Whole genome deoxyribonucleic acid microarray analysis of gene expression in ectopic versus eutopic endometrium

Kathleen M. Eyster, Ph.D.,[a,b,c] *Olga Klinkova, M.D.,*[a] *Vanessa Kennedy, B.S.,*[a] *and Keith A. Hansen, M.D.*[b,c,d]

[a] Division of Basic Biomedical Sciences, Sanford School of Medicine of the University of South Dakota, Vermillion; and [b] Women's Health Research Center, [c] Department of Obstetrics and Gynecology, and [d] Sioux Valley Hospital and USD Medical Center, Sanford School of Medicine of the University of South Dakota, Sioux Falls, South Dakota

Objective: To use DNA microarrays to identify differentially expressed genes in eutopic endometrium compared with ectopic endometrium.
Design: Prospective, cross-sectional, observational study.
Setting: University Medical Center and Research Laboratory.
Patient(s): Eleven women with endometriosis.
Intervention(s): None.
Main Outcome Measure(s): Differential gene expression.
Result(s): Seven hundred seventeen of the 53,000 probes on the whole human DNA microarrays were changed by twofold or greater in ectopic versus eutopic endometrium. Families of genes that were expressed differentially include genes that code for proteins associated with the immune system and inflammatory pathways, cell adhesion, cell-cell junctions, the extracellular matrix and its remodeling, cytoskeletal proteins, and signal transduction pathway components, among others.
Conclusion(s): The altered immune environment may allow survival of endometriotic cells that enter the abdominal cavity. Alterations of cell adhesion–associated genes may contribute to the adhesive and invasive properties of ectopic endometrium, and changes in signal transduction pathways support a change in the communication among cells of the endometrial explant compared with eutopic endometrium. These families of differentially expressed genes provide multiple opportunities for the development and testing of new hypotheses regarding endometriosis. (Fertil Steril® 2007;88:1505–33. ©2007 by American Society for Reproductive Medicine.)

Key Words: Endometriosis, differential gene expression, DNA microarray, phospholipase A_2

Endometriosis is defined by the presence of endometrial tissue growth outside of the uterus (1). It is a debilitating disease that affects up to 10% of women of reproductive age (1). Despite extensive research, the etiology of endometriosis remains obscure. The leading hypothesis regarding the etiology of endometriosis is that retrograde menstruation results in the entry of endometrial cells into the peritoneal cavity; these cells then implant and grow on tissues in the peritoneal cavity (1, 2). Alternative hypotheses are that endometriosis arises from metaplasia of the coelomic epithelium, from embryonic rests, or via lymphatic transport of endometrial cells in menstrual debris (1, 2). Another hypothesis is that the endometrium of women in whom endometriosis develops is fundamentally different from that of women in whom endometriosis does not develop (3).

There is also little understanding of the pathologic processes that are involved in endometriosis. Conventional cellular and molecular biology techniques have been used to study protein and gene expression patterns in endometriosis in an attempt to better understand the pathology of the disease. A variety of studies have examined gene expression in endometriosis one gene at a time; these studies have been well reviewed and summarized (4, 5). Examples of genes that have been reported to exhibit differential expression in eutopic versus ectopic endometrium include intercellular adhesion molecule (ICAM-1) (4), integrins (4), tissue inhibitor of metalloproteinases (TIMP-2) (4, 5), haptoglobin (6, 7), aromatase (4, 8), and 17β-hydroxysteroid dehydrogenase (17β-HSD) (9). Moreover, growth factors and cytokines that

Received June 24, 2006; revised and accepted January 11, 2007.
Supported by the Department of Obstetrics and Gynecology, Sanford School of Medicine of the University of South Dakota. The Genomics Core Facility at the University of South Dakota is supported by National Institutes of Health 2 P20 RR016479.
Reprint requests: Kathleen M. Eyster, Ph.D., Sanford School of Medicine of the University of South Dakota, 414 E. Clark St., Vermillion, SD 57069 (FAX: 605-677-6381; E-mail: Kathleen.Eyster@usd.edu).

regulate these proteins have been shown to be altered. For example, interleukin-6, interleukin-8, and tumor necrosis factor-α are cytokines involved in the regulation of angiogenesis and extracellular matrix proteins that have been reported to be elevated in the peritoneal fluid of women with endometriosis (4). In addition, cell cycle and apoptosis proteins that regulate the proliferation and death of endometrial cells may be altered (10, 11). Recently new mouse models for endometriosis and ovarian cancer have implicated K-ras in the development of endometriosis-like lesions in the mouse (12, 13).

The global gene expression analysis method of DNA microarray has also been applied to endometriosis. Our group used an earlier DNA microarray platform that contained only a fraction of the human genome (approximately 3,000 genes) to analyze gene expression in ectopic versus eutopic endometrium (14). Matsuzaki et al. (15) used laser capture microdissection to obtain epithelial and stroma cells from deep endometriosis and compared them with eutopic endometrium using microarrays that contained 1,176 genes. Similarly, Wu et al. (16) also used laser capture microdissection to obtain epithelial cells from endometriosis lesions and compared gene expression with epithelial cells from eutopic endometrium using microarrays containing 9,600 genes. Arimoto et al. used microarrays containing 23,040 genes to compare gene expression in ovarian endometrial cysts with that of eutopic endometrium (17). In contrast to these studies, Kao et al. (18) used DNA microarray technology to examine differential gene expression in eutopic endometrium from women with endometriosis and that of eutopic endometrium from women without endometriosis to examine the hypothesis that the endometrium of women with endometriosis is fundamentally different from that of women in whom the disease does not develop. However, their experiment was not designed to determine whether the differential expression of genes was a result of the presence of the endometriosis explants or a cause of the disease.

Collectively, the studies cited above support the hypothesis that endometriosis is the result of abnormal expression or regulation of certain key genes. In this project we used DNA microarrays containing 53,000 human genes/transcribed sequences to analyze gene expression patterns in ectopic and eutopic endometrium from the same patients as a means of identifying families of genes involved in the pathology of endometriosis.

MATERIALS AND METHODS

Consecutive subjects (N = 11) scheduled for surgery for infertility or pain were recruited to participate in this protocol. The study was approved by the Sanford School of Medicine of the University of South Dakota Institutional Review Board, and informed consent was obtained from all participants. None of the authors have any conflict of interest with the components of this study.

Ten patients were scheduled for surgery for infertility or pelvic pain during 6 to 12 days following the onset of menses. The remaining patient was scheduled for surgery 3 weeks after completing 6 months of leuprolide acetate (LA; TAP Pharmaceuticals, Lake Forest, IL). No patients were receiving hormone therapy at the time of the study. The patients ranged in age from 28 to 45 years (average age 32 years). Ten patients were white, and one was African American. Ten of the 11 patients were nulliparous.

Laparoscopy and hysteroscopy procedures were performed during the same surgical intervention. Endometriotic peritoneal implants or the cyst wall of endometriomas were removed at laparoscopy. Hysteroscopy and dilatation and curettage were performed to obtain a sample of eutopic endometrium from the same patient. The tissues were rinsed in lactated Ringer's solution and divided in half. One half of the tissue was immediately placed in an RNA protection medium, RNAlater (Ambion, Austin, TX), and placed on ice for transport to the research laboratory. The other half was sent to the pathology laboratory.

The diagnosis of endometriosis was confirmed by the presence of glands and stroma in the endometriotic lesions. The severity of endometriosis was classified with use of the American Society for Reproductive Medicine revised classification of endometriosis (19). The endometriomas of four patients were classified as severe, and two were classified as mild to moderate. The peritoneal explants of two patients were classified as severe, and three were mild to moderate. The eutopic endometrium was used to identify the phase of the uterine cycle. Although surgeries were scheduled for the follicular phase, nine of the 11 samples were found to be in the proliferative phase, one was early secretory phase, and one was benign inactive endometrium (previously taking LA). The one patient whose eutopic endometrium was classified as early secretory phase had ectopic endometrium classified as severe endometrioma. The patient who previously had been taking LA had ectopic endometrium that was classified as severe peritoneal explants; the pathologic diagnosis suggests that the ectopic endometrium was not quiescent although the eutopic endometrium was inactive.

Ribonucleic Acid Extraction

Eutopic and ectopic endometrium were obtained at surgery as described above. Total RNA was extracted from tissues with use of a modification of a method previously reported by our laboratory (20). Up to 30 mg of tissue was homogenized in 1 mL of TRI reagent (Molecular Research Center, Cincinnati, OH) with a Polytron homogenizer. Two hundred microliters of bromochloropropane and 60 μL 3M sodium acetate were added to the homogenate. After centrifugation the aqueous layer was removed, mixed with RLT buffer (Qiagen, Valencia, CA) and ethanol, and centrifuged through a Qiagen RNeasy column. The column was washed and the sample treated with ribonuclease-free deoxyribonuclease (Qiagen) on the column to remove any potentially contaminating