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Compartmentalized localization of 11β-HSD 1 and 2 at the feto-maternal interface in the first trimester of human pregnancy

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ABSTRACT

Glucocorticoids are engaged in a number of actions at the feto-maternal interface for the establishment of early pregnancy. However, excessive glucocorticoids can be deleterious to fetal development. Therefore, compartmentalized distribution of 11β-hydroxysteroid dehydrogenase 1 and 2 (11β-HSD1 and 2), which regenerates and inactivates cortisol respectively, would ensure an optimal cortisol concentration at the feto-maternal interface for the establishment of early gestation. However, the distribution pattern of 11β-HSD1 and 2 at the feto-maternal interface in early human pregnancy is not clearly defined. Here we showed that 11β-HSD1 distributed extensively on the maternal side including decidual stromal cells and epithelial cells but scarcely on the fetal side except for localization in the fetal blood vessels of the chorionic villi. In contrast, 11β-HSD2 was abundantly localized in syncytial layer of the chorionic villi and the decidual epithelium. In primary cultures, cortisol upregulated not only 11β-HSD1 expression in decidual stromal cells but also 11β-HSD2 expression in villous trophoblasts of early pregnancy. Further studies revealed that cortisol inhibited the expression of interleukin-1β and 6 in decidual stromal cells and villous trophoblasts, and stimulated expression of human chorionic gonadotropin in villous trophoblasts. Collectively, this study has revealed a compartmentalized distribution pattern of 11β-HSD 1 and 2 at the feto-maternal interface, both of which can be upregulated by glucocorticoids, suggesting that a coordinated interaction between 11β-HSD 1 and 2 may exist to ensure an optimal cortisol concentration at discrete locations at the feto-maternal interface for the establishment of early pregnancy.

1. Introduction

Glucocorticoids are essential for life [1]. Cortisol, as the major endogenous glucocorticoid in humans, regulates a number of vital processes including intermediary metabolism, vascular responsiveness and immune reactions [2,3]. In early pregnancy, glucocorticoids are engaged in a number of actions at the feto-maternal interface for the establishment of gestation [4]. The maternal decidua of the first trimester supplies an immune tolerant environment for the semi-allograft embryo [5,6]. Glucocorticoids are capable of inhibiting immune rejection responses during the peri-implantation window [7]. The chorionic villi of the first trimester contain three kinds of trophoblasts: syncytiotrophoblasts which provide a substance exchange interface between mother and fetus; extravillous trophoblasts (EVT) which express human leukocyte antigen-G (HLA-G), a tolerogenic molecule enabling EVT to escape from the mother's immune surveillance; and cytotrophoblasts which can differentiate into either syncytiotrophoblasts or EVT [8]. In the villous tissue, glucocorticoids promote HLA-G expression, accelerate trophoblast growth and invasion [9,10]. All these effects of glucocorticoids in the decidua and chorionic villi suggest that appropriate glucocorticoid abundance at the feto-maternal interface might be beneficial to early pregnancy.

Abbreviations: 11β-HSD1 and 2, 11beta-hydroxysteroid dehydrogenase 1 and 2; hCG, human chorionic gonadotropin; EVT, extravillous trophoblast; ST, syncytiotrophoblast; CT, cytotrophoblast; HLA-G, human leukocyte antigen-G; GR, glucocorticoid receptor; IUGR, intrauterine growth retardation; IL-1β, interleukin-1β; IL-6, interleukin-6; PRL, prolactin; IGFBP-1, insulin-like growth factor binding protein-1; qRT-PCR, quantitative real time PCR; DSC, decidual stromal cell; VT, villous trophoblast.

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However, excessive glucocorticoids *in utero* can also be deleterious to placental development and fetal growth [11–13] and even program the development of chronic diseases such as hypertension and type II diabetes in later life [14]. Thus, tightly-controlled glucocorticoid levels at the feto-maternal interface are important for the successful establishment of gestation and healthy development of the fetus. It is well known that 11β-hydroxysteroid dehydrogenase (11β-HSD) enzymes determine the local levels of biologically active glucocorticoids [15]. There are two isoforms of 11β-HSD recognized so far. 11β-HSD1 functions mainly as a reductase converting inactive cortisone to active cortisol [15], while 11β-HSD2 acts exclusively as an oxidase converting cortisol to cortisone [15]. However, limited information is available regarding the distribution of 11β-HSD1 at the feto-maternal interface in early pregnancy, although previous studies have revealed an upregulation of 11β-HSD1 during decidualization [16] and the presence of 11β-HSD2 in the decidua [17] and chorionic villi in the first trimester of human pregnancy [18,19]. Neither are we clear about the distribution of 11β-HSD1 and 11β-HSD2 in EVT, the crucial trophoblasts bridging the fetus and mother.

Glucocorticoids have been shown to induce 11β-HSD1 and 2 expressions in amnion fibroblasts and placental trophoblasts respectively at term, these proteins are responsible for appropriate cellular glucocorticoid levels at discrete locations during gestation [20–23]. However, how glucocorticoids regulate 11β-HSD1 and 2 expressions at the feto-maternal interface in early pregnancy remains unknown. Understanding the regulation of 11β-HSD1 and 2 by glucocorticoids may help us further understand their roles in early pregnancy. Here we hypothesized that 11β-HSD1 and 2 might adopt distinct distribution patterns at the feto-maternal interface and their expression might be subject to the regulation by glucocorticoids so that optimal concentrations of glucocorticoids could be maintained at the discrete compartments for the establishment of gestation. In this study, these issues were examined in the decidual and chorionic villous tissues obtained from the first trimester of human pregnancy.

2. Materials and methods

2.1. Tissue collection

Human first trimester (6–8 weeks) decidual tissue (n = 8) and chorionic villous tissues (n = 40) were obtained from women aging between 20 and 35 years old at the gestational age of 6–8 weeks (calculated from the first day of last menstruation), who underwent elective abortion. Written informed consent was obtained from the patients under a protocol approved by the Ethics Committee of Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University. Women with the history of spontaneous abortion, use of contraceptive and other complications were excluded from the study. The tissues were either fixed in 4% paraformaldehyde for immunohistofluorescence staining or processed immediately for decidual and villous trophoblast cell isolation.

2.2. Immunohistofluorescence staining of 11β-HSD1, 11β-HSD2 and glucocorticoid receptor

Immunohistofluorescence staining was performed on paraffin-embedded sections to examine the distribution patterns of 11β-HSD1, 11β-HSD2 and glucocorticoid receptor (GR) at the feto-maternal interface in early pregnancy. Briefly, the sections were permeablized with 0.4% Triton X-100 and then blocked with serum. The decidual sections were double stained with antibodies against 11β-HSD1 (1:200) (Abcam, Cambridge, UK) and cytokeratin (1:100) (Santa Cruz Biotechnology, Santa Cruz, CA), 11β-HSD2 (1:100) (Santa Cruz) and Vimentin (1:100) (Santa Cruz), or GR (1:100) (Santa Cruz) and cytokeratin (1:100) (Santa Cruz). The villous sections were double stained with antibodies against 11β-HSD1 (1:200) (Abcam) and HLA-G (1:200) (Abcam), 11β-HSD2 (1:100) (Santa Cruz) and HLA-G (1:200) (Abcam), or GR (1:100) (Santa Cruz) and HLA-G (1:200) (Abcam). After washing, the sections were incubated with Alexa Fluor 488-(green color) (1:100) (Proteintech, Chicago, USA) and Alexa Fluor 594-labeled secondary antibodies (red color) (1:100) (Proteintech). Nuclei were counterstained with DAPI (blue color) (1 μg/ml).

2.3. Isolation and culture of decidual stromal cells and villous trophoblasts

Decidual stromal cells and villous trophoblasts were isolated from the collected human decidual and chorionic villous tissues according to previously reported protocols [24–26]. Briefly, the decidual tissue was minced and subjected to 0.1% collagenase (type IV) (Sigma Chemical Co., St. Louis, MO) digestion twice. The tissue digest was then passed through a nylon sieve (38 μm). The cells were purified on discontinuous (40%:50%:55%) Percoll (GE Healthcare Bio-Sciences, Uppsala, Sweden) gradients. The stromal cells were plated for culture in Dulbecco Modified Eagle medium (DMEM)/F-12 (Gibco, Grand Island, NY) containing 10% fetal bovine serum (FBS) (Gibco) and 1% antibiotics (Gibco) at 37° in 5% CO_2/95% air. The purity of isolated cells was determined by immunocytofluorescence staining with an antibody against vimentin, showing ≥95% of the isolated cells were vimentin positive.

For isolation of villous trophoblasts, the fresh villous tissue was minced and digested with 0.1% trypsin (Sigma) and 0.02% DNase I (Sigma) in DMEM/F12. The dispersed trophoblasts were purified using discontinuous 10%–70% Percoll gradients at a stepwise increment of 10%. The cytotrophoblasts between densities of 1.049 g/ml and 1.062 g/ml were collected and plated for culture in DMEM/F-12 containing 10% FBS and 1% antibiotics at 37° in 5% CO_2/95% air. The plate for trophoblast culture was pre-coated with 1:10 diluted Matrigel (BD Biosciences, Franklin Lakes, NJ). The purity of isolated cells was determined by immunocytofluorescence staining with antibody against cytokeratin, showing ≥98% of the isolated trophoblasts were cytokeratin positive.

2.4. Treatment of decidual stromal cells and villous trophoblasts

To observe the effects of cortisol on 11β-HSD1 expression in decidual stromal cells or 11β-HSD2 expression in villous trophoblasts, two (decidual stromal cells) or three days (villous trophoblasts) after plating, the cells were treated in serum-free medium containing cortisol (0.01, 0.1, 1 μM) (Sigma), or cortisol (0.1 μM) in the presence or absence of RU486 (1 μM) (Sigma) for 24 h. Total cellular protein was extracted from the cells to analyze 11β-HSD1 and 11β-HSD2 with Western blotting. To examine whether cortisol affects decidualization of the stromal cells, total RNA was extracted from the decidual cells for analysis of prolactin (PRL) and insulin-like growth factor binding protein-1 (IGFBP-1) mRNA abundance, the well-recognized markers of decidualization, with quantitative real-time polymerase chain reaction (qRT-PCR). To study whether cortisol affects the expression of cytokines involved in immune rejection responses, interleukin-6 (IL-6) and interleukin-1β (IL-1β) mRNA abundance after cortisol treatment was analyzed with qRT-PCR. To investigate whether cortisol affects PRL and hCG abundance, the crucial hormone in early gestation maintenance, in villous trophoblasts, mRNA of PRL and β subunit of hCG (β-hCG) in the cells after cortisol treatment was analyzed with qRT-PCR, and culture medium was collected to measure secreted β-hCG with a chemiluminescence kit.