HCG increases trophoblast migration in vitro via the insulin-like growth factor-II/manose-6 phosphate receptor

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We have previously shown that both HCG and insulin-like growth factor-II (IGF-II) stimulate trophoblastic invasion. Furthermore, the invasion-promoting function of IGF-II resulted from IGF-II manose 6-phosphate receptor (IGF-II/M6PR) activation. Since HCG and IGF-II did not have an additive effect on cell migration of extravillous trophoblast (EVT) cell line, HTR-8 SVneo, we hypothesized that HCG actions are mediated via alterations in the expression and/or function of IGF-II axis. HCG treatment (50-50 000 mU/ml) of the HTR-8/SVneo cells did not alter the expression of either insulin-like growth factor-I or IGF-II mRNA or peptide synthesis, but caused (i) an increase in the 125I-IGF-II binding to EVT cells, and (ii) an increase in the externalization rate of the IGF-II binding sites without affecting their internalization. This effect was due to the increase in the number of IGF-II binding sites in the plasma membrane without any change in the IGF-II binding affinity. Although HCG did not influence the abundance of IGF-II/M6PR mRNA or protein, anti-IGF-II/M6PR antibody decreased HCG-induced migration of EVT, supporting the hypothesis that HCG might stimulate EVT migration by increasing IGF-II binding to the plasma membrane and subsequently by increasing the IGF-II effect probably mediated via the IGF-II/M6PR.

Key words: HCG/insulin-like growth factor-II/IGF-II receptor/trophoblast migration/receptor recycling

Introduction

A physiologically developed and functioning placenta is essential for the normal growth and development of the fetus. A poorly developed placenta can lead to preterm delivery, pre-eclampsia and other pregnancy disorders, and is therefore associated with higher perinatal morbidity and mortality (Khong et al., 1986; Hill and Han, 1997). The development of a haemochorial placenta involves invasion of extravillous trophoblast (EVT) into the uterine wall, and remodelling of the utero-placental vessels (Enders, 1968). Trophoblast invasion of the uterus is stringently controlled. An interaction of the trophoblast with growth factors, growth factor binding proteins and components of the extracellular matrix via cell surface receptors regulates its proliferation, the migration and invasion (Lala and Hamilton, 1996; Zygmunt et al., 1998a; Bischof et al., 2001). These factors may be both autocrine, as well as paracrine in their action (Graham and Lala, 1991).

We have reported that HCG, in addition to its function in the regulation of steroidogenesis, stimulates the migration and invasion of cytotrophoblastic JEG-3 cells in vitro (Zygmunt et al., 1998b). HCG stimulates its target cells by binding to specific receptors, which then leads to the activation of both adenyate cyclase and phospholipase C systems (Dufau, 1998). The mechanism of action of HCG on trophoblast invasion is still not fully understood.

We have also shown, that insulin-like growth factor-II (IGF-II) is expressed abundantly in the EVT in situ (Han et al., 1996), IGF-II stimulates EVT invasion in vitro (Hamilton et al., 1998) as well as IGF-II promoting angiogenesis (Herr et al., 2003). The intracellular signal transduction cascade of the IGF-II induced angiogenesis is transmitted via the IGF-II/M6PR with protein kinase C and G protein being involved (Herr et al., 2003). Recent studies in our laboratory have revealed that IGF-II stimulation of EVT migration is mediated by IGF-II/M6PR involving the inhibition of adenylate cyclase and stimulation of mitogen-activated protein kinase (MAPK) kinase pathway (McKinnon et al., 2001).

IGF-II/M6PR is a multifunctional transmembrane glycoprotein, which serves a dual function in certain cell types; in the transport of proteins containing a manose 6-phosphate (M6P) moiety to the lysosomes and in the regulation of embryonic growth, as IGF2/M6P gene disruption is generally lethal and leads to fetal overgrowth (Wang et al., 1994). IGF-II/M6PR mediated chemotaxis of endothelial cells occurs through a G protein-coupled pathway involving the activation of MAPK as shown for proliferin (Groskopf et al., 1997). IGF-II/M6PR is primarily localized in the intracellular compartment, and only 10% of the receptor is present on the cell surface (Kornfeld, 1992). The recycling pathways of IGF-II/M6PR are regulated by different signals, one of which is via the...
the phospholipase C/inositol-3-phosphate system (Kundra and Kornfeld, 1998).

Since IGF-II stimulation of trophoblast invasion is due to its migration-promoting actions mediated by IGF-II/M6PR (McKinnon et al., 2001) and IGF-II is synthesized by the trophoblast in situ (Han et al., 1996), we hypothesized that the action of HCG is indirectly mediated via the local expression of either IGF-II or IGF-II/M6PR in the EVT. We utilized an in vitro culture system of EVT, HTR-8/SVneo, to address this hypothesis.

Materials and methods

Cell line

HTR-8/SVneo (SV40 Tag immortalized human first trimester EVT cell line) was maintained in Roswell Park Memorial Institute 1640 (RPMI-1640) medium containing 10% fetal calf serum (FCS) and 1% penicillin–streptomycin in 5% CO₂ and 100% humidity at 37°C. The characterization of this cell line was reported previously (Graham et al., 1993). This cell line shares all the phenotypic and functional properties of the EVT in situ as well as non-transformed first trimester EVT cells derived from chorionic villous explants (Irving et al., 1995).

Migration assay

Millipore filters (8 µm pore size) were used as a membrane barrier in a Boyden chamber (Transwell, Costar, Cambridge, MA, USA; McKinnon et al., 1993; Herr et al., 2003) to test the migratory properties of the HTR-8/SVneo cell line. Different HCG concentrations (5–50,000 mU/ml), IGF-II (100 ng/ml), monoclonal anti-hIGF-IR blocking antibody (1:100–1:1000 dilution, R&D, Minneapolis, MN, USA) and polyclonal anti-IGF-II/M6PR blocking antisemur [1:200–1:25 dilution, a gift from Dr W.Sly, St Louis University, MO, and well-characterized previously (Nolan et al., 1987)], were used at the same concentrations as negative controls. Five wells were used for each concentration of HCG. After incubation (48 h) and fixation, cells which did not migrate through the millipore membrane were removed from the upper surface of the membrane with a cotton swab, and the cells on the underside of the membrane were stained with haematoxylin and eosin. Filters were examined microscopically, and the number of cells in eight microscope fields (magnification 200 ×) of each filter was counted, and the mean and SD were determined. The experiment was repeated at least five times.

IGF-I, IGF-II and IGF-II/M6PR mRNA, peptide and protein levels

Following a 24 h incubation in serum-free medium and a 24 h incubation with different concentrations of HCG (5–50,000 mU/ml), total mRNA was extracted from HTR-8/SVneo cells by the guanidine isothiocyanate denaturation and hot phenol extraction method (Chomczynski and Sacchi, 1987). IGF-I, IGF-II and IGF-II/M6PR mRNA were determined using northern blotting as described previously (Han et al., 1987). The insulin-like growth factor-I (IGF-I) and IGF-II cDNA probes, used for northern blotting, were kindly provided by Dr M.Jansen (University of Utrecht, The Netherlands).

The human IGF-II/M6PR cDNA (353 bp) was generated by RT–PCR of human placental total RNA using a 5′ sense primer corresponding to bases 1193 to 1170 (5′-CCTCCGCTCTGGCAAGTG-3′) and a 3′ antisense primer corresponding to bases 1193 to 1170 (5′-CCTCCGCTCTGGCAAGTG-3′) of published human IGF-II R cDNA (Morgan et al., 1987). The PCR product was subcloned into pGEM-T cloning vector (Promega Corp., Madison, WI, USA), and the sequence was confirmed by automated DNA sequencing.

IGF-I and IGF-II concentrations in the conditioned media were determined by radioimmunoassay following the separation of IGF from insulin-like growth factor binding protein (IGFBP) by acid ethanol extraction and sephadex G50 chromatography, as described previously (Hogg et al., 1993). Cellular IGF-II/M6PR protein abundance was determined by immunoblotting of cellular extracts with a polyclonal anti-bovine IGF-II/M6PR antiserum, which cross-reacts with the human IGF-II/M6PR (a gift from Dr S.Kornfeld, St Louis, MO, USA) and separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions (Messner et al., 1989).

125I IGF-II binding and cross-linking

HTR-8/SVneo cells were plated in 24-well tissue culture plates (Costar, Cambridge, MA) at 2 × 10⁵ cells/well. The cells were cultured for 24 h and changed to serum-free medium for 12 h. After washing with 0.1 mM HEPES buffer with bovine serum albumin (BSA) 10 mg/ml, pH 8.0, HCG treated (5000 mU/ml) and untreated cells were incubated with various concentrations (0.5 × 10⁻¹¹ to 5 × 10⁻⁸ M) of 125I-IGF-II in HEPES buffer, either alone (total binding) or in the presence of unlabelled IGF-II (3000 mU/ml for non-specific binding) at 4°C for 3 h. In the time-course binding studies, labelled IGF-II (5 × 10⁻⁷ M) was added to each well and incubated for varying lengths of time (15 min to 24 h) showing saturation of the binding after 6 h. After two washes with ice-cold phosphate-buffered saline (PBS), cells were solubilized with 0.1% SDS and 0.1 N sodium hydroxide, and the radioactivity was counted in a gamma counter. Specific binding was measured by subtracting non-specific binding from total binding. In the competition studies, 125I-IGF-II binding was determined in the presence of graded concentrations of unlabelled IGF-II (0–3000 ng/ml). All experiments were repeated four times.

For affinity cross-linking, HTR-8/SVneo cells were sub-plated at a cell density of 1 × 10⁶ cells/well in 6-well multi-well dishes in RPMI-1640 medium containing 10% FCS. The cells were grown to 80–90% confluence (48–72 h) and the medium was changed to serum-free RPMI-1640 (containing 10–5000 mU/ml HCG) for another 12 h. The cells were washed twice with 0.1 M HEPES buffer containing BSA 10 mg/ml (pH 8.0), and incubated with 125I-IGF-II (1 × 10⁶ cpm/dish) in the same HEPES buffer at 4°C for 16–18 h. The incubation solution was then aspirated, and the cultures were washed three times with cold HEPES buffer (pH 7.4) without BSA, to remove the unbound radiolabelled IGF-II. 125I-IGF-II, which was bound to the surface receptor protein, was affinity cross-linked with 0.1 mM disuccinimidyl suberate for 30 min at room temperature. The reaction was then stopped by adding 1 ml of 10 mM Tris–HCl. The cells were scraped from the bottom of the dish and solubilized in 2% SDS, 12.5 mM Tris, 0.002% bromphenol blue and 8% glycerol with reducing agent dithothreitol, 100 mM. Samples were boiled for 5 min prior to being separated on a 3–14% linear gradient SDS-PAGE. The gel was fixed and dried, and exposed to X-ray film (XAR, Kodak Laboratories, Rochester, NY, USA; Herr et al., 2003).

Immunoblotting

HTR-8/SVneo cell extracts were subjected to SDS-PAGE, using 6% polyacrylamide gel. The gel was electrophoresed to a polyvinylidene difluoride membrane for 2.5 h at 450 mA. After washing and blocking in 6% non-fat dry milk Tris Buffered Saline Tween (TBST) buffer (10 mM Tris–HCl, 100 mM NaCl and 0.1% Tween 20) for 1 h, membranes were immunoblotted overnight with anti-IGF-II/M6PR antibody in 1% non-fat dry milk solution (dilution 1:2000, antihuman monoclonal antibody, cross-reacting with the human receptor; Nolan et al., 1987) at 4°C. After three washes with TBST (for 10 min) the membranes were incubated with a secondary antibody (anti-rabbit immunoglobulin G horse-radish peroxidase enzyme conjugate, dilution 1:2000, Amersham, Piscataway, NJ) for 1 h. The membrane was washed in TBST buffer and developed with enhanced chemiluminescence substrate (Amersham, Piscataway, NJ). The membrane was exposed to X-ray film (XAR) for visualization of chemiluminescent bands.

125I IGF-II uptake and recycling

For the measurement of the 125I-IGF-II uptake, HTR-8/SVneo cells were grown in 24-well multi-well dishes at 80% confluence in RPMI-1640 medium containing 10% FCS. Before 125I-IGF-II was added to cells, endogenous IGF-II was removed by two sequential 1 h incubations with serum-free RPMI-1640. Cells were then incubated with 125I-IGF-II (5 × 10⁻⁷ M) alone, 125I-IGF-II and HCG (5000 mU/ml). 125I-IGF-II and wortmannin (10 µM) or 125I-IGF-II and HCG and wortmannin for 60 min at 37°C. Subsequently cells were washed four times in ice-cold PBS (to remove...
surface-bound $^{125}\text{I}-\text{IGF-II}$) and solubilized in 0.1% SDS and 0.1 N sodium hydroxide. Radioactivity in the lysates was measured by gamma counting. Non-specific binding/uptake was determined by incubating with an excess of unlabelled IGF-II (3000 ng/ml).

For the measurement of the $^{125}\text{I}-\text{IGF-II}$ recycling cells were grown in 24-well multi-well dishes to 80% confluence in RPMI-1640 medium containing 10% FCS, washed and incubated with $^{125}\text{I}-\text{IGF-II}$ as described above. Recycling was started by adding unlabelled IGF-II (100 ng/ml) alone or in combination with HCG (5000 mU/ml) or wortmannin (PI3 kinase inhibitor) diluted in dimethylsulphoxide to a final concentration of 10 μM (Sigma, Chemical Co., Oakville, ON) for 30 min at 37°C. Monolayers were washed four times with ice-cold PBS (to remove surface-bound $^{125}\text{I}-\text{IGF-II}$) and solubilized in 0.1% SDS and 0.1 N sodium hydroxide. Radioactivity in the lysates was measured by gamma counting (Shpetner et al., 1996; Kundra and Kornfeld, 1998).

**Statistics**

Statistical significance (two-tailed P value) of the experimental results were evaluated using a software program (GraphPad Software Inc., San Diego, CA). One-way analysis of variance (for single treatments) and the Kolmogorov-Smirnov test (testing for normality) were used as primary tests, and the post test was processed by Dunnet (parametric) where appropriate.

**Results**

**HCG and IGF-II effects on trophoblast migration**

Treatment of HTR-8/SVneo cells with different concentrations of HCG (5–50 000 mU/ml) resulted in a significant and dose dependent increase in the migration of EVT cells (Figure 1A). IGF-II alone (concentration 1–100 ng/ml) also significantly stimulated the migration of HTR-8/SVneo cells in a dose dependent manner (data not shown). When HCG (5000 mU/ml) and IGF-II (100 ng/ml) were combined, no additive or synergetic effect on migration of HTR-8/SVneo was seen ($P < 0.05$; Figure 1B).

**$^{125}\text{I}-\text{IGF-II}$ cross-linking**

Affinity cross-linking of $^{125}\text{I}-\text{IGF-II}$ to HTR-8/SVneo cells, revealed binding moieties of multiple sizes corresponding to the IGF-IR, IGF II/M6PR and IGF binding proteins (Figure 2A). The 230 kDa moiety was deduced to be the IGF-II/M6PR based on size and positive immunoreactivity to the anti-IGF-II/M6PR antiserum. The 130 kDa moiety was deduced to be the alpha subunit of the IGF-IR. The binding moieties between 20 and 60 kDa were deduced to be cell surface associated IGFBP. The identity of IGFBP has not been determined. Binding to the cell surface associated IGFBP was less than to either IGF-IR or IGF-II/M6PR. Binding to all moieties were specific as they were competed almost completely by 200 ng/ml of unlabelled IGF-II (lane 2), but not by insulin (lane 3). HCG treatment increased binding of $^{125}\text{I}-\text{IGF-II}$ to the IGF-II/M6PR (lane 4, 2.5-fold as shown by densitometry).

**HCG effects on the IGF-I and IGF-II mRNA and peptide synthesis**

To determine if HCG altered the expression of IGF-I and IGF-II genes in HTR-8/SVneo cells, we measured IGF-I and IGF-II mRNA abundance in the EVT cells and peptide concentrations in the conditioned media of cells treated with different HCG concentrations (5–50 000 mU/ml). No changes in the abundance of IGF-I and IGF-II mRNA were seen in the HTR-8/SVneo cells after treatment with various concentrations of HCG (Figure 3A). Conditioned media from HTR-8/SVneo cells contained 0.20 + 0.014 ng/ml of IGF-I and 1.23 + 0.26 ng/ml of IGF-II, respectively. Treatment with various concentrations of HCG did not alter IGF-I and IGF-II peptide concentrations in the conditioned media from HTR-8/SVneo cells. After the treatment with HCG (50 000 mU/ml) conditioned media from HTR-8/SVneo cells contained 0.21 + 0.028 ng/ml of IGF-I and 1.48 + 0.43 ng/ml of IGF-II, respectively (NS; $P > 0.05$).

**$^{125}\text{I}-\text{IGF-II}$ binding studies**

To examine if the increased $^{125}\text{I}-\text{IGF-II}$ binding to HTR-8/SVneo cells following HCG treatment was caused by the increased number of the binding sites or changes in their affinity, dose-dependent and competitive binding of $^{125}\text{I}-\text{IGF-II}$ were performed. This data showed that HCG treatment of the HTR-8/SVneo cells did not change the affinity of the $^{125}\text{I}-\text{IGF-II}$ binding ($K_{d_{HCG}} = 2.4 \times 10^4 \text{M}^{-1}$ versus $K_{d_{non-HCG}} = 2.9 \times 10^3 \text{M}^{-1}$, NS) but the number of the binding sites was increased 2.8-fold in the HCG pretreated cells (126 746 binding sites/cell) compared to HCG non-pretreated cells (44 128 binding sites/cells; $P < 0.05$; data not shown).

**Effects of HCG on the IGF-II/M6PR mRNA and protein synthesis**

To determine whether the increase in the number of IGF-II binding sites in the cytoplasmic membrane of HTR-8/SVneo cells was
due to increased synthesis of IGF-II/M6PR, IGF-II/M6PR mRNA abundance and IGF-II/M6PR protein in the cell, lysates were examined in cultures treated with different concentrations of HCG (5–50 000 mU/ml) using northern blotting and immunoblotting, respectively. IGF-II/M6PR mRNA detected at a size of 7.4 kb and after hybridization with 32P-labelled 18S probe (Figure 3A) was not increased by HCG treatment. Adjusting for protein loading control there was no increase by HCG treatment on protein levels of IGF-II/M6PR (Figure 3B).

Effects of HCG on the 125I-IGF-II uptake and recycling
To determine if the HCG-induced increase in IGF-II/M6PR number on the surface of the HTR-8/SVneo cells was due to the redistribution of the receptor between cell surface and intracellular compartment, we measured the uptake of the 125I-IGF-II without and with HCG treatment (5000 mU/ml). HCG treatment did not change 125I-IGF-II uptake as measured by the radioactivity of cell lysate after removal of cell surface bound 125I-IGF-II. However, HCG treatment decreased the accumulation of intracellular 125I-IGF-II. This effect was abolished by incubation of the cells with wortmannin (10 µM), a highly specific and potent inhibitor of the catalytic p110 subunit of PI-3 kinase (Shpetner et al., 1996; Kundra and Kornfeld, 1998; Figure 4).

Effects of IGF-IR and IGF-II/M6PR blocking antibodies on HCG-stimulated migration
To determine if the HCG stimulatory action on EVT migration was mediated by either IGF-II/M6PR or IGF-IR or both, blocking polyclonal anti-IGF-II/M6PR antiserum (dilutions 1:200–1:25) and blocking monoclonal anti-hIGF-IR antibody (dilutions, 1:100–1:1000) were added to the EVT migration assay. In previous studies, these concentrations have been shown to be inhibitory for IGF-IGF-II/M6PR interaction (Chomczynski and Sacchi, 1987; Zygmunt et al., 2002). Anti-IGF-II/M6PR antibody decreased the HCG (5000 mU/ml) induced migration of the HTR-8/SVneo cells in a dose dependent manner (Figure 5A; \( P < 0.05 \)), whereas anti-IGF-IR blocking antibody did not change HCG-induced migration (Figure 5B).

Discussion
We have shown that physiological concentrations of HCG enhanced the migration of EVT cell line HTR-8/SVneo, thus confirming our previous observation on the effect of HCG on the invasion and migration of trophoblastic JEG-3 cells (Zygmunt et al., 1998b). The data also supports the concept that HCG, in addition to its effects on placentation, plays a role in the paracrine regulation of trophoblast invasion and vascular adaptation to pregnancy (Tao et al., 1995; Zygmunt et al., 2002). The HTR-8/SVneo cell line used in this study
has been characterized to possess all the phenotypic and functional properties of EVT cells in situ. They express cytokeratin, W6/32, a distinct repertoire of integrins (alpha 1, alpha 3, alpha 5, alpha V, beta 1 and beta V) and hPL. In addition, they express HLA G when cultured on laminin or matrigel, and have invasive properties (Graham et al., 1993; Irving et al., 1995; Kilburn et al., 2000). They can also sustain prolonged growth in culture and bind to HCG similar to primary trophoblast cells and JEG-3 cells as revealed by affinity cross-linking. These properties make the HTR-8/SVneo a representative cell model for the study of trophoblastic invasion and migration.

Although it has been shown that HCG stimulates the activity of matrix metalloproteinases in trophoblast (El-Hendy et al., 1998; Zygmunt et al., 1998b), the precise mechanisms of HCG action on trophoblast are still not fully understood. The interaction of HCG with its receptor leads to the activation of both adenylate cyclase and phospholipase C systems (Dufau, 1998), which can then initiate a chain of events beginning with phosphatidylinositol biphosphate 2 breakdown, and the generation of phosphatidylinositol biphosphate 3 and diacylglycerol. The two latter mediators have been shown to cause the redistribution of the IGF-II/M6PR in different cell types (Brown et al., 1995; Korner and Braulke, 1996).
The mechanism by which HCG induces the redistribution of IGF-II/M6PR is not clear. The changes in the redistribution of IGF-II/M6PR can be caused by either recruitment of intracellular receptors, which do not normally recycle to the cell surface involving changes in the phosphorylation of the receptor (Hu et al., 1990).

The diacylglycerol analog and protein kinase C activator, phospholambanmyristyl acetate, also increases the number of the IGF-II/M6PR in the microvascular endothelial cells without affecting the binding affinity (Hu et al., 1990). It has been shown that a potent inhibitor of PI-3 kinase, wortmannin, completely abolishes the IGF-II induced redistribution of the IGF-II/M6PR in human hepatoma HepG2 cells (Korner and Braulke, 1996). Our studies also showed that the HCG treatment of the HTR-8/SVneo cells significantly decreased the intracellular accumulated $^{125}$I-IGF-II. This decrease might reflect an increase in the externalization rate of the IGF-II–IGF-II/M6PR complex. Incubation of the cells with wortmannin increased intracellularly accumulated IGF-II and abolished HCG-induced changes in the recycling of the ligand–receptor complex. We speculate that the externalization of IGF-II binding sites to the trophoblast cell surface in response to HCG treatment is accompanied by a parallel increase in the uptake and bioavailability of the receptor-bound IGF-II. The IGF-II/M6PR antibody may block more IGF-II/M6PR sites in HCG-treated cells, which then leads to the attenuation of IGF-II-mediated increase in the HTR-8/SVneo cell migration.

Another recently characterized link between HCG and IGFBP-1 also indicates an additional possible interaction of HCG with the IGFBP-system in the regulation of endometrial receptivity (Licht et al., 2002). It has also been suggested that HCG may be involved in IGFBP-1 gene expression during implantation (Kim and Fazleabas, 2004). In an independent in vivo study HCG administration during the secretory phase significantly up-regulated IGFBP-1, which correlates with endometrial differentiation and plays a crucial role in the induction of decidualization of endometrial cells (Jasinska et al., 2004).

Taken together, our data indicate a novel mechanism by which HCG may increase trophoblast migration and invasion via changes in the IGF-II/M6PR recycling, and not by influencing its gene expression. Knowledge of this interaction between two potent regulatory systems may have therapeutic potential in modulating the invasiveness of not only trophoblast cells but also of several other neoplastic cells (Meduri et al., 1997).

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References


Hill DJ and Han VKM (1997) In Le Roith Derek and Bondy Carolyn (eds), Growth Factors and Cytokines in Health and Disease, 3A; II, pp 26–31.


Hill DJ and Han VKM (1997) In Le Roith Derek and Bondy Carolyn (eds), Growth Factors and Cytokines in Health and Disease, 3A; II, pp 26–31.

