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hCG stimulates angiogenic signals in lymphatic endothelial and circulating angiogenic cells

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ABSTRACT

Human chorionic gonadotropin (hCG) has long been associated with the initiation and maintenance of pregnancy, where angiogenesis plays an important role. However, the function of hCG in angiogenesis and the recruitment of vascular active cells are not fully understood. In this study, the role of hCG and its receptor in circulating angiogenic and human endothelial cells, including lymphatic, uterine microvascular, and umbilical vein endothelial cells, was examined. Immunohistochemistry and immunoblot analysis were used to detect LH/hCG receptor expression and the expression of hCG-induced angiogenic molecules. HIF-1α was determined via ELISA and downstream molecules, such as CXCL12 and CXCR4, via real-time PCR. Chemotaxis was analyzed using Boyden chambers. Our results show that the LH/hCG receptor was present in all tested cells. Furthermore, hCG was able to stimulate LH/hCG-receptor-specific migration in a dose-dependent fashion and induce key angiogenic molecules, including HIF-1α, CXCL12, and CXCR4. In conclusion, our findings underscore the importance of hCG as one of the first angiogenic molecules produced by the conceptus. hCG itself alters endothelial motility, recruitment, and expression of pro-angiogenic molecules and may therefore play an important role in vascular adaption during implantation and early placental formation.

1. Introduction

Angiogenesis and remodeling of the spiral arteries are essential for placental development and function (Damsky and Fisher, 1998; Red-Horse et al., 2006).

The pregnancy-related hormone human chorionic gonadotropin (hCG) is found in the maternal plasma as early as hours after blastocyst implantation and is suggested to induce immune tolerance of the fetal allograft, increase endometrial angiogenesis, and support progesterone production of the corpus luteum (Strott et al., 1969; Zygmunt et al., 2002; Berndt et al., 2009; Cole, 2012). hCG

Abbreviations: hCG, human chorionic gonadotropin; HIF-1α, hypoxia inducible factor-1α; CXCL12, C-X-C motif chemokine 12; CXCR4, C-X-C chemokine receptor type 4; CAC, circulating angiogenic cells; LH, luteinizing hormone; LEC, lymphatic endothelial cells; UTM, uterine microvascular endothelial cells; HUVEC, human umbilical vein endothelial cells; GFM, growth factor medium; DFX, deferoxamine; CTB, cytotrophoblasts.

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has been associated with the initiation and maintenance of pregnancy, but its role in angiogenesis and recruitment of vascular active cells has been underestimated (Fazleabas et al., 1999; Herr et al., 2007).

Human chorionic gonadotropin appears to be involved in trophoblast invasion and in oxygen-independent induction of the hypoxia-inducible factor-1α (HIF-1α) in various cells, especially granulosa cells of the ovary (Herr et al., 2004; Van Den Driesche et al., 2008; Zhang et al., 2011). HIF-1α is usually stabilized by low oxygen tension, but there is also evidence to show that oxygen-independent HIF-1α functions are crucial for the differentiation processes of trophoblast cells (Maltepe et al., 2005). Additionally, the chemokine C-X-C motif chemokine 12 (CXCL12) and its receptor, C-X-C chemokine receptor type 4 (CXCR4), have been identified as HIF-1α transcriptional targets and are involved in cytotrophoblast mobility and the attraction of natural killer cells (Red-Horse et al., 2001; Hanna et al., 2003; Ceradini et al., 2004; Wu et al., 2004). Furthermore, trophoblast-derived molecules, such as members of the vascular endothelial and fibroblast growth factor family, can induce lymphangiogenesis in the decidual compartment of the placenta (Red-Horse et al., 2006).

Circulating angiogenic cells (CAC), previously known as endothelial progenitor cells, have been described to be increased in the maternal blood during pregnancy (Gussin et al., 2002; Sugawara et al., 2005). Several molecules, such as nitric oxide or relaxin, were investigated with regard to CAC migration, proliferation, mobilization, and function (Heiss et al., 2010; Segal et al., 2012). Until now, the possible role of hCG in the modulation of CAC function has to our knowledge not been examined.

The hCG receptor (LH/hCG receptor) belongs to the family of G-protein-coupled receptors and also binds to the luteinizing hormone (LH), suggesting cross-linked actions. Human CG binding to the LH/hCG receptor induces the formation of the ternary complex of ligand-receptor-G protein, which in turn activates the second messenger cAMP system (Ryu et al., 1996; Van Den Driesche et al., 2008). An example of LH-like hCG function is the triggering of ovulation with hCG in fertility treatment (Filicori et al., 2005). A common complication of this is the ovarian hyperstimulation syndrome, which is accompanied by ovarian cysts, ascites, or pleural effusion due to increased capillary permeability, and ovarian neoangiogenesis (Kumar et al., 2011).

The LH/hCG receptor is located in the human uterus, fallopian tube, ovary, placenta, fetal membranes, and some of the endothelial cells in these organs. Additionally, the LH/hCG receptor has been detected in extragonadal tissue, including human carcinoma cells of the prostate or breast, human skin, bone, and retina. However, the functions of hCG and its receptor in these organs are not yet fully understood (Rao, 2001; Tsampalas et al., 2010).

Therefore, we examined the possible role of hCG and the LH/hCG receptor in different types of endothelial cells, focusing on CAC and lymphatic endothelial cells (LEC) and key processes of angiogenesis, such as cell migration, and the expression of key pro-angiogenic molecules.

2. Materials and methods

2.1. Cell lines and primary cells

Human uterine microvascular endothelial cells (UTM) and human lymphatic endothelial cells (LEC) were purchased from Cambrex (East Rutherford, NJ, USA; now they are available at LONZA, Walkersville, MD, USA); human umbilical vein endothelial cells (HUVEC) and human choriocarcinoma cells (JAR) were purchased from ATCC (Manassas, VA, USA). The primary LEC were from female volunteers and there was no information available concerning the estrous cycle.

The HUVEC, LEC, and UTM were cultured in EBM-2 (Lonza, supplemented with 5% FBS), and the JAR cells in RPMI-1640 Medium (ATCC, supplemented with 10% FBS).

Primary cells were not used later than passage three. We used three different batches from each cell type, except for CACs, where cells were isolated from seven female subjects. All experiments were performed in triplicate.

2.2. Cell culture and characterization of blood-derived CAC

The CAC were differentiated ex vivo from peripheral blood mononuclear cells, as previously described (Hill et al., 2003). Briefly, these cells were isolated from peripheral blood samples. The healthy volunteers were recruited and blood was drawn at the University of California San Francisco (UCSF, $n=3$) and University Düsseldorf ($n=4$). Volunteers ($n=7$) were healthy, female, 25.1 ± 2.3 years old, and had normal values for body mass index, creatinine, C-reactive protein, fasting glucose, total cholesterol, hemoglobin, heart rate, and blood pressure. The women were in their follicular phase before ovulation took place. With regard to baseline demographic parameters, the subjects recruited at UCSF did not differ from those recruited at Düsseldorf. The study group included one smoker. The protocol was approved by the Institutional Review Board of UCSF and the University of Düsseldorf and all subjects gave written informed consent. Isolation of mononuclear cells was accomplished by density gradient centrifugation by the Ficoll method (Vacutainer CPT; Becton Dickinson, Franklin Lakes, NJ, USA). The mononuclear cells were cultured for seven days on fibronectin-coated (Sigma–Aldrich Chemie, Taufkirchen, Germany) plates with EBM-2 cell culture medium. Culture was preceded by one day of preplating to remove platelets and shed endothelial cells. We confirmed the endothelial-like phenotype on the day 7 adherent cells by fluorescent staining for lectin binding, acetylated low-density lipoprotein uptake (95% double positive cells), and CD31 (89% positive cells) (Asahara et al., 1997).

2.3. Immunofluorescence staining

For immunofluorescence, CAC ($n=4$) and LEC ($n=3$) were stained with antiserum containing the serum antibody that targets the LH/hCG receptor. This LH/hCG receptor serum antibody was made in male rabbits against a synthetic peptide, corresponding to amino acids 257–271