Human chorionic gonadotropin stimulates matrix metalloproteinases-2 and -9 in cytotrophoblastic cells and decreases tissue inhibitor of metalloproteinases-1, -2, and -3 in decidualized endometrial stromal cells

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Objective: To investigate the influence of hCG on trophoblastic matrix metalloproteinases (MMPs) -2 and -9 as well as endometrial tissue inhibitor of metalloproteinases (TIMPs) -1, -2, and -3.

Design: In vitro experiment.

Setting: Research laboratory at a university medical center.

Patient(s): Women undergoing legal abortions and premenopausal women undergoing hysterectomy for benign reasons.

Intervention(s): Human first trimester cytotrophoblasts and decidualized endometrial stromal cells were incubated with recombinant hCG.

Main Outcome Measure(s): Trophoblastic MMP-2 and -9 were analyzed by enzyme-linked immunosorbent assay (ELISA) and zymography, and endometrial TIMP-1, -2, and -3 were measured by real time reverse transcriptase–polymerase chain reaction and ELISA.

Result(s): HCG increases the secretion of MMP-2 and -9 in cytotrophoblasts dose dependently. This effect occurs after 4 hours of incubation and becomes less pronounced after 24 hours. In contrast, TIMP-1, -2, and -3 are significantly reduced by hCG in endometrial stromal cells in a time- and dose-dependent manner.

Conclusion(s): These results suggest a regulatory role of hCG on the MMP/TIMP system at the implantation site. By increasing trophoblastic MMP secretion and reducing endometrial TIMP expression, hCG may be an important tool for the invading embryo to regulate the depth of its implantation. (Fertil Steril® 2008;90:1390–5. ©2008 by American Society for Reproductive Medicine.)

Key Words: Trophoblast, endometrium, implantation, hCG, MMP, TIMP

Invasion of trophoblast into the maternal endometrial stroma is essential for successful implantation and placentation (1–3). This highly regulated process is mediated by the spatiotemporal expression of matrix metalloproteinases (MMPs), which characterize the transiently invasive property of trophoblastic cells (4, 5). MMP-2 and -9 are considered the two essential proteases in the extracellular matrix remodeling that takes place during trophoblastic invasion (6–8). In contrast to tumor invasion, trophoblastic invasion into the maternal endometrium is a highly coordinated and controlled process, requiring a subtle interaction between trophoblast and endometrium (9). Several cytokines such as leukemia inhibitory factor (LIF), interleukin (IL)-1β , IL-6, insulin-like growth factor binding protein (IGFBP)-1, and tumor necrosis factor (TNF)-α are potential regulators of trophoblastic invasion (10–12). The activity of MMPs is directly inhibited by specific tissue inhibitors of metalloproteinases (TIMPs) (13). TIMP-1 and -2 bind in a 1:1 ratio to the active sites of MMPs. TIMP-1 preferentially binds to MMP-9, whereas TIMP-2 has a high affinity for MMP-2 (5). TIMP-3 is not so well characterized but does regulate MMP activity (13, 14). TIMP-1, -2, and -3 are expressed in the human endometrium, where they show characteristic cyclical changes of expression (5). Members of the TIMP family expressed in the endometrium during implantation represent a substantial barrier to invasion (15).

One of the first known hormonal signals of the embryo during implantation is hCG (16). In addition to its classical role in rescuing the maternal corpus luteum at the onset of pregnancy, the early appearance of hCG suggests a paracrine role of this hormone during the first steps of implantation. In agreement with these considerations, we and other investigators have been able to show that hCG has paracrine effects on the trophoblast and the maternal endometrium and regulates angiogenesis in the developing placenta (17–23). Both human endometrial cells and first trimester trophoblast have been shown to express functional hCG/LH receptors (24, 25). Using the intrauterine microdialysis technique, we were able to show an influence of locally applied hCG on

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endometrial PRL, LIF, vascular endothelial growth factor, MMP-9, and macrophage colony stimulating factor during the luteal phase in the human female in vivo (18). In addition, we observed an inhibitory influence of hCG on endometrial IGFBP-1 (21, 26), a factor that has been shown to directly regulate cytotrophoblast (CTB) invasion (27, 28). Moreover, we were able to show that hCG in vitro stimulates the invasive potential of the CTB cell line JEG-3, which suggests an autocrine control of the invasion process (29).

On the basis of these observations, we examined the effects of hCG on the MMP/TIMP-system at the fetomaternal interface during early implantation. As a model, we used primary cultures of human first trimester CTB as well as human decidualized endometrial stromal cells (ESCs) to test the influence of recombinant hCG on trophoblastic MMP-2 and -9 and endometrial TIMP-1, -2, and -3, the most important members of the MMP/TIMP family at the implantation site.

MATERIALS AND METHODS

Preparation, Culture, and Treatment of Human CTBs

CTBs were isolated, purified, characterized, and cultured as described elsewhere (30). Briefly, trophoblastic tissue that was obtained with written informed consent from patients undergoing legal abortions (7–12 weeks of gestation) was digested by trypsin, and the CTBs were separated from blood cells and syncytiotrophoblast on a discontinuous Percoll gradient. Contaminating leukocytes were removed by immunopurification with an antibody to CD45 (Dako Diagnostics, Zug, Switzerland) coupled to magnetic particles (Dyna Beads, Dynal, Geneva, Switzerland). The CTBs were cultured overnight in Dulbecco's minimal essential medium (DMEM) containing 2 mM L-glutamine, 4.2 mM magnesium sulphate, 2.5 mM HEPES, 1% gentamycin, 1% amphotericin B, 100 μg/mL streptomycin, and 100 μg/mL penicillin in the presence of 10% fetal bovine serum (FBS). The next morning, the medium was changed to serum-free DMEM, and the cells were incubated in the presence or absence of increasing concentrations (5–30 IU/mL) of recombinant hCG (Sigma, Taufkirchen, Germany). The supernatants were collected after 4 and 24 hours of incubation and stored at -20°C until assayed.

Preparation, Decidualization, and Treatment of Human ESCs

After approval by the local ethics board, endometrial tissue was obtained with informed consent from premenopausal women undergoing hysterectomy for benign reasons. All the patients had regular menstrual cycles and were considered to be healthy except for the uterine leiomyoma. Tissue samples were washed with phosphate-buffered saline (PBS) twice, minced in small pieces, and digested for 1 hour at 37°C in 0.5% collagenase (200 IU/mg) in DMEM/F-12 medium without phenol red. Separation of ESCs was performed by filtration through a 180-μm nylon membrane followed by a 40-μm nylon sieve. The ESCs that passed the 40-μm sieve were thoroughly washed with PBS, seeded in 75-cm^2 culture flasks, and incubated in DMEM/F-12 without phenol red containing 10% charcoal-stripped FBS and 1% gentamycin. The purity of ESC cultures was tested by flow-cytometric analysis of intracellular vimentin expression after standard procedures.

After reaching 80% of confluence, cells were detached with trypsin and seeded in 24-well culture plates at a density of 1×10^5 cells/well to perform experiments in quadruplicates. For all experiments, ESCs were used from a single separation and passaged only once. Decidualization in vitro was induced by incubating the cells in culture medium containing 30 nM 17β-estradiol and 1 μM P (both from Sigma) for 9 days. Decidualization was demonstrated by measuring a significant increase of IGFBP-1 and PRL mRNA and protein as described recently (21). Decidualized ESCs were incubated in the presence or absence of increasing concentrations (0.01–100 IU/mL) of recombinant (r-) hCG (Sigma). The supernatants were collected after 24 and 48 hours of incubation and stored at -20°C until assayed. Cells were detached with trypsin and used for the extraction of total RNA.

Cell Viability Assay

To exclude any influence of hCG on the viability of ESCs, we performed the following assay: ESCs were plated in 96-well plates at 1×10^4 cells/well and allowed to reach confluence. Decidualization and incubation with various concentrations of r-hCG were performed as described above in replicates of six. Cell viability was determined by adding 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT; Sigma) to measure metabolic activity of the cells. After an incubation period of 6 hours in the presence of MTT, 10% sodium dodecyl sulfate (Roth, Karlsruhe, Germany) was added, and the absorbance (A_{550}) was measured after incubating overnight at 37°C.

Enzyme-linked Immunosorbent Assays (ELISAs) for MMP-2 and -9

MMP-2 and -9 in cell culture supernatants of CTB were measured using our own ELISAs as described and validated elsewhere (11).

Zymography

The gelatinolytic activity of MMP-2 and MMP-9 was measured by zymography using bovine gelatine as the substrate as described elsewhere by our group (31). Zymograms were scanned in an Apple Onescanner, and the surface of the digestion bands was measured by Kodak digital science one-dimensional image analysis software (Eastman Kodak, Rochester, NY) on a Macintosh iBook computer (Apple, Cupertino, CA). The surface area of the digestion band was expressed as a percent of an internal activity standard run on each zymogram. The activity standard consisted of a mixture of supernatants from fibroblasts and U937 cell cultures (0.75 and 0.25 mL, respectively). Each individual result was then