Overexpression of lysine-specific demethylase 1 in ovarian endometriomas and its inhibition reduces cellular proliferation, cell cycle progression, and invasiveness

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Objective: To investigate whether lysine-specific demethylase 1 (LSD1) is aberrantly expressed in endometriomas and whether treatment with tranylcypromine, an LSD1 inhibitor, has any effect on cell viability, cell cycle, and invasiveness.
Design: Laboratory study using human tissues.
Setting: Academic hospital.
Patient(s): Forty-two ectopic endometrial tissue samples, their homologue eutopic endometrial tissue samples, and 70 control endometrial tissue samples.
Intervention(s): Immunohistochemistry analysis of LSD1 of all human tissue samples, and Western blot analysis, quantitative real-time reverse-transcription polymerase chain reaction analysis, cell viability assay, cell cycle analysis, and invasion assay of eutopic and ectopic endometriotic stromal cells and normal endometrial stromal cells.
Main Outcome Measure(s): Immunostaining levels of LSD1, gene and protein expression levels, cell viability, cell cycles, and invasiveness.
Result(s): The expression of the LSD1 gene and protein in endometriosis was elevated. Treatment of endometriotic stromal cells with tranylcypromine statistically significantly reduced the cellular proliferation, cell cycle progression, and invasiveness.
Conclusion(s): Because DNA and histones are intimately intertwined and work in concert in transcription regulation, conceivably histone demethylation activity of LSD1 could be wide ranging. The inhibition of LSD1 activity by tranylcypromine and the resultant inhibition of proliferation, cell cycle progression, and invasiveness suggest that LSD1 may be a candidate therapeutic target for endometriosis. (Fertil Steril® 2014;101:740–9. ©2014 by American Society for Reproductive Medicine.)
Key Words: Endometriosis, invasiveness, lysine-specific demethylase 1, monoamine oxidase inhibitor, tranylcypromine

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Endometriosis, characterized by the presence and growth of functional endometrial-like tissues outside the uterine cavity, is a common and benign gynecologic disorder with poorly understood etiopathogenesis and pathophysiology (1). It is a leading cause of disability in women of reproductive age, responsible for dysmenorrhea, pelvic pain, and subfertility (2). In the last decade or so, evidence has been accumulating that epigenetic regulation of gene expression is dysregulated in endometriosis (3). The dysregulation ranges from aberrant DNA methylation in homeobox A10 (HOXA10) (4–6), progesterone receptor isoform B (PR-B) (7, 8), estrogen receptor β (ERβ) (9), SF-1 (10), E-cadherin (11), aromatase (12, 13), and cyclooxygenase 2 (COX-2) (14), to aberrant methylation of microRNA miR-196b (15) and to aberrant expression of genes involved in DNA methylation (16–18) and histone

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deacetylation (19–21). Very recently, it has been reported that CCAAT/enhancer-binding protein α is epigenetically silenced by histone deacetylation in endometriosis (22). Along this line, it has been shown by numerous in vitro and in vivo studies that endometriosis is possibly treatable by rectifying epigenetic aberrations through pharmacologic means (3, 23–26). In fact, valproic acid, a histone deacetylase (HDAC) inhibitor, and dopamine agonists are the only two classes of a drug that have been pilot-studied in humans with promising results (27).

Epigenetic regulation of gene transcription is mediated by a diverse family of protein complexes, including chromatin remodeling and transcription factors (28). One important way of chromatin remodeling is through posttranslational modification of histones, including acetylation, phosphorylation, methylation, and ubiquitination. The combination of various histone modifications at a given promoter specifies whether gene promoters are in an active or a repressive conformation (29). In particular, histone lysine methylation has recently been emerged as a key determinant of transcriptional regulation (30). The first protein showing histone lysine (K) demethylase (KDM) activity identified in mammals is a flavin-containing amino oxidase (AO) that specifically demethylated mono- or di-methylated lysine 4 at histone H3 (H3K4me1 and H3K4me2) and has been named lysine-specific demethylase 1 (LSD1) or KDM1A (31). Although dysregulation of genes responsible for histone demethylation including LSD1 has been shown extensively to be involved in cancer development (32), whether these genes are dysregulated in endometriosis is completely unknown.

LSD1 catalyzes lysine demethylation via a flavin adenine dinucleotide (FAD)-monoamine oxidase (MAO) mechanism (33); as such, it can be inhibited by compounds related to the MAO inhibitor (MAOI) class of pharmaceuticals (34). LSD1 has been shown to be intimately involved in DNA methylation (35), to interact with nuclear hormone receptors such as ER (36), and to have a potential role in the repression of E-cadherin and thus in the epithelial-mesenchymal transition (EMT) (37) and in regulation of vascular-endothelial growth factor A (VEGF-A) (38). More importantly, it has been reported recently there is a global H3K4 and H3K9 hypomethylation in endometriosis (39). In view of these studies, we hypothesized that LSD1 may be dysregulated in ovarian endometriomas. Thus, we determined the LSD1 immunoreactivity in eutopic and ectopic endometrium from women with endometriomas and in control endometrium. With evidence that LSD1 is dysregulated in endometriosis, we examined whether treating endometriotic stromal cells with a MAOI has any effect on cellular proliferation, cell cycle progression, and invasion.

MATERIALS AND METHODS

Patients and Specimens

Ectopic and paired eutopic endometrial tissue samples were obtained after informed consent from 42 cycling, premenopausal women with laparoscopically and histologically diagnosed ovarian endometriomas who received no hormonal treatment at least 3 months prior to the surgery. The specimens were fixed with 10% formalin neutral buffer solution for immunohistochemistry analysis. Among them, 10 ectopic endometrial tissue samples were also used for primary culture of eutopic endometrial stromal cells (UESCs) and ectopic endometrial stromal cells (EESCs), which were used for Western blot analysis, proliferation and invasion assays, and cell sorting.

As controls, endometrial tissue samples were obtained after informed consent from 70 cycling, premenopausal women who had been age- and menstrual phase-matched with the endometriosis patients. The controls were undergoing surgery for benign gynecologic disorders but were free of endometrial abnormalities, endometriosis, adenomyosis, uterine fibroids, or gynecologic cancers. Among these patients, 42 (60.0%) had ovarian teratoma or other benign cysts but no endometrioma, 9 (12.9%) had recurrent cervical intraepithelial neoplasia III and were undergoing laparoscopic surgery, 11 (15.7%) had a septate uterus, and 8 (11.4%) were being treated for subfertility and underwent both hysteroscopic and laparoscopic examinations. Among this group, 9 endometrial tissue samples were also used for primary culture of normal endometrial stromal cells (NESCs).

For all recruited participants, information on the phase of the menstrual cycle (determined by histologic endometrial dating), severity of dysmenorrhea (on a verbal descriptor scale, namely, none, mild, moderate, and severe), parity, size of the largest endometrioma (for patient group only), bilaterality, and revised American Society for Reproductive Medicine (ASRM) staging were retrieved for patients with endometriosis and controls. This study was approved by the institutional ethics review board of Shanghai Obstetrics and Gynecology Hospital.

Immunohistochemistry and Histochemistry

Tissue samples of control endometrium (n = 70), ectopic endometrium (n = 42), and their homologue eutopic endometrium (n = 30) were fixed with 10% formalin and paraffin-embedded. Serial 4-μm sections were obtained from each block, with the first resultant slide stained for hematoxylin and eosin (H&E) to confirm the pathologic diagnosis, and the subsequent slides stained for LSD1. Routine deparaffinization and rehydration procedures were performed.

For antigen retrieval, the slides were heated at 98°C in an EDTA-Tris buffer (pH 9.0) for a total of 30 minutes and cooled naturally to room temperature. Sections were then incubated with the LSD1 primary antibody (1:100; Novus Biologicals) overnight at 4°C. After the slides were rinsed, the horseradish peroxidase (HRP)-labeled secondary anti-rabbit antibody Detection Reagent (Shanghai Sun BioTech Company) was incubated at room temperature for 30 minutes. The bound antibody complexes were stained for 3 to 5 minutes or until appropriate for microscopic examination with diaminobenzidine and then counterstained with hematoxylin (30 seconds) and mounted. A human invasive breast cancer tissue sample was used as the positive control. For the negative control, a normal human endometrium tissue sample was incubated with rabbit serum instead of the primary antibody.

Nuclear immunostaining results for LSD1 were evaluated using a semiquantitative scoring system, as reported previously

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