Aberrant expression and localization of deoxyribonucleic acid methyltransferase 3B in endometriotic stromal cells

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Objective: To define the expression and function of DNA methyltransferases (DNMTs) in response to decidualizing stimuli in endometriotic cells compared with healthy endometrial stroma.
Design: Basic science.
Setting: University research center.
Patient(s): Premenopausal women with or without endometriosis.
Intervention(s): Primary cultures of stromal cells from healthy endometrium (E-IUM) or endometriomas (E-OSIS) were subjected to in vitro decidualization (IVD) using 1 μM medroxyprogesterone acetate, 35 nM 17β-estradiol, and 0.05 mM 8-Br-cAMP.
Main Outcome Measure(s): Expression of DNMT1, DNMT3A, and DNMT3B in E-IUM and E-OSIS were assessed by quantitative real-time polymerase chain reaction and immunoblotting. Recruitment of DNMT3B to the promoters of steroidogenic factor 1 (SF-1) and estrogen receptor α (ESR1) was examined by chromatin immunoprecipitation.
Result(s): IVD treatment reduced DNMT3B messenger RNA (74%) and protein levels (81%) only in E-IUM; DNMT1 and DNMT3A were unchanged in both cell types. Significantly more DNMT3B bound to the SF-1 promoter in E-IUM compared with E-OSIS, and IVD treatment reduced binding in E-IUM to levels similar to those in E-OSIS. Enrichment of DNMT3B across 3 ESR1 promoters was reduced in E-IUM after IVD, although the more-distal promoter showed increased DNMT3B enrichment in E-OSIS after IVD.
Conclusion(s): The inability to downregulate DNMT3B expression in E-OSIS may contribute to an aberrant epigenetic fingerprint that misdirects gene expression in endometriosis and contributes to its altered response to steroid hormones. (Fertil Steril® 2015;104:953–63. ©2015 by American Society for Reproductive Medicine.)
Key Words: Endometriosis, DNA methyltransferase, decidualization, estrogen receptor, steroidogenic factor-1

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The human endometrium consists primarily of epithelial and stromal cells that undergo continued cycles of growth and shedding to maintain fertility. This cycle requires remarkable plasticity in the endometrial stroma, which must renew, as well as differentiate, in response to the sex steroids. The differentiation of estrogen-primed, uterine, stromal cells into the stromal cells of pregnancy is termed decidualization, and is a feature common to most placental mammals (1). However, in humans and menstruating primates, decidualization is initiated in response to ovarian hormones, independently of an implanting blastocyst (2). Full differentiation and maintenance of the decidua is contingent on pregnancy, because the absence of a conceptus provokes shedding of the predecidualized endometrial layer and

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menstruation, and the possibility that the evolution of spontaneous decidualization necessitated menstruation has been suggested (3). This intriguing observation led us to propose that the gene regulatory pathways that coordinate spontaneous decidualization in menstruating primates are the key pathways dysregulated in endometriosis (4).

Endometriosis affects 5%–10% of women of reproductive age. It is a chronic and recurrent disease characterized by the presence of endometrium-like tissue outside the uterine cavity, primarily on the ovaries and pelvic peritoneum (5, 6). The main symptoms are severe dysmenorrhea, chronic pelvic pain, dyspareunia, and commonly, infertility (5, 6). Although estrogen is crucial for the establishment and maintenance of endometriosis, the exact origin of the disease remains enigmatic (6–8).

Most evidence supports Sampson's model of endometriosis, suggesting that the disease arises from innate or acquired defects in endometrial tissue that has been shed to distal sites by retrograde menstruation (9–11). However, many cases of the disease cannot be explained by an endometrial origin, and nearly all women experience retrograde menstruation, a fact that is difficult to reconcile with only a fraction developing the disease (12, 13). Recent studies characterizing epigenetic defects in endometriosis may help explain this link. The identification of genes whose expression is correlated with aberrant DNA methylation in endometriotic cells has undergone explosive growth (4,14–23), including many of the nuclear hormone receptors such as estrogen receptor alpha and beta (ESR1 and ESR2) and steroidogenic factor-1 (SF-1). In addition to supporting a role for epigenetic defects in the pathogenesis of endometriosis, many of these genes remarkably serve key roles during decidualization (4, 24, 25).

Methylation of DNA is initiated and maintained by three DNA methyltransferases (DNMTs)—DNMT1, DNMT3A, and DNMT3B—which catalyze the addition of methyl groups to the 5′ carbon of cytosine in targeted cytosine-phosphate-guanine dinucleotides (CpGs) (26). Unlike cancer, in which the frequent hypermethylation of CpG islands (CGIs) is observed against a backdrop of global hypomethylation, experiments comparing global methylation in healthy endometrium with endometriosis have identified more focused deviations in methylation (4, 27).

Our recent work demonstrated that CpGs near transcriptional start sites, but distal to classical CpG islands, were significantly altered in endometriotic cells; however, the mechanism by which specific CpGs are altered is unknown. Studies examining the DNMTs and their binding partners in the endometrium have identified highly variable expression levels throughout the menstrual cycle, but consensus has not been reached on the levels of functional protein in decidualizing stroma (28–32). Additionally, few genome-wide changes in methylation have been observed during decidualization; thus, the effects of the DNMTs in the endometrium may be more tightly coordinated across key genes affecting stromal cell function (14, 32).

Ovarian steroids have been reported to alter the expression of DNMTs as well, in both the eutopic endometrium of women with endometriosis, and in endometriotic lesions. Again, no consensus has been reached on how the DNMTs are expressed in the diseased stromal cells. We and others have suggested that these DNMTs might affect key targets associated with disease progression (33).

We hypothesize that hormonally driven changes in expression of DNMTs alter their genomic distribution in endometriotic cells, which may underlie the pathologic epigenetic defects observed in diseased tissues. Without prior knowledge of how DNMTs affect early stages of the disease, we sought to characterize DNMTs in stromal cells that were clearly healthy or diseased, and compared normal eutopic endometrium (E-IUM) to established endometriosis (E-OSIS). Herein, we characterize the expression of all three DNMTs in vitro, identifying altered DNMT3B expression in E-OSIS. The functional outcomes of these findings were explored by defining DNMT3B occupation at known, differentially methylated loci in 2 key genes affecting endometriosis: *ESR1* and *SF-1*.

MATERIALS AND METHODS

Tissue Acquisition

The Northwestern Institutional Review Board for Human Research approved this study (1375-005). Written, informed consent from each subject was obtained before surgery. Eutopic endometrium was obtained from subjects who did not have surgically confirmed endometriosis (average age: 43.8 ± 4.2 years) and were undergoing hysterectomy for benign conditions (cervical dysplasia or uterine leiomyoma). Ectopic endometriotic tissue from the cyst walls of surgically diagnosed ovarian endometriomas was obtained immediately after surgery from subjects whose average age was 40.5 ± 2.4 years. No subject received any preoperative hormonal therapy, and all surgeries were performed during the proliferative stage of the patients' menstrual cycle. Endometriosis was confirmed in the E-OSIS samples by histologic examination.

Isolation and Culture of Primary Stromal Cells

Unless otherwise specified, all chemicals and reagents were obtained from Sigma. The E-IUM and E-OSIS stromal cells were isolated from normal endometrium and endometriotic lesions as previously described, with minor modifications (34, 35). Tissues were first dissected by a pathologist. Following evaluation of tissues for pathology, the stromal and glandular tissues were carefully dissected from surrounding uterine (for E-IUM) or ovarian endometriotic (for E-OSIS) tissues. Tissues were minced and digested, first with collagenase and DNase at 37°C for 30 minutes, and second with collagenase, DNase, pronase, and hyaluronidase for an additional 30 minutes. Stromal cells were separated from epithelial cells by progressive filtration through 70- and 20-μm sieves. After this step, cells were dispensed for adherent growth and maintained in DMEM/F12 1:1 (Gibco BRL), supplemented with 10% FBS, 1,000 units/mL penicillin G, 0.1 mg/mL streptomycin sulfate, and 0.25 μg/mL Amphotericin B. Adherent cells were grown in a humidified atmosphere, with 5% CO_2 at 37°C.