Differential expression of genes in eutopic and ectopic endometrium from patients with ovarian endometriosis

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Objective: To investigate whether genes that had been found to be differentially expressed in deep-infiltrating endometriosis and matched eutopic endometrium in our previous complementary DNA microarray study also are differentially expressed in ovarian endometriosis and matched eutopic endometrium.
Design: Prospective study.
Setting: University hospital in France.
Patient(s): Patients with ovarian endometriosis.
Intervention(s): During surgery, paired samples of tissue representing ovarian endometriosis and eutopic endometrium were obtained from 12 patients.
Main Outcome Measure(s): Expression levels of messenger RNA for heat shock protein 90 alpha (HSP90A), chicken ovalbumin upstream promoter transcription factor 2 (COUP-TF2), prostaglandin E_2 receptor subtype EP3 (PGE_2EP3), tyrosine kinase receptor B (TrKB), and 17β-hydroxysteroid dehydrogenase type 2 (17βHSD2; epithelial cells) and of platelet-derived growth factor receptor alpha (PDGFRA), protein kinase C beta 1 (PKCβ1), Janus kinase 1 (JAK1), mitogen-activated protein kinase kinase (MKK7), Sprouty2, μ-opioid receptor (MOR), and 5HTT (stromal cells) from ovarian endometriosis and matched eutopic endometrium were determined by using laser capture microdissection and real-time reverse-transcription polymerase chain reaction (RT-PCR) techniques.
Result(s): Expression of PDGFRA, PKCβ1, JAK1, HSP90A, COUP-TF2, MOR, and 17βHSD2 was significantly higher in ovarian endometriosis than in eutopic endometrium, whereas that of Sprouty2 and PGE_2EP3 was significantly lower. There was no significant difference in mitochondrial RNA expression of MKK 7, TrKB, and 5HTT.
Conclusion(s): Ovarian endometriosis might share several common molecules with deep-infiltrating endometriosis that act to sustain endometriotic lesions, whereas molecules involved in local endocrine control might be different between these two types of endometriosis. (Fertil Steril® 2006;86:548–53. ©2006 by American Society for Reproductive Medicine.)

Key Words: Deep-infiltrating endometriosis, endometrium, laser capture microdissection, ovarian endometriosis

Our recent DNA microarray analysis that used samples obtained by laser capture microdissection (LCM) identified several genes that might be involved in the pathophysiology of deep-infiltrating endometriosis (DIE) (1).

Of these candidate genes, heat shock protein 90 alpha (HSP90A), which actively participates in steroid-induced signal transduction (2), was up-regulated in endometriotic epithelial cells, whereas two potential negative regulators of aromatase expression, chicken ovalbumin upstream promoter transcription factor 2 (COUP-TF2) and prostaglandin E_2 receptor subtype EP3 (PGE_2EP3), were down-regulated.

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In addition, our recent study demonstrated that aromatase messenger RNA (mRNA) expression was significantly higher in epithelial cells than stromal cells in both eutopic and ectopic endometrium that was obtained from patients with DIE. Expression of 17β-hydroxysteroid dehydrogenase type 2 (17βHSD2) mRNA was not detected in either epithelial or stromal cells from 50% of the patients with DIE (3).

Platelet-derived growth factor receptor alpha (PDGFRA) and members of the downstream RAS/RAF/mitogen-activated protein kinase (MAPK) signaling pathway were expressed differentially between stromal cells from DIE and cells from matched eutopic endometrium (EE; e.g., up-regulation: PDGFRA, PKCβ1, JAK1; down-regulation: Sprouty2, MKK7).

Furthermore, three potential candidate genes were identified that might be involved in endometriosis-related pain: tyrosine kinase receptor B (TrKB) in endometriotic epithelial cells and serotonin transporter (5HTT) and μ-opioid receptor (MOR) in endometriotic stromal cells all were up-regulated.

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Endometriosis is a heterogeneous disease based on location and clinical outcome. Different theories of histogenesis that have been proposed for the different forms of endometriosis are based on location (4). It is, therefore, necessary to investigate whether these potential candidate genes identified in our previous study also are differentially expressed in other forms of endometriosis such as ovarian endometriosis (OE).

Additional results will provide more information to identify endometriosis gene expression markers that can be used for development of effective strategies for prevention and treatment, as well as to gain a better understanding of the pathophysiology and disease etiology.

In the present study, we investigated mRNA expression levels of these potential candidate genes in OE and matched EE by using LCM and real-time polymerase chain reaction (PCR) techniques.

MATERIALS AND METHODS

Patients

Patients undergoing laparoscopy for OE were recruited for this study beginning in May 2001 in the Polyclinique de l'Hotel Dieu, CHU Clermont-Ferrand (Clermont-Ferrand, France). All tissue samples were obtained with full and informed patient consent. The research protocol was approved by the Consultative Committee for Protection of Persons in Biomedical Research of the Auvergne region of France.

All patients did not receive hormonal treatments, such as GnRH agonist or sex steroids, and did not use intrauterine contraception for ≥6 months before surgery. Recruited patients had regular menstrual cycles (between 26 and 32 days), had their menstrual history confirmed, and had serum 17β E_2 and P levels measured just before surgery.

The endometrial dating criteria as described by Noyes et al. (5) in 1950 and the menstrual history were used to assess the menstrual cycle phase. Endometrial tissue biopsies were performed just before operation with an endometrial suction catheter (Pipelle; Laboratoire CCD, Paris, France).

Finally, a total of 12 patients (6 patients during the proliferative phase and 6 during the secretory phase) were selected for the present study. All patients had complaints of pain and infertility before surgery.

Samples of OE and the matched EE were divided into two portions. The first tissue portion was fixed in 10% formalin–acetic acid and embedded in paraffin for histopathological examination. The second portion was immediately collected in RNAlater (Ambion, Cambridgeshire, UK) and stored at −20°C until further analysis was performed.

Laser Capture Microdissection

From each fresh-frozen tissue sample, 8-μm-thick frozen sections were prepared for endometriotic tissues, and 10-μm-thick frozen sections, for the matched EE. Sections were mounted on positively charged slides (Super frost Plus; Menzel GmbH, Braunschweig, Germany). Hematoxylin and eosin staining on frozen sections was performed by using the National Cancer Institute protocol (http://cgap-mf.nih.gov/Protocols/index.html), with some minor modifications as described elsewhere (1).

Briefly, slides were fixed in 70% ethanol for 15 seconds and stained with hematoxylin and eosin, followed by dehydration in two 15-second washes in 95% ethanol, two 60-second washes in 100% ethanol, and two final washes in xylene for 3 minutes each. Slides were air-dried for 5 minutes and were stored in a desiccator for no more than 1 hour.

Glandular epithelial cells and stromal cells from endometrial or endometriotic tissues were isolated from the slides by using the PixCell II LCM System (Arcturus, Plaisir, France) according to the manufacturer's instructions. A 7.5-μm and 15-μm beam diameter were used for epithelial cells and for stromal cells, respectively. Microdissected cells were collected on optically transparent LCM Macro caps for EE and on LCM HS caps for endometriotic tissue (Arcturus).

Extraction and Quantification of RNA

After LCM, RNA extraction was performed by using the Picopure RNA extraction kit (Arcturus). The caps were placed in microcentrifuge tubes (Eppendorff, le Pecq, France) containing lysis buffer and were incubated at 42°C for 30 minutes.

After centrifugation, the caps were removed, and RNA was isolated by using the Picopure RNA extraction protocol. To eliminate potential genomic DNA contamination, RNA samples were treated with DNaseI (15 U; Qiagen, Courtaboeuf, France) at reverse transcriptase for 15 minutes. Finally, total RNA was resuspended in 11 μL of RNase-free water and was kept at −80°C until needed.

Quantities of RNA were measured by using the RiboGreen RNA Quantitation Kit (Molecular Probes Europe BV, Leiden, the Netherlands). All procedures were performed according to the manufacturer's instructions.

Quantitative Real-Time Reverse-transcriptase PCR

Quantitative real-time reverse-transcription PCR with a LightCycler (Roche, Mannheim, Germany) was performed on nonamplified total RNA from microdissected tissues. Total RNA (10 ng) was subjected to a reverse-transcription reaction by using Superscript II Reverse Transcriptase (Invitrogen).

Quantitative real-time PCR was performed in a Light Cycler system by using the Fast Start DNA master SYBR green I kit as recommended by the manufacturer (Roche). In a total volume of 20 μL, each reaction contained 2 μL of SYBR Green I reaction mix (consisting of Taq DNA-polymerase reaction buffer, deoxyribonucleotide triphos-