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Hormones and Human Trophoblast Differentiation

A Review

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In the human, fetal cytotrophoblastic cells play a key role in the implantation process and in placental development. With the progression of placentation, two pathways of differentiation lead to the formation of two distinct phenotypes. In the villous trophoblast (fusion phenotype), the trophblast differentiates from the fusion of mononuclear cytotrophoblastic cells into a syncytium, the syncytiotrophoblast. Bathing the maternal blood, the syncytiotrophoblast is involved in maternal-fetal exchanges and in placental endocrine functions. In the extravillous trophoblast (proliferative/invasive phenotype), the cytotrophoblastic cells proliferate and migrate into the decidua, remodeling the pregnant endometrium and its vasculature. This review summarizes our current knowledge of the key step of villous differentiation—the cell-cell fusion of the cytotrophoblastic cells—and on the invasion process of extravillous trophoblast. Experimental evidence demonstrates that the genetic differentiation/invasion programs of cytotrophoblastic cells could be modulated by their environment: oxygen, extracellular matrix, and soluble factors (cytokines, growth factors, and hormones). Cytotrophoblastic cells fusion and the functional differentiation of villous trophoblast are specifically stimulated by estradiol, glucocorticoids, and human chorionic gonadotropin (hCG) whereas progesterone is ineffective. Because these hormones are temporally secreted in large amounts and present at the fetomaternal interface, they are in good position to play a physiologic role in trophoblast differentiation. hCG may be important very early in pregnancy, when production of this glycoprotein is maximal, whereas estrogen increasingly produced by the fetoplacental unit and cortisol secreted from the fetal adrenal may be implicated in the end-stage maturation and aging of the trophoblast.

Key Words: Human placenta; hormones; trophoblast; differentiation.

Introduction

In mammals, the placenta that forms by an implantation process in the maternal organism allows the development of the embryo and the fetus by exchanging ions, metabolites, and waste. Furthermore, the human placenta is characterized by the extent and specificity of its hormonal production (steroids and protein hormones), essential for the development of pregnancy, and by an extensive invasion of the trophoblastic cells into the maternal uterus leading to a hemochorial placentation. At 7 to 8 d postconception, the blastocyst invades the uterus, and the formation of the placenta is the result of a complex series of interactions between fetal trophoblast and maternal cells in the decidua of the uterus. This process involves the proliferation, the invasion, and the differentiation of extraembryonic trophoblastic cells, which are the stem cells from which the different trophoblast populations of the placenta are derived. With progression of placentation, two pathways of differentiation (Fig. 1) lead to the formation of two distinct trophoblastic cell populations, and by d 21 after ovulation, the definitive structural and functional units of the placenta are already present: the "floating villus" and the "anchoring villus" (Fig. 2) *(1)*.

In the *villous phenotype*, the cytotrophoblastic cells of the floating villi (in the intervillous space) remain attached to the villous basement membrane, forming a monolayer of epithelial cells that proliferate and differentiate by fusion to form a syncytiotrophoblast covering the entire surface of the villus. The trophoblastic epithelium (syncytiotrophoblast and cytotrophoblast) surrounds a core of connective tissue including fetal vessels, fibroblasts, and macrophages. The syncytiotrophoblast is engaged in absorptive, exchange, and specific endocrine functions; thus, villous trophoblast is the functional barrier between maternal blood and fetal stroma.

In the *extravillous phenotype*, cytotrophoblastic cells of the anchoring villi proliferate, detach from the basement membrane, and aggregate into cell columns to attach to the uterine wall. From there, individual cells migrate into the decidua and the myometrium, remodeling the pregnant endometrium and its vasculature. Indeed, some of the extravillous cytotrophoblastic cells (EVT) invade the uterine arterioles, destroy the media, and replace the endothelial cells, thus creating low-resistance, large-diameter blood vessels. Alternatively, many extravillous cytotrophoblastic cells scattered through the decidua and the myometrium differentiate into

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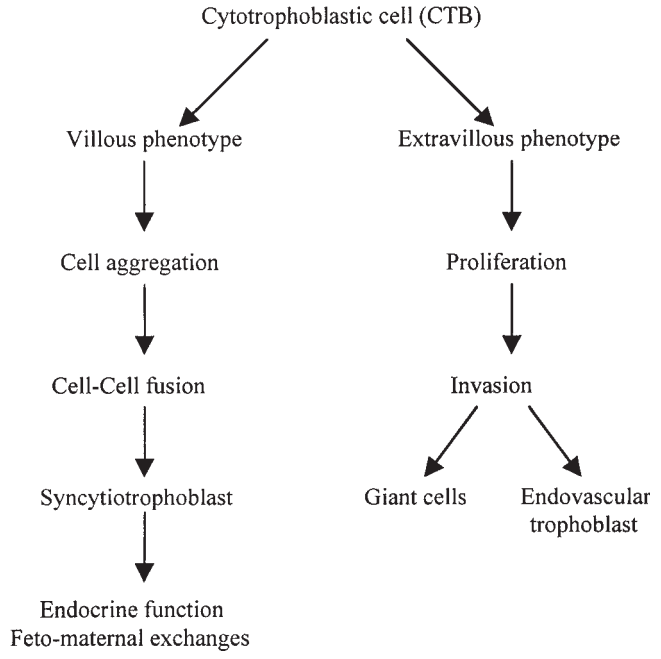

Fig. 1. The two differentiation pathways of human cytotrophoblast.

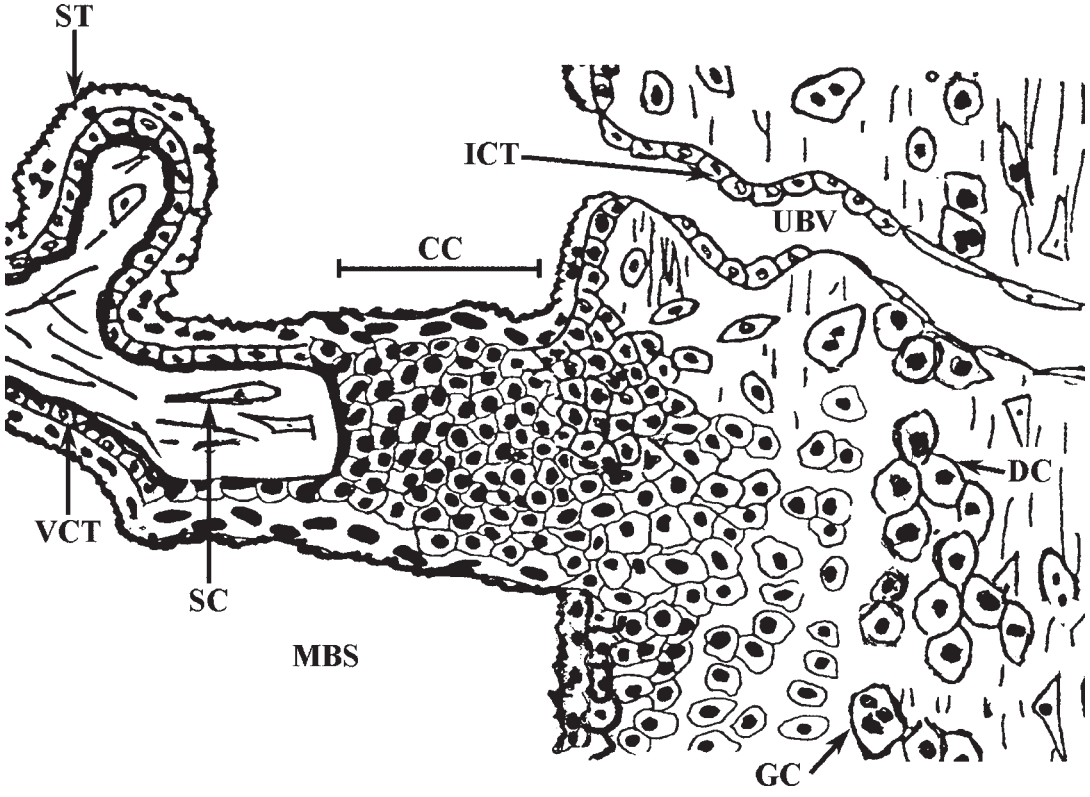

Fig. 2. Diagram of a longitudinal section of villus at fetomaternal interface. The chorionic floating villus (**left**) is bathed with the maternal blood in the maternal blood space (MBS). In the floating villus, villous cytotrophoblastic cells (VCT) differentiate by fusion into syncytiotrophoblast (ST). In the anchoring villus (**right**), the cytotrophoblastic cells proliferate, detach from the basement membrane and aggregate into cell column (CC), and then invade the decidua and the uterine blood vessels (UBV). The infiltrating cells eventually differentiate into multinucleated placental giant cells (GC). SC, stromal core of the floating villus; DC, decidual cell; ICT, invasive cytotrophoblast. (Adapted from refs. *1* and *65*.)

multinucleated placental giant cells (Fig. 2). The trophoblastic endovascular invasion is of major importance for feto-placental physiology. Indeed, during the first trimester, plugs of cytotrophoblasts block the small arteries that supply the placental site, and the early placental environment is hypoxic, preventing exposure of the conceptus from excessively high

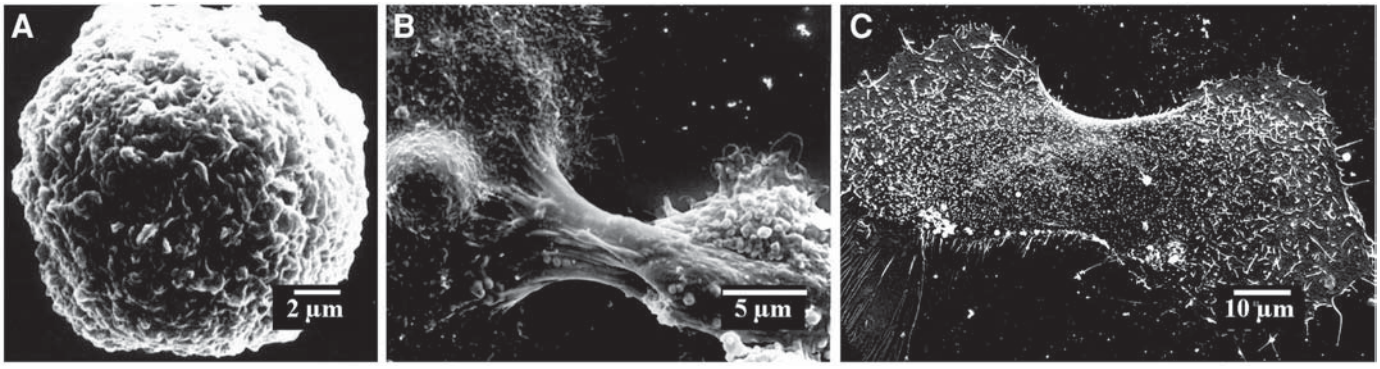

Fig. 3. Villous trophoblast differentiation: morphologic changes in isolated cytotrophoblastic cells cultured on plastic dishes in presence of FCS. Scanning electron micrographs show cytotrophoblastic cells at different times of culture. (**A**) Isolated cytotrophoblastic cells before plating. (**B**) After 1 d, pseudopodia of cytotrophoblastic cells are making contact with a neighboring cytotrophoblastic cells. (**C**) After 3 d, a large syncytiotrophoblast is observed with a central nuclear mount and the extending cytoplasm.

oxygen levels during this critical stage of development. In addition, insufficient invasion of the uterine wall is implicated in the pregnancy disorder preeclampsia, in which the mother shows signs and symptoms such as hypertension, proteinuria, and edema and which is associated in some cases with fetal intrauterine growth retardation. In contrast to tumoral invasion, this trophoblastic invasion is precisely regulated, confined spatially to the endometrium, the first third of the myometrium, and the associated spiral arterioles and temporally to early pregnancy *(2)*.

The molecular mechanisms that direct cytotrophoblastic cells into one or the other differentiation pathways is the subject of intensive research. In vitro studies have shown that the future of cytotrophoblastic cells depends on the surrounding environment, and several types of regulators have been investigated: cytokines, growth factors, extracellular matrix (ECM), oxygen tension, and hormones. The aim of this review is not to be an exhaustive catalog of all the potential regulators but to describe some aspects of trophoblast differentiation and to analyze the influence of fetoplacental hormonal production on the phenomenon.

Morphologic and Functional Differentiation of Villous Trophoblast

In situ, the villous trophoblastic epithelium has two compartments. The inner compartment (cytotrophoblast) is proliferative and transforms from a continuous to an incomplete cellular layer as gestation advances. The outer compartment faces the intervillous space and is a syncytium continuum: the syncytiotrophoblast. Functional evidence of cytotrophoblastic fusion to form syncytium was convincingly presented by [^{3}H]thymidine labeling studies in human placenta *(3)* and by time-lapse photography analysis of in vitro cultured isolated cytotrophoblastic cells *(4)*. Recently, morphologic aspects of this differentiation pathway have been described in the broader context of continuous trophoblast turnover, including continuous proliferation of cytotrophoblastic stem cells, recruitment of postmitotic cells into syncytiotrophoblast after membrane fusion, progression toward apoptotic cell death, and extrusion of groups of apoptotic nuclei surrounded by syncytial plasma membrane into the maternal circulation as syncytial knots *(5)*.

Because of the variability of the placenta, animal models are not suitable and isolation of villous trophoblastic cells has been used to study villous differentiation. Indeed, in the presence of fetal calf serum (FCS), purified first-trimester and term mononucleated cytotrophoblastic cells migrate, aggregate, and fuse together to form a nonproliferative multinucleated syncytiotrophoblast with pregnancy-specific hormonal production (human chorionic gonadotropin [hCG], human chorionic somatomammotropin [hCS], and estrogens) (Fig. 3). This recapitulates the important activities accomplished by normal cytotrophoblastic cells during in vivo maturation. Interestingly, this process of fusion is associated with a concomitant increase in cellular levels of cyclic adenosine monophosphate (cAMP) *(6)*, required for the synthesis of numerous specific trophoblast proteins, and a decrease in basal Ca^{2+} activity *(7)*. This cell fusion is the key step of the formation of the syncytiotrophoblast, and recently several factors have been found to be involved in the process.

Connexin 43 (Cx43) expression and gap junctional intercellular communication (GJIC) are directly implicated. Indeed, gap junction channels, consisting of proteins called connexins, connect the cytosol of adjacent cells, allowing the exchange of ions and small molecules between the coupled cells. We have demonstrated *in situ* the presence of Cx43 mRNA and of Cx43 protein localized between cytotrophoblastic cells and between cytotrophoblastic cells and syncytiotrophoblast *(8)*. Furthermore, in vitro, using the fluorescence recovery after photobleaching method (gap-FRAP), we have demonstrated the presence of a functional gap junctional intertrophoblastic communication during trophoblast fusion *(9)*. This GJIC can be inhibited by heptanol (a junctional uncoupler), leading to a dramatic decrease in syncytiotrophoblast formation and of hormonal production. Recently,

using an antisense strategy, we demonstrated that the treatment of cytotrophoblastic cells by a Cx43 antisense induced a clear decrease in trophoblastic cell fusion and functional differentiation *(10)*. Thus, the ability of villous cytotrophoblast to develop a transient GJIC is a prerequisite for the formation of syncytiotrophoblast.

Another membrane event implicated in fusion is the phosphatidylserine flip. Phosphatidylserine, a phospholipid normally confined to the inner layer of the plasma membrane, but prior to fusion, this translocates to the outer layer and facilitates intermembrane fusion. Adler et al. *(11)* have shown that incubation with an antiphosphatidylserine antibody inhibited the forskolin-induced syncytial fusion of choriocarcinoma cells. According to Huppertz et al. *(12)*, this phosphatidylserine flip is a consequence of activation of initiator caspase (e.g., caspase 8) leading to the concept that the molecular machinery of early apoptosis is involved in the fusion process.

Implication of endogenous retrovirus genome in trophoblastic fusion has been suggested *(13,14)*, and recently a role of endogenous retroviral envelope glycoprotein encoded by the HERV-W (syncytin) was demonstrated *(15)*. Syncytin is expressed in the syncytiotrophoblast and an antisyncytin antiserum can inhibit fusion of a human trophoblastic cell line expressing endogenous syncytin.

In addition, trophoblastic fusion probably involves other molecules such as ADAM (a disintegrin and metalloproteinase domain). Indeed, ADAM 12 (meltrin α) is involved in the fusion process of myoblast, and recent microarray analysis demonstrated that ADAM 12 was one of the most upregulated genes during in vitro trophoblast differentiation *(16)*.

The assessment of morphologic differentiation is not obvious. The use of phase contrast microscopy, staining techniques, or electron microscopy is not sufficient, because it is difficult to appreciate whether a structure is an aggregate of cells or a truly multinucleated syncytium. Syncytium formation has also been followed by the distribution of desmoplakin immunostaining associated with nuclear staining *(17, 18)*. Indeed, the staining of desmoplakin is localized at the intercellular boundaries in aggregated cells and disappears with syncytium formation. The use of the gap-FRAP method has allowed us to differentiate between truly multinucleated syncytium and noncommunicating aggregated cells. This method is currently used to monitor morphologic trophoblast differentiation *(19)*.

The hallmark of differentiation into syncytium is not only cell fusion but also temporal expression of specific genes leading to the acquisition of specific hormonal production. In vivo, numerous growth factors and steroid and peptide hormones are produced by the syncytiotrophoblast, and some polypeptide hormones are specific to human pregnancy such as hCG, hCS, and human placental growth hormone. Therefore, in vitro functional differentiation has been mainly assessed by the measurement of hormonal production in the culture medium of trophoblast (hCG, hCS) *(4,16, 20)* but also by means of various methods (Northern blotting, reverse transcriptase polymerase chain reaction [RT-PCR], real-time PCR) measuring the levels of expression of specific genes (hCG, hCS, HERV-W) *(21,22)*.

Recently, molecular biology technologies have allowed researchers to explore the fascinating topic of the genetic control of trophoblast development. Using subtractive cDNA library of in vitro differentiating trophoblast, a remarkable shift in the spectrum of gene expression was demonstrated. While expression of protooncogenes c-*myc*, c-*fos*, and c-*jun* and histone 2A decreased, a spontaneous increase in expected syncytial genes (αhCG, pregnancy-specific 1β glycoprotein, 3β-hydroxysteroid dehydrogenase, plasminogen activator inhibitor type I) was observed. Furthermore, an increase in other genes was also detected: for instance, keratin 19, calreticulin, heat-shock protein 27, serum and glucocorticoid-regulated kinase, superoxide dismutase 2 *(23)*. Using cytotrophoblastic cells cultured in the presence of human maternal serum, the critical role for transcription factors AP-2 and NF-IL-6 was demonstrated *(24)*. Recently, cDNA microarray technology has allowed researchers to observe multiple kinetic patterns of accumulation and decline in gene transcript levels and to implicate genes not previously known to play roles in villous differentiation (e.g., prostate differentiation factor, carcinoembryonic antigen gene family 6, cytochrome P-450 XIA, ADAM 12) *(16)*. Of the 6918 genes analyzed, 141 genes were induced (e.g., αhCG, βhCG, hCS, syndecan 1, fibronectin 1) and 256 were downregulated (e.g., superoxide dismutase 2, insulin-like growth factor binding protein 10 [IGFBP-10], integrin-α2) by more than twofold. Classification of genes into functional categories (cell and tissue structure, cell cycle and apoptosis, intercellular communication, metabolism, and regulation) has allowed us to hypothesize that the simultaneous activation, repression, or degradation of mRNA from within a given functional group is necessary to accomplish the marked cell morphology changes that occur during differentiation.

Factors Regulating Villous Trophoblast Differentiation

Both functional and morphologic approaches are required to convincingly prove the formation of mature phenotype. Hormonal characteristics could represent simply secretagogue or induction effects of the in vitro added factor. Indeed, many hormones, growth factors, cytokines, peptides (leukemia inhibitory factor [LIF], fibroblast growth factor, inhibin, activin, gonadotropin-releasing hormone), and retinoid receptor ligands are known to affect trophoblastic hormonal secretion *(25–27)*. Therefore, few factors demonstrate the full range of biochemical and phenotypic effects and thus qualify as regulators of differentiation. It must be pointed out that in vitro, the presence of serum is required for complete villous differentiation. Isolated cytotrophoblastic cells maintained in serum-free conditions can-

Table 1
Main Factors Modulating
In Vitro the Formation of Syncytiotrophoblast

Stimulating action/reference	Inhibiting action/reference
EGF *(29,31)*	TGF-β1 *(36,37)*
hCG *(19,46)*	LIF *(38)*
cAMP *(9,18)*	Hypoxia *(40,41)*
GM-CSF *(32)*	Endothelin *(7)*
Macrophages and macrophage-conditioned media *(33,34)*	15ΔPGJ2 *(39)*
Dexamethasone *(63)*	
Estradiol *(57)*	

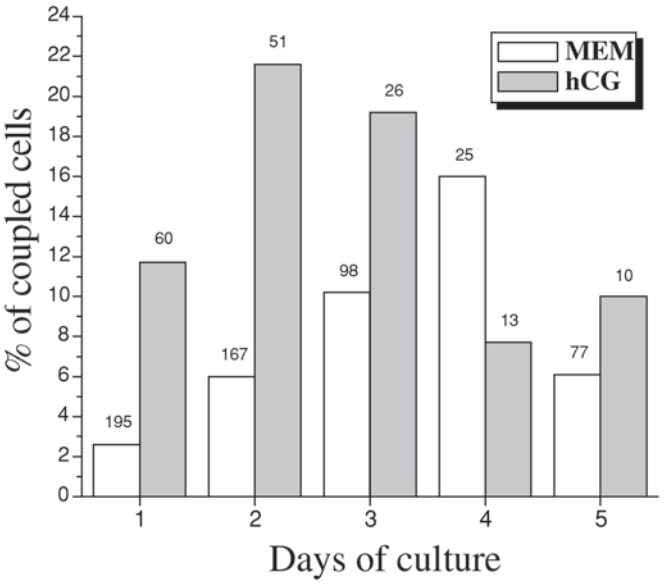

Fig. 4. Stimulation by hCG of villous trophoblast differentiation. Evolution of GJIC vs time in control conditions and under exogenous hCG is shown. In the presence of 500 mIU/mL of hCG in the culture medium, the percentage of coupled cells was increased at all stages of culture, and the highest proportion of coupled cells was observed after 2 d of culture vs 4 d in control medium. Numbers of intercellular contact analyzed by means of gap-FRAP technique are indicated on top of the bars. MEM, minimum essential medium.

not aggregate or fuse *(28)* or display a small degree of spontaneous differentiation *(29)*. Furthermore, term cytotrophoblastic cells cultured in human pregnancy sera progress to a more advanced stage of differentiation than those cultured in FCS *(20,30)*. Therefore, the action of soluble factors present in the serum has been investigated in many centers (Table 1). Regarding the nonhormonal factors, epidermal growth factor (EGF) *(29,31)*, granulocyte macrophage colony-stimulating factor (GM-CSF) *(32)*, the presence of macrophage *(33)* or of macrophage-conditioned media *(34)*, and vascular endothelial growth factors *(35)* induce differentiation, whereas transforming growth factor-β1 (TGF-β1) *(36,37)*, endothelin *(7)*, LIF *(38)*, 15-deoxy-Δ-prostaglandinJ2 (15ΔPGJ2) *(39)*, and hypoxia *(40,41)* impair the process. Interestingly, the overexpression of copper zinc dismutase impairs trophoblastic fusion, supporting the implication of the cellular oxidative status in differentiation of trophoblast *(22)*.

Influence of Fetoplacental Hormonal Production

Human Chorionic Gonadotropin

hCG, a heterodimeric glycoprotein, is composed of α- and β-subunits. The α-subunit, common to all glycoprotein hormones, is a polypeptide of 92 amino acids with two N-linked oligosaccharides and is encoded by a single gene on chromosome 6q21.1-23. The specific β-subunit is a polypeptide of 145 amino acids with two N-linked and four O-linked amino acids encoded by a cluster of genes: six CGβ genes, one CGβ pseudogene, and one LHβ gene, on chromosome 19q13.3. The hCG subunits are already transcribed in eight-cell embryos, and the trophoblast is able to secrete the hormone before hCG becomes measurable in the maternal serum near time of implantation, i.e., 8–10 d after ovulation *(42,43)*. The concentration of hCG in serum increases exponentially, reaching a peak at approx 6 wk after ovulation; it subsequently declines and reaches a nadir at the beginning of the second trimester. During the first 6 wk of pregnancy, hCG is essential for the maintenance of pregnancy through its luteotrophic effect, extending the corpus luteum progestational function.

In addition to this major role, hCG has been implicated as an intracrine, autocrine, paracrine, and endocrine regulator of human fetoplacental function and as a regulator in various nongonadal tissues *(44,45)*. Shi et al. *(46)* demonstrated, for the first time, that hCG is able to enhance the differentiation of isolated cytotrophoblastic cells into syncytiotrophoblast. Furthermore, the differentiated trophoblast from term placenta expresses full-length hCG/luteinizing hormone (LH) receptors, and hCG is able to self-regulate the mRNA levels of its own subunits in a biphasic manner: a stimulation at moderate concentration and an inhibition at high concentration *(47)*. The stimulating effect of hCG on trophoblast differentiation was confirmed by the study of GJIC (Fig. 4). Using gap-FRAP, it was demonstrated that hCG specifically enhanced, via a cAMP–protein kinase pathway, the GJIC and subsequent differentiation while the presence of a polyclonal hCG antibody decreased basal GJIC as well as the response to exogenous hCG *(19)*. Consequently, a direct role for cAMP-dependent protein kinase in stimulating trophoblastic fusion was demonstrated *(9,18)*. Because trophoblast differentiation is stimulated by hCG and because hCG production is regulated by numerous factors, the effect of a precise factor could be the result of hCG production

and/or a self-sufficient effect. Furthermore, it is possible that the control of hCG production by endocrine agents is indirect, reflecting the factor's action on trophoblast differentiation.

Steroid Hormones

During human pregnancy, progesterone (P_4) and estradiol (E_2) are increasingly produced by the fetoplacental unit, in an unchanged ratio until term. This production is very intense and appears to have major roles in maintaining the uterus in a quiescent state to ensure continued pregnancy to term. For the synthesis of progesterone, the syncytiotrophoblast utilizes maternal lipoprotein-carried cholesterol (low-density lipoprotein pathway) as a major substrate *(48)* via various lipoprotein receptors *(49,50)*. The synthesis of P_4 also involves the activity of cytochrome P450 cholesterol side chain cleavage enzyme (P450 scc) and of 3β-hydroxysteroid dehydrogenase. In contrast to other steroidogenic organs, the human placenta does not express cytochrome P450 17α-hydroxylase and is unable to utilize steroids such as pregnenolone or progesterone as substrate for the production of androgen for estrogen synthesis. Thus, placental estrogen synthesis depends on a source of androgen precursor (dehydroepiandrosterone sulfate [DHEA-S]) produced from the maternal adrenal gland (50%) and from the expanded zone from the fetal adrenal gland (50%). Fetal adrenal DHEA-S may also undergo 16α-hydroxylation in the fetal liver, leading to formation of 16α- DHEA-S, the androgen precursor for estriol (E_3). DHEA and DHEA-S diffuse from the fetal blood to the syncytiotrophoblast, are hydrolyzed by a steroid sulfatase, and then are aromatized by the syncytiotrophoblastic cytochrome P450 aromatase to form estrogen.

Numerous previous studies have shown that E_2 can modulate placental hormonal production (for review, see ref. *25*). Furthermore, in cultured syncytiotrophoblast, lipoprotein uptake and P450 scc activity were enhanced by E_2 *(51)*. Estradiol receptor mRNA was demonstrated by RT-PCR in villous trophoblast, and the estradiol receptor protein was localized in the nuclei of cultured human syncytiotrophoblast *(52)*. All of these data clearly demonstrate that the syncytiotrophoblast is an estrogen-sensitive element. The action of P_4 on trophoblast is more doubtful; various effects are perhaps due to a glucocorticoid-like action of the steroid. Progesterone could increase, decrease, or have no effect on hCG release (for reviews, see refs. *25* and *53*), and it has been demonstrated that P_4 regulates osteopontin expression *(54)*. Furthermore, the presence of P_4 receptor (PR) in trophoblast has been a subject of debate. Using Western blotting analysis, Karalis et al. *(55)* found a glucocorticoid receptor (GR) but not a PR in villous trophoblast in culture. On the other hand, using immunocytochemistry, binding, and RT-PCR studies, a PR was demonstrated *(56)*.

We have demonstrated that E_2 stimulated gap junctional coupling, Cx43 expression, hCG and hCS production, and formation of the syncytiotrophoblast. The E_2 effect was dose dependent and specific because it was inhibited by tamoxifen. In the presence of an efficient concentration of hCG antibody, E_2 still stimulated hCS expression, suggesting a self-sufficient effect of the steroid *(57)*. Physiologic concentrations of P_4 were ineffective in modulating trophoblast differentiation. These results are consistent with a recent in vivo study in the baboon in which administration of androstenedione elevated serum E_2 threefold and increased the ratio of syncytiotrophoblast/cytotrophoblast volumes on d 60 by 50% of that normally observed on d 100 *(58)*.

Cortisol is another steroid largely produced during gestation. Maternal free cortisol levels increase during pregnancy and near parturition. Furthermore, the progressive maturation of fetal hypothalamic pituitary axis induces an increased production of cortisol by the fetal adrenal. GR has been identified in trophoblastic cells *(55)*, and glucocorticoid treatment profoundly affected placental metabolism; hormonal production; and expression of several genes including hCG *(59)*, ECM *(60)*, integrins *(61)*, and corticotrophin-releasing hormone (CRH) *(62)*. Dexamethasone specifically increased gap junctional coupling of trophoblast, Cx43 expression, hormonal production, and syncytiotrophoblast formation. Furthermore, an excess of hCG antibody with dexamethasone did not significantly affect the stimulatory effect of the glucocorticoid, suggesting a sufficient effect of the steroid *(63)*.

Factors Regulating Extravillous Differentiation

As previously mentioned, placental development is dependent on trophoblast invasion of the uterine endometrium and blood vessels. Immunohistochemical studies on placental bed biopsies have revealed the patterns of expression of proto-oncogenes, HLA-G, cell adhesion molecules, and connexins *(8,64)*, and an "integrin switching" was demonstrated during trophoblast invasion *(1,65,66)*. Recently, expression of factors that may influence cell migration/invasion of extravillous cytotrophoblastic cells has been shown: basic helix-loop transcription factors, inhibitor of DNA-binding protein 2 *(67)*, NEUROD 1, and NEUROD 2 *(68)*. During recent years, culture methods have led to the discovery of factors affecting extravillous cytotrophoblastic cells invasion. Indeed, isolated first-trimester cytotrophoblastic cells exhibit an ability to migrate, aggregate, and penetrate a barrier of ECM such as Matrigel®. This activity depends on integrin-ECM interactions as well as matrix metalloproteinase (MMP) activity *(69–71)*. Thus, the components of endometrial ECM are potent regulators of trophoblastic invasion. Local production of factors by decidual tissue could also be implicated such as IGFBP, tumor necrosis factor, interleukin, inhibitor of metalloprotease, or members of the TGF-β family *(71,72)*. Recently, it was reported that peroxisome proliferator activated receptor gamma (PPARγ)/retinoid X receptor alpha (RXRα) ligands modulate trophoblast invasion. Both synthetic (rosiglitazone) and natural

(15ΔPGJ2 *[12,14]*) PPAR agonists inhibit extravillous cytotrophoblast invasion in a concentration-dependent manner and synergize with pan-RXR agonists *(73)*.

An increasing body of evidence suggests that oxygen is a key regulator of extravillous differentiation. Because of the presence of plugs of cytotrophoblasts, which obliterate the tips of the uteroplacental arteries, the environment of early placenta is hypoxic *(74)*. The plugs are subsequently displaced and blood flow begins at approx 11 wk of pregnancy. As a result, partial pressure of oxygen increases from ~20 mmHg at 8 wk of gestation to 55 mmHg at 10–12 wk of gestation *(74)*. Using placental villous explants cultured on three-dimensional ECM, it has been shown that trophoblastic cells are sensitive to oxygen; first-trimester villi explanted in 20% oxygen form new columns at their tips *(75)*, whereas low-oxygen tension (2 to 3%), comparable with that present during early gestation, maintains trophoblast in a proliferative noninvasive phenotype *(76)*. Furthermore, it was demonstrated that the expression of hypoxia-inducible factor-1α (HIF-1α), which is known to activate the transcription of genes in response to hypoxia, has a developmental profile. It has been suggested that the oxygen-regulated early events of the villous trophoblast differentiation were mediated by HIF-1α through TGF-β3 *(77)*.

Up to now, very few studies have investigated the influence of hormone production on trophoblastic invasion. Because the phenomenon is known to be regulated by a variety of proteases, the influence of hormones was tested on the in vitro production of MMP. It was shown that P_4 downregulates the production of MMP-9 in first trimester cytotrophoblastic cells *(78)* and that leptin increases the secretion of MMP-2 and enhances the activity of MMP-9 *(79,80)*.

Like the villous trophoblast, the invasive extravillous cytotrophoblast, and particularly the endovascular trophoblast, express hCG/LH receptors *(81)*. Yagel et al. *(82)* have shown that hCG inhibits invasion of first-trimester cytotrophoblastic cells in a dose-dependent manner by preventing the initiation of the collagenic cascade. By contrast, Zygmunt et al. *(83)* observed that hCG increased the Matrigel invasion of a choriocarcinoma cell line (JEG-3). Further studies are needed to appreciate the real action of hCG. On the other hand, extravillous cytotrophoblastic cells express hCS *(21,76)*, suggesting a possible autocrine/paracrine role for this pregnancy-specific hormone in trophoblastic invasion.

Conclusion

During recent years, numerous in vitro studies have demonstrated that the genetic differentiation program of the cytotrophoblastic stem cell could be modulated by the balanced actions of ECMs and various soluble factors that become operative at temporally discrete times in gestation. Because these regulating factors have been tested in vitro, it is evident that extrapolating these results to the in vivo situation cannot be done easily. Nevertheless, steroid hormones and hCG are secreted in large amounts and are thus present at the fetomaternal interface in a good position to play a physiologic role in the trophoblast differentiation. We hypothesized that hCG may be particularly important very early in pregnancy, when production of this glycoprotein is maximal, for villous trophoblast differentiation and perhaps extravillous trophoblast invasion. During this period, cytochrome P-450 aromatase is not expressed in sufficiently high levels by the syncytiotrophoblast to form significant amounts of estrogen. Estrogen is increasingly produced by the fetoplacental unit, and during late pregnancy cortisol is secreted from the fetal adrenal. According to Majzoub and Karalis *(84)*, fetal cortisol paradoxically stimulates the placental CRH gene expression. This trophoblastic CRH, via the umbilical vein, promotes fetal pituitary-adrenocorticotropin secretion and adrenal steroidogenesis, thus increasing cortisol secretion and DHEA synthesis. Therefore, estrogen and glucocorticoid may be implicated in the final stages of gestation. Despite the large body of recent data, a complete understanding of in vivo regulation of trophoblast differentiation and invasion is still lacking.

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