERK inhibition and that cAMP activates ERK pathway in a PKA-independent manner. On the other hand cAMP may activate MAP kinase through members of the Ras superfamily. In this mechanism, cAMP binds to guanine nucleotide exchange factor and activates Rap1A, which then increases MAPK phosphorylation [52]. These possible interrelationships are described in the model based in reported data [49] shown in Fig. 3.

4.4. Regulation of leptin expression in placental cells by estradiol

Steroids play an important role in the growth, differentiation, metabolism, reproduction, and morphogenesis of higher organisms

and humans and are particularly required for the development and maintenance of reproductive tissues [53]. The most potent and dominant estrogen in humans is 17β-estradiol (E_2) but lower levels of the estrogens estrone and estriol are also present [54]. Human placenta is known to synthesize estrogens during pregnancy, in association with cytotrophoblast invasion.

Some years ago, Chardonnens et al. [24] observed that E_2 increased leptin production in cultured human cytotrophoblastic cells from first trimester placenta. Besides, O'Neil et al. [55] demonstrated that estrogen could activate the LEP promoter in choriocarcinoma JEG-3 cells through ERalpha and suggested that regulation of leptin biosynthesis may depend on the existence of a functional ER. Recently we observed a significant upregulation of leptin expression in BeWo cells at the transcriptional level using physiological E_2 concentrations. Moreover, treatment with the antiestrogen ICI 182,780 completely blocked E_2 induction of leptin promoter activity and also inhibited basal leptin expression in human placental explants, suggesting a role of endogenous estradiol production in the autocrine control of leptin synthesis [56].

Estrogen, acting via ERα or ERβ, modulates gene expression through multiple mechanisms. The classical activation of an ERE involves ligand-bound receptor binding at a specific palindromic sequence of DNA within the promoters of estrogen-responsive genes [57]. Genomic effects of estrogen could also be explained by an ERE-independent mechanism, because ERs can modulate the function of other transcription factors through protein–protein interactions in the nucleus. It was reported that ligand-bound ER mediates gene transcription from AP1 enhancer elements, when complexed with the AP1 transcription factors FOS and JUN [58]. It was also seen that genes containing GC-rich promoter sequences are regulated in a similar manner through the interaction of ERs with the SP1 transcription factor [59].

Our results support the involvement of ERα in E_2-induced placental leptin expression and suggest that a minimal region spanning up to −1951 bp to −1847 bp is sufficient to evidence such induction. *In silico* analysis of this region of the leptin promoter revealed potential consensus half sites for ER element, a putative binding site for SP1 transcription factor and a placental enhancer region, PLE, previously described [56].

Recent evidence suggests that along with gene regulation, estradiol also mediates rapid cellular effects (non-genomic or extranuclear pathways) [60]. These membrane-initiated actions may indirectly influence gene expression, through the activation of signal transduction pathways that eventually act on target transcription factors. Activation of the MAPK signaling pathway by E_2 has been reported in several cell types, including endothelial, bone, and neuroblastoma cells. It is also known that ER directly associates with PI3K and that E_2 activates this signaling pathway in endothelial, breast cancer, and liver cells [54]. Both MAPK and PI3K signal transduction pathways can be also activated in placental explants following E_2 treatment. Furthermore, the stimulatory effect of E_2 on leptin expression was blocked with PD98059 or by the overexpression of dominant negative mutant forms of MEK or MAPK, suggesting that this pathway is involved in E_2-induced leptin expression [56].

The nature of the plasma membrane binding sites for estrogens is currently under intense debate and investigation. However, to date, both classic ERα and ERβ and nonclassical ER (e.g. ER-X, and GPR30) have been identified at the membrane of several target cells [54]. Particularly, using the complex E-BSA that prevents E_2 from entering the cell due to the large size of the conjugated molecule, it was demonstrated that E_2 regulation of leptin expression in placental cells could be partially mediated by estrogen membrane receptors [56], and their nature is currently being investigated. Our
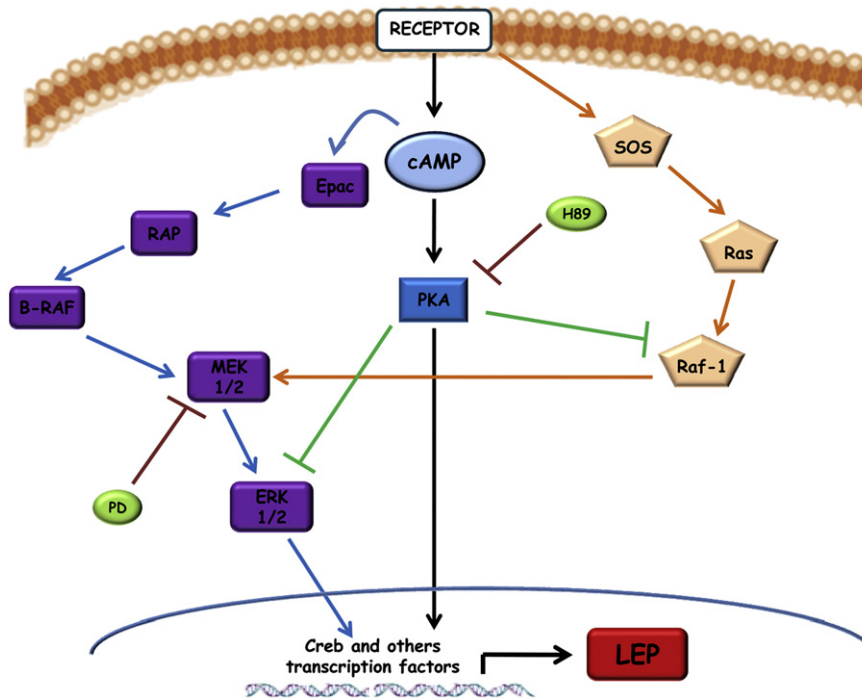
Fig. 3. *Cross talk between PKA and MAPK signaling pathways is involved in cAMP effect on leptin expression in placenta.* Proposed model of the signaling pathways involved in cAMP stimulation based on current data and its relation with leptin expression. Open arrows indicate stimulation; flat arrows, inhibition. Epac, exchange protein activated by cAMP; RAP, Small Ras-like GTPase Rap.

unpublished results suggest the presence of ERα bound to the plasmatic membrane of BeWo cells.

In conclusion, regulation of placental leptin expression by estradiol is a multifactorial process, and may depend on the presence and concentration of estrogen receptors, transcription factors,

co-regulatory proteins and signal transducers, among others, as illustrated in Fig. 4.

5. Conclusions

We have reviewed some aspects of the multiple roles that leptin has in reproduction, and in particular in human placental physiology. The regulation of leptin expression in placenta has also been discussed. In conclusion, leptin triggers major leptin receptor signaling pathways in trophoblast cells and exerts an autocrine antiapoptotic and proliferative effect in human placenta. Moreover, leptin stimulates protein synthesis by activating the translational machinery. We have reviewed the regulation of leptin expression by different molecules that have important functions during pregnancy. HCG upregulates leptin gene in human trophoblastic cells involving the MAPK signal transduction pathway. cAMP also upregulates leptin gene in human placenta probably involving crosstalk between the PKA and the MAPK pathways. On the other hand, 17β-estradiol enhances leptin expression through genomic and non-genomic actions. More experiments are required to demonstrate the exact mechanisms involved in the regulation of leptin expression. It is clear that leptin plays a key role in reproductive biology. The versatile and expanding list of activities and signaling cascades that are involved in its biology mean that much more remains to be studied. The results discussed in this review provide insights in the action of leptin in human placenta and in the regulatory mechanisms of its expression. This could be important in the understanding of several pathologies of pregnancy, such as recurrent miscarriage, gestational diabetes, intrauterine growth retardation and preeclampsia, where the expression of leptin is altered.
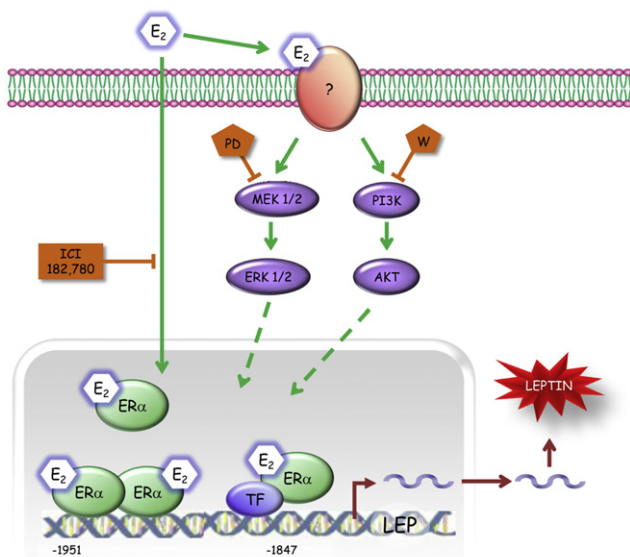

Fig. 4. *E$_2$ has a stimulatory effect on endogenous leptin expression at the transcriptional level.* Proposed model of E$_2$ mechanisms involved in leptin-induced expression based on current data. E$_2$ action involves estrogen receptor alpha (ERα) and probably involves membrane receptors too, whose nature is currently unknown. Proposed model of signaling pathway involved in E$_2$-induced leptin expression is shown. PD98059 (PD), Wortmannin (W), Transcription Factors (TF).

Conflict of Interest Statement

The authors state they have no conflict of interest.

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Disclosure statement

The authors have nothing to disclose.

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