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Human Chorionic Gonadotropin Expression in Human Trophoblasts from Early Placenta: Comparative Study Between Villous and Extravillous Trophoblastic Cells

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Abstract

Human trophoblast differentiates into two pathways: extravillous cytotrophoblasts (EVCT) that invade the uterus wall and villous cytotrophoblasts (VCT) that fuse to form the syncytiotrophoblast (ST) involved in placental exchanges and endocrine function. It is established that hCG is produced and secreted by the ST into the maternal compartment where it plays a key endocrine role and stimulates ST formation in an autocrine manner. Herein, we investigated hCG expression in early placentas by immunohistochemistry using different antibodies. We then compared hCG secretion by primary cultures of VCT and EVCT isolated from the same first trimester human chorionic villi. In situ hCG was immunodetected in EVCT all along their invasive differentiating pathway except in cells near the stromal core of the proximal column. hCG expression was confirmed in vitro by immunocytochemistry and hCG secretion quantified in cell supernatants. Interestingly, whereas hCG secretion increased during VCT differentiation into ST (from 60 to 350 UI/L/μg DNA), EVCT secretion remained constant and at a high level during the same culture period (160 UI/L/μg DNA). Our data demonstrated that in addition to the ST, invasive EVCT also expressed and secreted high levels of hCG, suggesting a specific paracrine and/or autocrine role for hCG from EVCT origin.

Keywords: Cytotrophoblast; Syncytiotrophoblast; Placental hormone; Differentiation; Invasion

1. Introduction

After implantation, human trophoblasts follow two differentiation pathways: the villous and the extravillous cytotrophoblasts that display different phenotypes and functions (cf. Fig. 2A). The mononucleated villous cytotrophoblasts (VCT) that cover the floating chorionic villi aggregate and fuse to form a multinucleated syncytiotrophoblast (ST), which is involved in the exchange of gases and nutrients between the mother and the fetus. Moreover, the ST represents the endocrine tissue of the placenta, secreting large amounts, unparalleled in other mammals, of protein hormones including human chorionic gonadotropin hormone (hCG) [1]. The trophoblasts located at the tip of the anchoring villi contacting the uterine wall follow a different differentiation pathway. These trophoblasts, named extravillous cytotrophoblasts (EVCT), proliferate to form multilayered columns of cells and then invade the decidua up to the upper third of the myometrium. At the deciduo-muscular junction, EVCT undergo a final differentiation into multinucleated giant cells. Trophoblastic infiltration of the arterial wall is accompanied by dramatic structural changes of the vascular media such as the loss of elastic fibres

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and smooth muscle cells which are usually attributed to proteolytic activities of the invasive endovascular cells [2]. Hence, EVCT are directly involved in the anchoring of chorionic villi in the uterus, and in the essential remodeling of the uterine arterioles to provide adequate supply of maternal blood to the intervillous space necessary for fetal growth [3–7].

Human chorionic gonadotropin hormone, specific to humans and great apes, is a glycoprotein composed of an alpha-subunit (α hCG), which is common to other peptide hormones such as LH (luteinizing hormone), FSH (follicle stimulating hormone) and TSH (thyroid stimulating hormone), and a beta-subunit (β hCG), which confers the biological specificity of the hormone [8,9]. α hCG is encoded by a single gene and β hCG by six genes of which the CGβ5 gene is expressed predominantly in the placenta [10,11]. After implantation, hCG is the first trophoblast signal detected in the maternal blood and is used as a diagnostic marker of pregnancy. hCG and its free β-subunit are detected in the maternal blood from the second week of pregnancy with peak levels at 12 weeks and then gradually decrease; α hCG levels increase progressively up to term [12]. Maintenance of pregnancy during the first trimester depends on the synthesis of hCG, which prevents regression of the corpus luteum [13] allowing the maintenance of ovarian progesterone secretion [1]. In addition to its well-established endocrine role, hCG plays an autocrine/paracrine role in human trophoblast differentiation [14,15].

Although the synthesis and secretion of hCG by the villous trophoblast is well established and documented [16], presence of hCG in the cytotrophoblast from extravillous origin remains controversial since opposite results were reported by different authors [17–19]. Therefore, in the present study we first investigated in situ the localization of hCG at the feto-maternal interface using different antibodies. Then, we examined the in vitro expression and secretion of hCG in cultured villous and extravillous cytotrophoblasts isolated from the same chorionic villi of first trimester human placentas, and compared quantitatively the hCG secretion of each cytotrophoblastic cell type.

2. Materials and methods

2.1. Materials

Placental tissues from patients who voluntarily and legally chose to terminate pregnancy during the first trimester (8–12 weeks of gestation) were obtained from Broussais Hospital (Paris, France, $n = 14$). All patients gave informed consent. We also obtained paraffin sections of an implantation site (placenta and myometrium) at 16 weeks of gestation from the pathology department of "La Citadelle Hospital" (Liège, Belgium). Hysterectomy was performed because of cervical carcinoma stage IIa diagnosed at 15 weeks.

2.2. Immunohistochemistry

Samples from 8 to 12-week placentas were either fixed by incubation in 4% formalin for 4–24 h at room temperature and then embedded in paraffin ($n = 3$), or embedded in TissueTek, frozen in isopentan and kept at −80 °C until use ($n = 4$). Paraffin sections of the 8–12-week placentas and of the 16 weeks of gestation implantation site (placenta and myometrium obtained from Belgium) were dewaxed in xylene and rehydrated in ethanol/water. Sections were then incubated for 45 min in citrate buffer at 90 °C for antigen retrieving. Immunostaining was performed with a streptavidin–peroxidase immunostaining kit (Peroxidase, Dako, LSAB® + Kit DAKO©, Trappes, France). Samples were permeabilized for 4 min in 0.3% Triton X-100 (for cytokeratin 7 (CK7) and Ki67 immunodetections only) and non-specific binding was blocked by incubation for 5 min in a blocking reagent containing 3% H_2O_2 and then in 3% serum albumin in PBS buffer for 30 min. The sections were incubated with several antibodies directed against different hCG epitopes, with an anti-Ki67 antibody and with an anti-CK07 antibody for 30 min at room temperature.

Characteristics for each antibody are detailed in Table 1. Sections were washed in PBS and incubated with a biotinylated secondary antibody for 15 min. They were then washed three times in PBS and incubated with streptavidin conjugated to horseradish peroxidase for 15 min. The sections were washed in PBS and staining was detected by incubation for 2 min with the DAB (3,3′-diaminobenzidine) chromogen. Controls were performed by incubating the sections with non-specific IgG at the same concentration as the primary antibody. Preparations were counterstained with hematoxylin blue (except for Ki67 staining) and mounted in an aqueous mounting medium (Aqueous Mounting Medium, DAKO©, Trappes, France), then examined and photographed with an Olympus BX60 microscope.

2.3. Isolation and purification of villous (VCT) and extravillous (EVCT) cytotrophoblasts

Seven different isolations of EVCT and VCT were performed using seven independent human chorionic villi from first trimester legally induced abortion. EVCT and VCT were each time isolated from the same chorionic villi by differential trypsin digestion according to the methods of Tarrade et al. [20] with slight modifications. Chorionic villi were washed, dissected and rinsed in Ca^{2+}, Mg^{2+}-free HBSS supplemented with 100 UI/mL penicillin and 100 μg/mL streptomycin.

For EVCT isolation, the tissue was incubated in HBSS (5 mL/g) containing 0.125% trypsin (Difco Laboratories, Detroit, Michigan), 4.2 mM $MgSO_4$, 25 mM Hepes, 50 U/mL Dnase type IV (Sigma, Saint-Quentin Fallavier, France) for 35 min at 37 °C without agitation. After tissue sedimentation, the supernatant containing isolated EVCT was filtered (100 μm) and stored in the presence of 10% (v/v) fetal calf serum (FCS) to inhibit trypsin activity. The tissue was washed 5–6 times with warm HBSS and supernatant fractions were pooled. Cells were centrifuged at 300 × g for 10 min, washed twice in

Table 1
Antibodies used for immunohistochemistry and immunocytochemistry

Antibody	Species	Isotype	Antigen	Dilution	Source
M 7018 (OV-TL 12/30)	Mouse	IgG1κ	Cytokeratin 7	1:200	Dako, Trappes, France
HT13	Mouse	IgG1	α hCG and total hCG	1:200	Generous gift of J.M. Bidard IGR, Villejuif, France
FB12	Mouse	IgG1	β hCG and total hCG	1:500	Generous gift of J.M. Bidard IGR, Villejuif, France
A0231	Rabbit	Polyclonal	β hCG and total hCG	1:4000	Dako, Trappes, France
Ki67	Mouse	IgG1κ	Ki67	1:100	Dako, Trappes, France