Immune-inflammation gene signatures in endometriosis patients

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Objective: To determine if the molecular profiles of endometriotic lesions contain informative measures of inflammation and immune dysfunction that may contribute to better understanding of the interplay between immune dysfunction and inflammation and their contribution to endometriosis pathogenesis.
Design: Immune and inflammation transcriptomic analysis with the use of the Nanostring nCounter GX Human Immunology V2 platform (579 human immune and inflammation–related genes and 15 housekeeping genes).
Setting: Academic university and teaching hospital.
Intervention(s): None.
Patient(s): Stage III–IV endometriosis patients with infertility (n = 8) and fertile disease-free control women undergoing tubal ligation (n = 8). Menstrual stage was matched to secretory phase in all participants.
Main Outcome Measure(s): Immune and inflammation transcriptomics quantification from ectopic endometriotic lesions and matched eutopic endometrium from patients. Endometria of fertile women served as control subjects.
Result(s): Our results displayed endometriotic lesions as molecularly distinct entities compared with eutopic endometrium and endometrium of control samples; 396 out of 579 screened immune and inflammation–related genes were significantly different in ectopic tissues compared with control endometrium. Most importantly, eutopic endometrium of the patients displayed a unique molecular profile compared with the control endometrium (91/579 genes were significantly different), particularly of genes involved in regulation of cell apoptosis and decidualization.
Conclusion(s): We characterize differential expression of immune-inflammation genes in endometriosis patients, and show molecular distinction of eutopic endometrium of patients compared with control fertile women. (Fertil Steril® 2016;■:■–■. ©2016 by American Society for Reproductive Medicine.)
Key Words: Angiogenesis, endometriosis, immune genes, infertility, inflammation

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Endometriosis is a disease driven by the inflammatory peritoneal environment (1, 2). Characterized by the growth of endometrial-like tissue in ectopic locations, endometriosis affects millions of women worldwide with chronic pelvic pain and subfertility (3). Despite decades of research, pathogenesis of endometriosis is incompletely understood, and multiple theories exist regarding its etiology (4). Since Sampson's theory of retrograde menstruation, which proposed endometrial fragments as the potential source of endometriosis (5), numerous studies have documented the high prevalence of retrograde menstruation in women with endometriosis (6). However, that theory does not adequately explain disease prevalence, because women with and without endometriosis commonly demonstrate retrograde menstruation (7, 8), but the disease is present in only 2%–10% of women (9). Indeed, dysregulation of the immune response toward endometriotic lesions has been noted in patients, including increased inflammatory cytokines and overreactive macrophages and neutrophils in the peritoneal cavity (10, 11). Additionally, autoimmune diseases are commonly diagnosed in endometriosis patients (12), which further strengthens the notion of dysfunctional immune regulation in women with endometriosis. To provide improved therapeutic options and to increase the diagnostic power of this detrimental disease, it is imperative that we attempt to dissect the molecular profile of endometriosis pathogenesis (13). To this end, we

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hypothesized that endometriotic lesions have a distinct molecular profile that will provide insights into biologic mechanisms and pathways that govern the immune dysfunction and inflammation in women with endometriosis.

In this study, we profiled the expression of 579 genes involved in immunology and inflammation with the use of the nCounter GX Human Immunology v2 Kit (Nanostring Technologies), from three tissue sources: pelvic endometriotic lesions, matched eutopic endometrium from the patients, and endometrium samples from fertile (disease-free) women as control samples. All of our samples were matched to the secretory phase of the menstrual cycle. To our knowledge, this study is the first to attempt molecular profiling of differential immune and inflammation gene expression between matched ectopic tissues and eutopic endometrium from patients, ectopic and control endometrium, and eutopic and control endometrium that are also matched by menstrual cycle. Our results demonstrate dysregulation of a number of specific pathways involving leukocyte activation and cytokine-cytokine receptor interaction in endometriosis samples. In addition, our data provides insights into immune gene expression profile of eutopic endometrium of women with endometriosis compared with control endometrium samples.

MATERIALS AND METHODS

Ethics Approval

Human eutopic endometrial and ectopic endometriosis tissue samples were collected from endometriosis patients, and endometrial samples were collected from control subjects comprising healthy women after informed consent with the use of a protocol approved by the Institutional Review Committees at Greenville Health Systems, Greenville, South Carolina, and the University of North Carolina, Chapel Hill, North Carolina. Ethics approval for this study was provided by the Health Sciences Research Ethics Board, Queen's University, Kingston, Ontario, Canada.

Sample Collection from Patients and Control Women Undergoing Laparoscopic Surgery

Matched human endometrium (n = 8) and endometriosis samples (n = 8) from patients with stage III–IV endometriosis were provided by Greenville Hospital Systems, Greenville, South Carolina, after informed consent from patients. The mean age and body mass index (BMI) of the patient group were 33.1 ± 7.3 years and 24.2 ± 4.0 kg/m^2, respectively. Out of the eight patients, four patients were nulligravida, three were primigravida, and one was multigravida. The stage of endometriosis was determined based on the revised American Society of Reproductive Medicine criteria (14). From each endometriosis patient undergoing laparoscopic removal of the disease, the eutopic endometrium samples were obtained by means of Pipelle sampling. The patient samples used in this study comprised women diagnosed with infertility and/or pelvic pain, and all of the women, including patients and fertile control subjects, were free from hormonal therapy for 3 months before the collection of samples. For control samples, endometrial biopsies (n = 8) were obtained by means of Pipelle sampling from healthy fertile women who underwent tubal ligation at the University of North Carolina. The mean age and BMI of the control group were 26 ± 5.8 years and 23.4 ± 2.6 kg/m^2, respectively. All eight healthy control subjects had no history of pregnancy. All samples were snap-frozen with the use of liquid nitrogen and then stored at −80°C until further use. All patients and healthy control subjects were at the secretory phase of the menstrual cycle when samples were obtained.

Gene Expression Profiling with the Use of Nanostring nCounter GX Human Immunology v2 Kit

Total RNA was isolated from all samples with the use of Norgen Biotek Total RNA isolation kit (no. 17200) per the manufacturer's instructions. In brief, tissues were homogenized in 600 μL lysate buffer by means of an electrical mortar and pestle and then were centrifuged at 15,000 rpm for 1 minute at 20°C. The resulting supernate was collected, mixed with 70% ethanol, and passed through the columns provided with the kit. The concentration and quality of RNA from each sample was assessed with the use of a Nanodrop 2000 Spectrophotometer (Thermo Scientific). All samples were normalized to 25–30 ng/μL with the use of RNAse-free distilled water. Nanostring nCounter analysis system–based gene expression profiling was performed on 100 ng total RNA from each sample as previously reported (14). Briefly. All RNA samples were subjected to analysis by means of nCounter Human Immunology v2 Panel consisting of 579 immune and inflammation–associated genes and 15 housekeeping genes as control samples in a prebuilt panel. The samples were subjected to overnight hybridization reaction at 65°C, where 5 μL of total RNA samples were combined with 20 μL of nCounter Reporter probes in hybridization buffer and 5 μL nCounter capture probes for a total reaction volume of 30 μL. Post-hybridization of probes with targets of interest in the samples, the abundance of target molecules, was quantified with the use of the nCounter Digital Analyzer and assessed with the use of the nSolver platform.

Statistical Analysis, Marker Selection Analysis, and Hierarchic Clustering with the Use of GENE-E Software

The nCounter human Immunology v2 panel included 15 housekeeping genes—eight negative and six positive control samples—which were used for background subtraction and normalization of the raw mRNA transcript counts of all samples. With the use of nSolver data analysis software provided by Nanostring technologies, the raw data were normalized with the use of the geomean of all six positive control subjects, in addition to the geomean of the housekeeping genes that displayed percentage coefficient of variation (%CV) of ≤55. The list of ten housekeeping genes used in the normalization process is shown in Supplemental Table 1 (Supplemental Tables 1–6 and Supplemental Figs. 1–7 are available online at