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Cell-type specific expression and regulation of apolipoprotein D and E in human endometrium

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ABSTRACT

Objective: To assess the expression and regulation of antilipoprotein D (ApoD) and antilipoprotein E (ApoE) in human endometrium.
Study design: Endometrial biopsies from healthy, regularly cycling women were collected during the late proliferative and mid-secretory phase. mRNA gene expression of ApoD and ApoE was determined using real-time PCR in whole tissue, in isolated stromal (ESC), epithelial (EEC) and $CD45^+$ leukocytes (EIC), as well as after hormonal stimulation of ESC and EEC in vitro. Protein expression was analyzed using immunohistochemistry.
Results: ApoD and ApoE mRNA was expressed in all cell types examined. A rise in ApoD mRNA expression was seen in whole endometrium, ESC, and EEC in the secretory phase, as well as after hormonal stimulation of ESC and EEC in vitro. ApoE mRNA was significantly upregulated in whole endometrium of secretory phase biopsies, while its expression was not altered by progesterone in vitro. Immunohistochemistry of whole endometrial tissue localized ApoD mainly in ESC and EEC. While ApoE was localized slightly in ESC, it was particularly noted on the surface of secretory phase endothelial cells.
Conclusion: We demonstrate for the first time the cell-type and cycle dependent expression of ApoD and ApoE within human endometrium, suggesting their role in endometrial modulation.

1. Introduction

Endometrium is a very plastic tissue undergoing cyclical changes throughout the reproductive life span of healthy women. Many prior studies have given insight into functional changes within the endometrium [1–4], including gene and protein arrays [5–7]. Most importantly those endometrial changes are thought to prepare the maternal tissue for the complex fetomaternal communication during implantation [8].

Apolipoproteins (Apo) are mainly known as lipid transporters regulating the metabolism of lipoproteins and their uptake in tissues. In addition they serve as enzyme co-factors and receptor ligands [9,10]. Several apolipoprotein members have additional functions in regulation of lipid and vitamin homeostasis, cellular migration, neurodevelopment, and synaptic plasticity in other tissues [11]. Several of these functions are also found to be relevant in the reproductive tract [12–14]. ApoJ, for example, increases during early nidation and potentially protects uterine and fetal tissues from attacks by complement factors and reactive catabolites [15]. In addition, ApoA-1 actively inhibits implantation and needs to be repressed by HCG, a process that seems to be disturbed in women with endometriosis, possibly contributing to their reduced implantation rates [14].

Two other apolipoproteins, ApoD and ApoE, were differentially expressed during the menstrual cycle of human endometrium in a prior microarray study comparing proliferative with secretory phase endometrium, suggesting a potential role in the plasticity of the endometrium [5]. The microarray study from Altmae et al. [8] also favors ApoD upregulation in secretory endometrium as a potential factor in fetomaternal communication, since the embryo has a high demand for membrane formation during this sensitive period. While ApoD is expressed in many tissues, it is not a typical lipoprotein, but rather belongs to the lipocalin family and functions as a multi-functional transporter involved in cell-to-cell ligand transportation [10,16]. In guinea pigs, ApoD has been

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found to be significantly upregulated in fallopian tubes and ovaries during gestation. The authors assumed that ApoD may be involved in the retention of steroid hormones within reproductive tissue [17]. ApoE, on the other hand, mediates cholesterol uptake into cells and may also be involved in immune modulation [18]. Finally, stage-specific variation of ApoE mRNA expression in seminiferous epithelium suggests involvement in spermatogenesis [19].

Since ApoD and E are only assumed to be expressed in endometrium [5], the aim of this study was to confirm the ApoD and E expression in human endometrium, as well as their suggested (up)regulation in mid-secretory compared to late-proliferative phase endometrium [5] on the gene and protein levels. Furthermore, their localization within endometrial tissue compartments and their potential hormonal regulation in vitro was investigated.

2. Material and methods

2.1. Patients

Endometrial biopsies were collected from healthy, regularly cycling women, undergoing laparoscopy or hysterectomy for non-endometrial reasons, after informed consent under the approved Ethics protocol of the Heidelberg University. Proliferative phase sample biopsies were collected at cycle day 10 to 13, and for the mid-secretory sample biopsies were chosen at cycle day 19 to 23. Exclusion criteria were hormone therapy during the last three months, endocrinopathies, cancerous lesions and irregular menstrual bleeding. Histological examination of the endometrial biopsies was performed by the Department of Pathology, Heidelberg University, to confirm the adequate cycle phase according to Noyes' criteria [20]. The biopsies were then either shock frozen in liquid nitrogen, put in Tissue tek® compound blocks or processed for individual cell type isolation and in vitro culture.

2.2. Cell isolation

Endometrial stromal (ESC) and epithelial cells (EEC) were separated as described earlier [21]. Briefly, endometrial tissue was cut into small pieces using a scalpel, followed by digestion at 37 °C for 60 min with collagenase (Gibco, Karlsruhe, Germany), DNAse I (Roche Diagnostics, Mannheim, Germany), and hyaluronidase (Sigma-Aldrich, Taufkirchen, Germany), then filtered with a 180 μm filter. In a second filtration step (40 μm filter) EEC were collected. Cells were then either used for cell culture experiments or further separated as follows: in order to gain isolated cell fractions for mRNA analysis, the remaining ESCs were separated from CD^{45+} leukocytes (EIC) via positive selection using specific magnetic spherical particles of ferric oxide coated with antibodies against leukocyte common antigen (CD45) according to the manufacturer's protocol (Lot Nr. 11153D, DynalBiotech GmbH, Hamburg, Germany). The Dynabeads were bound to the target cells by an antigen-antibody-reaction, and the separation was performed via exposure to a magnetic field. After washing with PBS, isolated CD^{45+} cells were shock frozen. The remaining ESC were exposed to a bead mix containing Dynabeads® CD45, CELLection™ Epithelial Enrich (Prod No. 16203) and Dynabeads® CD31 Endothelial Cell (Prod. Nr. 11155D), followed by a two-hour culture in stromal cell media to remove further contaminating cells. Individual cells (ESC, EEC, EIC) were shock frozen in liquid Nitrogen.

2.3. Endometrial epithelial cell culture

After digestion and filtration EEC ($n = 6$) were cultured in 24-well plates (Costar, USA) in DMEM (Gibco) with 1% FCS (Perbio Science, Thermo Fischer, Bonn, Germany) at 37 °C and 5% CO_2, and incubated with 17β-estradiol (10 nM, Sigma-Aldrich), progesterone (1 μM, Sigma-Aldrich) and EGF (20 ng/ml, Sigma-Aldrich) for 72 h. Thereafter, TRIZOL (Gibco) was added and cells were shock frozen in liquid nitrogen.

2.4. Endometrial stromal cell culture and decidualisation

Isolated ESC ($n = 6$) were cultured in medium containing DMEM, MCDB-105 (Sigma-Aldrich), and 10% FCS until confluent, then passaged twice. The purity of these cells has been proven previously [22]. Thereafter, cells were cultured in 6-well plates until ≈80% confluency was reached. Serum-free DMEM/MCDB-105 medium, 17β-estradiol (10 nM), progesterone (1 μM), and EGF (20 ng/ml) were used for 12–14 days until decidualisation was confirmed by double sandwich ELISA (Siemens Diagnostics, Eschborn, Germany) of secreted prolactin. Then TRIZOL was added and cells were shock frozen.

2.5. mRNA analysis

Shock frozen whole tissues ($n = 4$ proliferative and $n = 4$ secretory), fresh isolated cell fractions ($n = 4$ proliferative and $n = 3$ secretory) and cultured cells ($n = 6$ each) were thawed and mRNA was isolated using TRIZOL according to the manufacturer's instructions. Reverse transcription (RT) was performed with 1 μg of total RNA per 20 μl reaction, followed by real-time PCR in triplicates using Taqman primers from Applied Biosystems (Darmstadt, Germany) for ApoD (TaqMan, Hs00155794_ml) and ApoE (TaqMan, Hs001711681_ml) running 40 cycles (10 min 95 °C, 15 sec 95 °C, 1 min 60 °C) on a 7500 Fast real-time PCR System (Applied Biosystems, Darmstadt, Germany), according to the manufacturer's protocol. Ribosomal protein L0 (RPL0) was used as the housekeeping gene. Ct values were normalized to RPL0 of the individual sample. For quantification purposes ΔΔCT was analyzed between normalized values of the individual proliferative and secretory phase samples, as well as between treated and untreated cells.

2.6. Immunohistochemistry

Immunohistochemical staining ($n = 8$ each cycle phase) was performed as previously described [1]. Frozen sections (10 μm thick) were stained using commercially available kits (Histostain-Plus Kit; Zymed Laboratories Inc., San Francisco, USA). Sections were incubated with 10% non-immune goat serum and then incubated with the primary human anti-ApoD monoclonal mouse antibody 1:1000 (ab 130388, Abcam, Cambridge, United Kingdom) or monoclonal human anti-ApoE mouse antibody 1:1000 (ab 1906, Abcam) at 4 °C over night. For control staining the primary antibody was substituted by unspecific IgG mouse antibody 1:1000 (DakoCytomation, Denmark). After rigorous washings, slides were incubated with secondary anti-mouse biotinylated antibody (Zymed labs. Inc. San Francisco, USA) followed by incubation with streptavidin peroxidase conjugate for 10 min. After washing with PBS, slides were incubated with 3,3′-diaminobenzidinie tetrahydrochloride substrate kit (Zymed labs. Inc.) for 5 min at room temperature. For counterstaining hematoxylin (Merck GmbH, Darmstadt, Germany) was used. The staining pattern was evaluated by two independent researchers (JJ, AG) using a four-point scaling system (0–3).

2.7. Statistical analysis

Changes in gene expression were assessed using the normalized CT values and comparison among the groups was performed using