Identification of global transcriptome abnormalities and potential biomarkers in eutopic endometria of women with endometriosis: A preliminary study

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Abstract. The etiology and pathophysiology of endometriosis remain unclear. The aim of the current study was to identify a candidate pathogenic gene, as well as potential biomarkers of endometriosis using messenger RNA (mRNA) sequencing (mRNA-seq). Twenty-three eutopic endometria from women with endometriosis and 20 endometria from control subjects were investigated. Eight eutopic endometria and five normal endometria were selected for mRNA-seq. Differentially expressed genes (DEGs) were identified and functional analysis was conducted. Validation of certain DEGs was performed in the remaining cases and control subjects by reverse transcription-quantitative polymerase chain reaction (RT-qPCR). A total of 72 DEGs (66 upregulated and 6 downregulated) were identified in samples from women with endometriosis and compared with the control subjects. High DEGs included those involved in various functions, such as extracellular matrix (ECM) remodeling, angiogenesis, cell proliferation and differentiation. Enriched by these DEGs, 100 Gene Ontology terms were identified as significantly important, particularly 'ECM' and 'endogenous stimulus'. Validation using RT-qPCR indicated that matrix metallopeptidase 11, dual specificity phosphatase 1, Fos proto-oncogeneand serpin family E member 1 were significantly upregulated and adenosine deaminase 2 was significantly downregulated in the eutopic endometrium of patients with endometriosis. The identified DEGs may be involved in the pathogenesis of endometriosis and may be potential biomarkers in the eutopic endometrium. The current study provides a comprehensive, but preliminary insight for elucidating the mechanisms of endometriosis, which require further in-depth studies for confirmation.

Introduction

Endometriosis is characterized by the presence of endometrial tissue (glandular and stromal) abnormally outside the uterine cavity (1). According to certain statistics, there is an average of 10.4 years elapse from the first onset of symptoms to diagnosis, and 74% of patients receive at least one false diagnosis, which results in economic costs comparable with certain serious chronic diseases (2). Despite its significant impact on the quality of life and financial burden on patients, the etiology and pathophysiology of endometriosis remain unclear. Various theories have been proposed to explain the pathogenesis; however, none have interpreted it comprehensively (3). Sampson's retrograde menstruation theory is the most widely accepted, which proposes that fragments of the eutopic endometrium are implanted into the peritoneum or pelvic organs during menstruation through reflux via the fallopian tubes (4). Subsequently, extensive studies have been conducted to identify the differences between the eutopic and normal endometrium; indeed, various studies have demonstrated a distinct expression pattern in the eutopic endometrium compared with the normal endometrium, but the evidence is insufficient (5). In addition, laparoscopy remains the gold standard for the diagnosis of endometriosis and, to date, a histological examination is necessary for confirmation. However, this diagnostic method is a surgically invasive inspection requiring general anesthesia, which carries surgery-associated risks, such as hemorrhage, infection and adhesion formation, and requires an experienced surgeon (6). As a result of these drawbacks,

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non-invasive biomarkers using serum, urine and endometrial tissue as research samples have emerged; however, these current diagnostic tests for endometriosis lack sensitivity and specificity, and are difficult to repeat (7). Therefore, a thorough and comprehensive description of molecular differences between the eutopic endometrium in a patient with endometriosis and a control subject with a normal endometrium is essential to understand the pathogenesis of this disorder, and identify sensitivity and specificity biomarkers.

In the current study, it was hypothesized that the eutopic endometrium in endometriosis patients contains aberrant expression genes and exhibits dysregulated pathways that predispose itself to implant, invade and migrate outside the uterus. Messenger RNA (mRNA) sequencing was performed to detect the transcriptome expression profiling of eutopic endometrium in women with endometriosis compared with normal endometrium from healthy control subjects. Through global mRNA expression profiling, the aim was to identify candidate pathogenic genes and pathways that are implicated in the pathogenesis of endometriosis, as well as potential biomarkers of this common, clinically significant, but complex disorder.

Materials and methods

Ethics statement. The study protocol was approved by the Local Ethics Committee of Chinese People's Liberation Army (PLA) General Hospital (Beijing, China), and each patient was involved in the study after providing written informed consent.

Patient samples. Twenty-three eutopic endometria from patients with endometriosis were obtained from the Chinese PLA General Hospital between February and September 2016. Among them, eight eutopic endometria were randomly selected and prepared for mRNA sequencing (mRNA-seq), and the remaining 15 samples were used for validation. All patients were confirmed to have endometriosis by histological examination and diagnosed as being of moderate to severe stage (stage III-IV) according to the revised American Fertility Society (rAFS) classification (8) during laparoscopic surgery. None of the patients received hormone therapy prior to sampling. Twenty women without endometriosis, who underwent laparoscopic surgery for examination or hydrotubation, were included in the control group. Five endometria were randomly selected for sequencing analysis and 15 endometria were used for validation. Regions potentially exhibiting endometriotic lesions were confirmed as being negative by biopsy.

The eutopic and normal endometria were obtained via curettage prior to the laparoscopic procedure. Only patients in the secretory phase of the menstrual cycle, which was confirmed by the method of Noyes *et al* (9) were included in the study. There were no significant differences between the ages and body mass index values of the patient and control groups.

Tissue processing, RNA extraction and quality control. All tissue samples were divided into two parts: One-half was fixed and prepared for pathological examination to identify the endometrial phase of the menstrual cycle and the other half was placed in RNAlater solution (Sigma-Aldrich; Merck KGaA,

Darmstadt, Germany) at 4°C for 24 h, and subsequently transferred to -80°C until use. Total RNA was extracted using the single-step acid guanidinium thiocyanate-phenol-chloroform method (10). The quality and purity of RNA were examined using a Nanodrop 8000 spectrophotometer (Thermo Fisher Scientific, Inc., Waltham, MA, USA) and Bioanalyzer 2100 (Agilent Technologies, Inc., Santa Clara, CA, USA). Samples with RNA integrity number ≥8 were included.

mRNA sequencing and data analysis. Three micrograms of RNA per sample was prepared for library construction. The Ribo-Zero Gold kit (Epicentre; Illumina, Inc., San Diego, CA, USA) and NEB Next Ultra RNA Library Prep kit (New England BioLabs, Inc., Ipswich, MA, USA) were used for rRNA removal and library construction according to the manufacturer's instructions. For high-throughput sequencing, paired-end 150-bp sequencing of the cDNAs was performed using the Illumina HiSeq4000 system (Illumina, Inc.), which was conducted by Annoroad Genomics (Beijing, China). Raw data were processed with Perl scripts to ensure the quality of data used for further analysis. Bowtie2 (v2.2.3; https://sourceforge.net/projects/bowtie-bio/files/bowtie2/) was used for building the genome index, and clean data was mapped to the human genome build (hg19) using Tophat (v2.0.12; https://tophat.cbcb.umd.edu/). Read counts of each gene were counted by HTSeq (v0.6.0; http://www-huber.embl.de/users/anders/HTSeq/doc/overview.html), and reads per kb of a gene per million reads (RPKM) were subsequently calculated to estimate the expression level of genes in each sample. DEGseq (v1.18.0; http://www.bioconductor.org/packages/release/bioc/html/DEGseq.html) was used for analyzing differentially expressed genes (DEGs) with the following parameters: False discovery rate (FDR) ≤0.05 and fold-change (FC) ≥2 or ≤0.5.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Five mRNAs, including matrix metallopeptidase 11 (MMP-11; ENSG00000099953), dual specificity phosphatase 1 (DUSP1; ENSG00000120129), Fos proto-oncogene, AP-1 transcription factor subunit (FOS; ENSG00000170345), serpin family E member 1 (SERPINE1; ENSG00000106366), and adenosine deaminase 2 (ADA2; ENSG00000093072) were selected for validation analysis, and GAPDH served as an mRNA endogenous control. The primers are presented in Table I. cDNA synthesis was conducted using a RevertAid™ First Strand cDNA Synthesis kit (Thermo Fisher Scientific, Inc.). The relative mRNA expression was determined by RT-qPCR according to the THUNDERBIRD™ SYBR qPCR Mix (Toyobo, Co., Ltd., Osaka, Japan). qRT-PCR was performed on an ABI PRISM 7500 (Applied Biosystems; Thermo Fisher Scientific, Inc.). The relative gene expression was calculated using ABI PRISM 7500 version 2.0.6 software (Applied Biosystems; Thermo Fisher Scientific, Inc.) according to the $2^{-\Delta\Delta Cq}$ method (11).

Functional analysis. To exploit the functional roles of DEGs, DAVID (https://david.ncifcrf.gov/home.jsp) was used, which integrated the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases to analyze biological function. Finally, the enrichment values of the GO terms,

Table I. Reverse transcription-quantitative polymerase chain reaction primers.

Primer	Length, bp	Sequence
Matrix metallopeptidase 11	93	Forward: GCTGCCTTCCAGGATGCTGAT
		Reverse: GCCTTCCAGAGCCTTCACCTT
Dual specificity phosphatase 1	85	Forward: GCCACCATCTGCCTTGCTTAC
		Reverse TGCTTCGCCTCTGCTTCACA
Fos proto-oncogene, AP-1 transcription factor subunit	245	Forward: CGAGATTGCCAACCTGCTGAAG
		Reverse: CCATGCTGCTGATGCTCTTGAC
Serpin family E member 1	262	Forward: TTCAGGCTGACTTCACGAGT
		Reverse: CCAGATGAAGGCGTCTTTCC
Adenosine deaminase 2	237	Forward: GGCTGTCATCGCAGAATCCATC
		Reverse: AGCATCAGAGCATCCAGAATGTTC

Table II. Summary of the mRNA sequencing data following filtering and mapping.

Sample	Total raw reads	Total Q30 (%)	Total clean reads	Mapped reads	Unique map reads	MultiMap reads
EU1	108,063,442	94.38	106,300,690	99,490,109	98,103,400	1,386,709
EU5	115,796,574	94.93	114,245,924	108,073,952	106,633,173	1,440,779
EU6	88,538,384	94.76	87,285,608	81,742,080	80,563,047	1,179,033
EU7	125,048,606	94.97	123,155,952	116,419,348	114,821,450	1,597,898
EU11	112,650,384	94.35	110,637,684	102,884,399	101,501,805	1,382,594
EU18	89,731,074	94.42	88,482,524	83,389,862	82,191,074	1,198,788
EU19	105,616,842	94.57	104,296,544	97,924,454	96,502,461	1,421,993
EU21	112,190,626	94.68	110,717,296	103,453,282	102,134,657	1,318,625
N2	119,170,846	94.89	117,104,548	109,135,053	107,668,064	1,466,989
N8	100,224,148	95.00	98,937,330	92,856,399	91,660,813	1,195,586
N12	95,701,846	94.50	94,451,810	88,290,492	87,035,145	1,255,347
N16	106,411,788	94.92	104,457,624	97,033,960	95,631,762	1,402,198
N19	116,983,022	93.76	114,885,072	107,817,828	106,214,767	1,603,061

obtained using the hypergeometric test, were considered significantly enriched when the q-value (adjusted as a P-value) was 0.05.

Results

mRNA filtering and mapping. mRNA sequencing generated 1,396,127,582 reads, with an average of 107,204,492 reads per sample in the eutopic endometrium group and 107,698,330 reads per sample in the normal endometrium group. A fastQC quality test demonstrated that 1,374,958,606 (98.48%) reads had a Q-score ≥30, and thus were considered for further analyses. Of these reads, 93.71% were mapped to the hg19 and 98.61% were uniquely aligned. The detailed filtering and mapping data are presented in Table II.

Identification of DEGs and RT-qPCR findings. Among the 13 samples, there were 40,576 mRNAs with an RPKM value of 1 in at least one sample. The transcriptome expression profiling of eutopic endometria and normal endometria exhibited very similar expression levels in the evaluated mRNAs, which directly indicated the homology of the two sequenced groups

(Fig. 1A). On the basis of the above-mentioned criteria of DEGs, 72 DEGs were identified with 66 upregulated genes and 6 downregulated genes (Table III and Fig. 1B).

Two DEGs, ADA2 and MMP-11, were significantly different in the current study, but were not previously selected for further validation. FOS, which exhibited contradictory results in previous studies, was selected. In addition, two DEGs, SERPINE1 and DUSP1, which have been associated with endometriosis in cell lines and animal models, but have not been reported in human tissue, were simultaneously selected. Although the counts of DEGs in mRNA sequencing were relatively low in the two groups, the RT-qPCR analysis indicated easily detectable expression levels. Data analysis indicated that the results from RT-qPCR were consistent with the mRNA sequencing data (Fig. 2).

Functional analysis. To gain an overall understanding of the functional roles in these DEGs, GO term and KEGG pathway analysis were conducted. The results revealed that significantly enriched GO terms under the cellular component (CC) category were extracellular matrix (ECM;

Table III. Differentially expressed genes (n=72) in eutopic endometrium samples from women with endometriosis versus healthy control subjects.

Gene symbol	Ensemble ID	Change	Fold-change	False discovery rate
RP11-319E12.2	ENSG00000251459	Upregulated	120.99	2.32E-07
IGFBP1	ENSG00000146678	Upregulated	115.01	1.79E-03
SERPINB2	ENSG00000197632	Upregulated	72.95	1.51E-04
FOSB	ENSG00000125740	Upregulated	66.12	2.01E-19
EREG	ENSG00000124882	Upregulated	52.02	2.05E-09
MMP27	ENSG00000137675	Upregulated	40.93	1.69E-04
MMP10	ENSG00000166670	Upregulated	31.41	4.01E-04
LEFTY2	ENSG00000143768	Upregulated	30.59	1.37E-08
WIF1	ENSG00000156076	Upregulated	30.56	1.38E-04
CDC20B	ENSG00000164287	Upregulated	25.21	6.06E-09
LRRC15	ENSG00000172061	Upregulated	25.20	3.17E-24
LINC01411	ENSG00000249306	Upregulated	24.95	7.67E-03
RPL10P9	ENSG00000233913	Upregulated	21.83	3.10E-02
IGKV1-12	ENSG00000243290	Upregulated	21.19	2.04E-05
FAM159B	ENSG00000145642	Upregulated	20.34	2.34E-02
INHBA	ENSG00000122641	Upregulated	15.33	1.19E-15
FOXN4	ENSG00000139445	Upregulated	14.90	1.71E-02
RGS1	ENSG00000090104	Upregulated	14.80	1.19E-15
MMP3	ENSG00000149968	Upregulated	14.71	4.40E-02
FOS	ENSG00000170345	Upregulated	14.54	2.55E-24
IGHV1-2	ENSG00000211934	Upregulated	14.08	3.82E-04
NR4A1	ENSG00000123358	Upregulated	13.80	8.80E-17
EGR3	ENSG00000179388	Upregulated	13.24	1.19E-15
VGFA	ENSG00000112715	Upregulated	13.10	8.07E-17
RP11-459E5.1	ENSG00000253125	Upregulated	12.77	8.50E-06
ARC	ENSG00000198576	Upregulated	12.72	3.82E-04
EPYC	ENSG00000083782	Upregulated	12.28	4.50E-02
KCNF1	ENSG00000162975	Upregulated	10.88	1.33E-02
AREG	ENSG00000109321	Upregulated	9.49	6.67E-03
FOSL1	ENSG00000175592	Upregulated	9.42	1.87E-08
VGF	ENSG00000128564	Upregulated	9.14	3.29E-02
NPTX1	ENSG00000171246	Upregulated	9.11	2.50E-02
ATF3	ENSG00000162772	Upregulated	8.99	3.83E-08
SERPINE1	ENSG00000106366	Upregulated	8.17	2.00E-04
IL11	ENSG00000095752	Upregulated	7.97	7.57E-04
IGFN1	ENSG00000163395	Upregulated	7.79	2.78E-05
ASIC2	ENSG00000108684	Upregulated	7.40	1.31E-02
NR4A3	ENSG00000119508	Upregulated	7.30	4.57E-06
CRYGN	ENSG00000127377	Upregulated	7.25	9.11E-03
AP000349.2	ENSG00000280178	Upregulated	7.07	4.06E-13
MTUS2	ENSG00000132938	Upregulated	6.87	5.42E-03
ZCCHC12	ENSG00000174460	Upregulated	6.82	1.05E-03
MMP11	ENSG00000099953	Upregulated	6.77	5.21E-13
ARSI	ENSG00000183876	Upregulated	6.46	9.06E-03
LOC101929415	ENSG00000254254	Upregulated	5.90	4.56E-02
RP11-613D13.8	ENSG00000244953	Upregulated	5.72	2.98E-03
KRT17	ENSG00000128422	Upregulated	5.56	9.34E-05
EGR1	ENSG00000120738	Upregulated	5.47	1.43E-09
CYR61	ENSG00000142871	Upregulated	5.38	2.70E-09
GEM	ENSG00000164949	Upregulated	4.72	7.61E-06
C11orf96	ENSG00000187479	Upregulated	4.57	2.05E-04

Table III. Continued.

| Gene symbol	Ensemble ID	Change	Fold-change	False discovery rate
PTHLH	ENSG00000087494	Upregulated	4.51	3.73E-02
EGR2	ENSG00000122877	Upregulated	4.41	1.33E-02
DUSP1	ENSG00000120129	Upregulated	4.35	2.05E-04
LOC102724428	ENSG00000275993	Upregulated	4.35	8.21E-03
PAMR1	ENSG00000149090	Upregulated	4.23	1.34E-03
TNFRSF12A	ENSG00000006327	Upregulated	3.83	5.70E-03
DUSP5	ENSG00000138166	Upregulated	3.66	1.60E-03
SLC47A1	ENSG00000142494	Upregulated	3.62	8.33E-03
ADAMTS16	ENSG00000145536	Upregulated	3.39	1.45E-02
CAB39L	ENSG00000102547	Upregulated	3.38	1.65E-03
HES1	ENSG00000114315	Upregulated	3.23	6.51E-03
HTRA3	ENSG00000170801	Upregulated	3.17	1.60E-03
LTBP2	ENSG00000119681	Upregulated	2.92	6.92E-03
LOC284454	ENSG00000267519	Upregulated	2.89	4.88E-02
RGCC	ENSG00000102760	Upregulated	2.74	4.03E-02
LRRC26	ENSG00000184709	Downregulated	109.57	2.98E-03
S100A7	ENSG00000143556	Downregulated	40.68	5.10E-08
PWP2	ENSG00000241945	Downregulated	18.29	8.21E-03
GUCY1B2	ENSG00000123201	Downregulated	11.64	6.54E-03
CTD-2384B11.2	ENSG00000225407	Downregulated	7.54	6.54E-03
ADA2	ENSG00000093072	Downregulated	3.23	1.30E-02
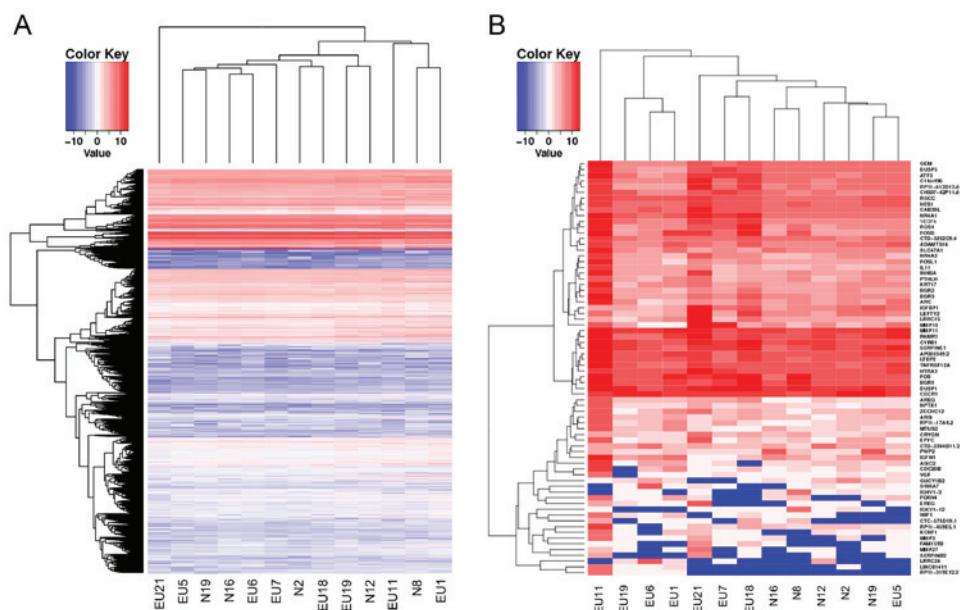
Figure 1. Hierarchical clustering analysis. (A) Unsupervised hierarchical clustering of the 40,576 expressed mRNAs and (B) supervised hierarchical clustering analysis of 72 differentially expressed genes between the eutopic endometrium samples from endometriosis patients and healthy control subjects.

GO:0031012), proteinaceous ECM (GO:0005578) and extracellular space (GO:0005615). The molecular function (MF) category included nine enriched terms, particularly in metalloendopeptidase activity (GO:0004222), growth factor activity (GO:0008083), and RNA polymerase II core promoter proximal region sequence-specific DNA binding transcription factor activity (GO:0000982). The biological process (BP) category contained 88 enriched terms, the top three of which were response to endogenous stimulus (GO:0009719), cellular response to endogenous stimulus (GO:0071495) and response to cyclic adenosine monophosphate (GO:0051591). In addition, certain GO terms that are commonly observed in tumor-like diseases were also significantly enriched in the current results, such as growth (GO:0040007), angiogenesis (GO:0001525)
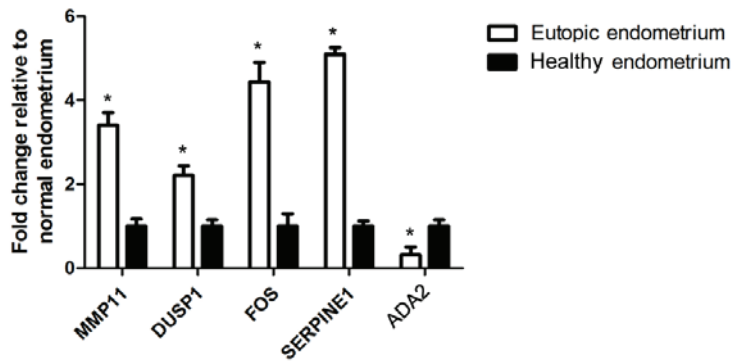
Figure 2. The expression levels of five selected mRNAs in eutopic endometrium samples from women with endometriosis (n=15) and healthy control endometrial tissue samples (n=15) following reverse transcription-quantitative polymerase chain reaction. *P<0.01 vs. healthy endometrium.
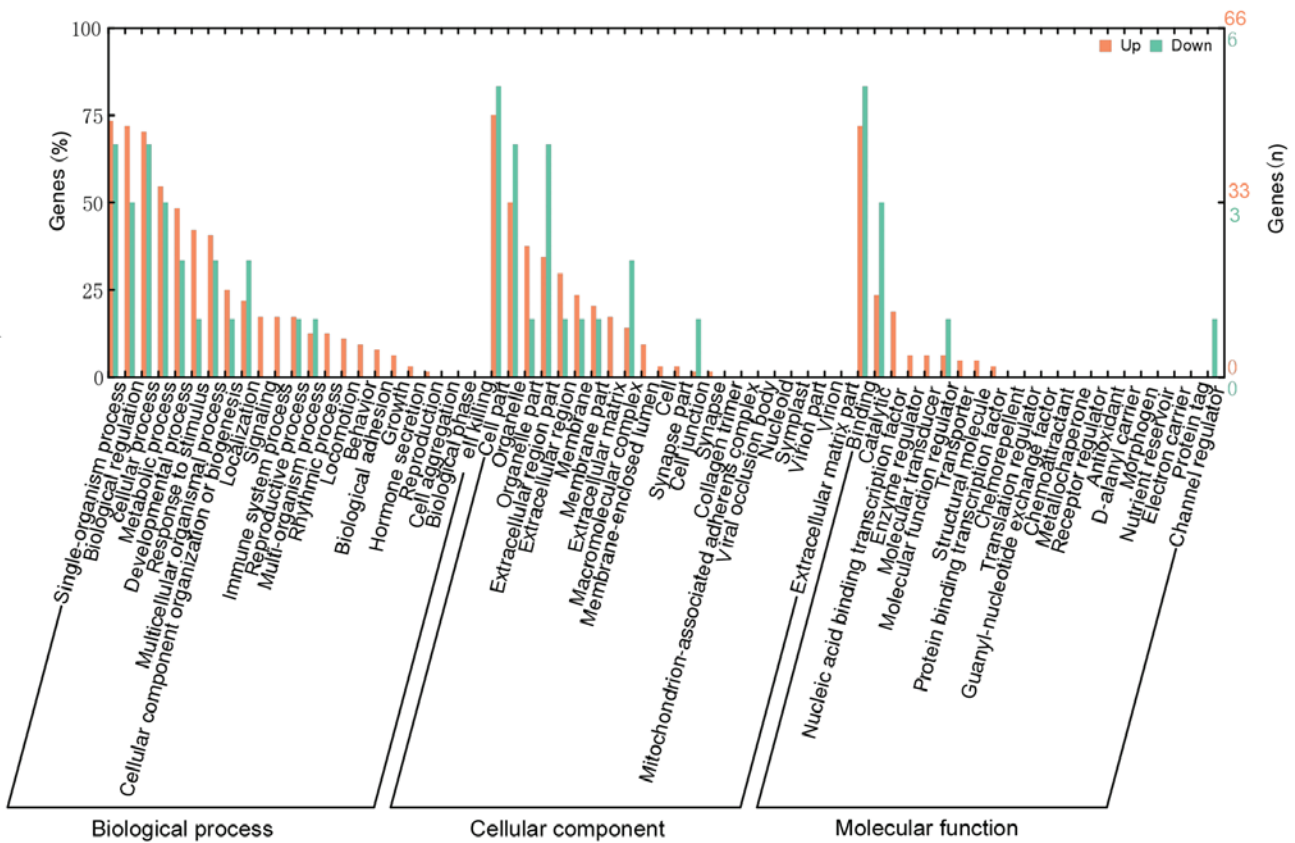

Figure 3. Gene Ontology annotations for the differentially expressed genes in the eutopic endometrium samples of women with endometriosis compared with the healthy control subjects. Upregulated genes are presented in red while downregulated genes are in green.

and cell migration (GO:0016477). These results are presented in Fig. 3 and Table IV. Due to the limited number of DEGs, none of the pathways were identified to be significantly enriched in the KEGG analysis with the above-mentioned thresholds.

Discussion

To date, the majority of studies that focused on the eutopic endometrium of women with endometriosis were hypothesis-based studies, which evaluated a limited number of previously susceptible genes (7). A recent systematic review from 1984 to 2010 summarized >200 potential endometrial biomarkers in the endometrium, but did not identify a standard biomarker in a clinical study (12). In addition, various studies using a microarray-based method have identified hundreds of potential pathogenic genes and pathways; however, these studies present few overlapping results (13-15). This inconsistency may be caused by various factors, including a small number of samples, poorly defined controls, different rAFS stages, different phases of the menstrual cycle, methodology limitations, various types of endometriosis, and interference by coexistent diseases. Therefore, future studies should continue to search for the important DEGs and focus on these confounding factors. To the best of our knowledge, the current study is the first to present the genome-wide gene expression profiling of the eutopic endometrium in women with endometriosis using

Table IV. Top 10 enriched GO terms of DEGs in the endometrium from women with endometriosis compared with healthy control subjects.

Category	GO ID	Description	Q value	n	Involved DEGs
BP	GO:0009719	Response to endogenous stimulus	4.47E-07	21	CAB39L, SERPINE1, AREG, HES1, NR4A3, LTBP2, DUSP1, EGR1, INHBA, EGR2, NR4A1, EREG, FOSB, VGF, LEFTY2, IGFBP1, MMP3, HTRA3, FOSL1, EGR3, VEGFA
BP	GO:0071495	Cellular response to endogenous stimulus	1.54E-06	17	CAB39L, SERPINE1, HES1, LTBP2, DUSP1, EGR1, INHBA, EGR2, NR4A1, EREG, FOSB, LEFTY2, IGFBP1, MMP3, HTRA3, EGR3, VEGFA
BP	GO:0051591	Response to cyclic adenosine monophosphate	1.54E-06	8	AREG, DUSP1, EGR1, EGR2, FOSB, VGF, FOSL1, EGR3
CC	GO:0031012	Extracellular matrix	4.5E-06	11	EPYC, MMP11, SERPINE1, LTBP2, MMP27, CYR61, LEFTY2, ADAMTS16, MMP3, MMP10, AP000349.2
BP	GO:0001525	Angiogenesis	1.2E-05	9	TNFRSF12A, RGCC, SERPINE1, NR4A1, EREG, CYR61, S100A7, EGR3, VEGFA
BP	GO:0046683	Response to organophosphorus	1.49E-05	8	AREG, DUSP1, EGR1, EGR2, FOSB, VGF, FOSL1, EGR3
BP	GO:0014074	Response to purine-containing compound	1.95E-05	8	AREG, DUSP1, EGR1, EGR2, FOSB, VGF, FOSL1, EGR3
BP	GO:0048646	Anatomical structure formation involved in morphogenesis	3.16E-05	17	TNFRSF12A, PTHLH, RGCC, SERPINE1, NR4A3, DUSP1, INHBA, EGR2, NR4A1, EREG, DUSP5, FOXN4, CYR61, S100A7, ADAMTS16, EGR3, VEGFA
BP	GO:0010243	Response to organonitrogen compound	5.42E-05	14	CAB39L, AREG, HES1, NR4A3, DUSP1, EGR1, EGR2, EREG, FOSB, VGF, IGFBP1, MMP3, FOSL1, EGR3
BP	GO:0009725	Response to hormone stimulus	8.50E-05	14	CAB39L, AREG, HES1, NR4A3, DUSP1, EGR1, INHBA, EGR2, EREG, FOSB, VGF, IGFBP1, FOSL1, EGR3

GO, Gene Ontology; DEG, differentially expressed gene; BP, biological process; CC, cellular component.

a transcriptome sequencing technique. Considering that the genome profiling of normal endometria demonstrated marked molecular differences between samples obtained from the proliferative and secretory phases of the menstrual cycle, only mid- and late-secretory phase endometria were investigated in the patient and control groups, which represents most closely the reflux endometrium. To avoid other confounding factors, the endometriosis patients included were restricted to those in the moderate to severe stages (stages III-IV) and only those patients with ovarian endometriosis without combined diseases were enrolled. Finally, 72 DEGs enriched in 100 functional GO terms were identified. The top enriched terms in each category were ECM (GO:0031012) in CC, metalloendopeptidase activity (GO:0004222) in MF, and cellular response to endogenous stimulus (GO:0009719) in BP. Notably, various DEGs may be candidates for potential biomarkers in the eutopic endometrium of women with endometriosis.

It has been reported that human endometrium undergoes cyclic tissue remodeling during the menstrual period, during which several MMPs and ECM-associated proteins are activated (16). These proteins are suggested to facilitate the degradation and invasion of ECM and facilitate with the attachment of reflux endometrial tissue to the peritoneum and ovarian surface. In the current study, four MMP members, MMP-3, MMP-10, MMP-11, and MMP-27, were identified as upregulated in eutopic endometrium (17). Gilabert-Estellés et al (18) and Ramón et al (19) demonstrated that eutopic endometria from women with endometriosis have increased expression levels of MMP-3, which is consistent with the present result. Uzan et al (20) examined the immunohistochemical expression of MMP-11, although no difference was identified between the patient and control groups. Cominelli et al (21) suggested that MMP-27 is maximally expressed during the menstrual phase in the normal endometrium and no difference in ectopic versus eutopic endometria was observed; however, the authors did not compare between eutopic and normal endometria. Prior studies reported that the overexpression of SERPINE1 (also termed PAI-1) may result in the impairment of the fibrinolytic system, rendering the woman prone to endometriosis (22). Braza-Boïls et al (23) demonstrated that the protein expression

levels of SERPINE1 were significantly higher in endometriotic lesions than in control endometrial tissue samples, but identified no difference between eutopic and control endometria. Unlike other members mentioned above, MMP-10, epiphycan, latent transforming growth factor β binding protein 2, cysteine rich angiogenic inducer 61, left-right determination factor 2, ADAM metallopeptidase with thrombospondin type 1 motif 16, and AP000349.2 have received little attention in endometriosis research and therefore require further confirmation.

The FOS gene family comprises four members, namely FOS, FosB proto-oncogene, AP-1 transcription factor subunit (FOSB), FOS like 1, AP-1 transcription factor subunit (FOSL1) and FOSL2. Their encoded proteins dimerize with the Jun family members to form the group of AP-1 proteins, and are involved in various physiological and pathological processes, such as cell proliferation, apoptosis, and differentiation and transformation (24). In the present study, abnormally high expression levels of FOS, FOSB, and FOSL1 were observed in eutopic endometria from patients with endometriosis, which were predominantly enriched in response to endogenous stimulus terms, nucleic acid-binding transcription factor activity terms, and response to hormone stimulus terms. FOS, as an early response gene, is critical in estrogen-mediated proliferation of endometrial cells (25). Pan *et al* (26) reported that FOS protein expression levels in eutopic and ectopic endometria samples from females with endometriosis were significantly higher than those in the endometria samples from healthy control subjects; however, the findings of Morsch *et al* (27) were not similar. Therefore, the FOS gene was selected to validate RT-qPCR in the present study, and the result was consistent with the results of Pan *et al* (26). To the best of our knowledge, the association between FOSB and FOSL1, and endometriosis have not yet been reported. As with FOS, the early growth response (EGR) family of transcription regulatory factors was predicted to be key in cellular growth and differentiation (28). Three members, EGR1, EGR2 and EGR3 were identified to be highly expressed by sequencing, which were also significantly enriched in response to endogenous stimulus terms, response to gonadotropin stimulus terms, and growth terms. Birt *et al* (29) reported the overexpression of EGR1 in endometriotic animal models and inferred that EGR1 may affect downstream protease pathways impeding ovulation in endometriosis. The roles of EGR1and EGR3 in angiogenesis has also recently been recognized and were considered to regulate certain important angiogenic factors, such as vascular endothelial growth factor A (VEGFA), fibroblast growth factor 2, and C-X-C motif chemokine ligand 1. Angiogenesis is considered to be pivotal to the implant and growth of endometriotic lesions in the pelvic microenvironment (30). The endometrium, which contains robust stem cell populations and striking regenerative ability, is a rich source of angiogenic factors (31). In the present study, enriched angiogenesis and blood vessel development terms were also observed. Aberrant upregulation of VEGFA in eutopic endometria demonstrated concordance with two earlier results: Taylor *et al* (32) emphasized the importance of VEGFA in the endometrium of women with endometriosis, as it may be activated by inflammatory-, oxidative-, hormonal- and endoplasmic reticulum-stress signals. Bourlev *et al* (33) reported a high expression level of VEGFA in the eutopic endometrium of women with endometriosis, as well as high concentrations

of VEGFA in the peritoneal fluid. In addition, dysregulation of various angiogenic factors, including regulator of cell cycle, TNF receptor superfamily member 12A, nuclear receptor subfamily 4 group A member 1, epiregulin, cysteine rich angiogenic inducer 61, and S100 calcium binding protein A7, was observed in the present study in women with endometriosis when compared with those without endometriosis. Although these preliminary data require further characterization, the current findings provide novel information for future experimental studies.

In addition to gaining an improved understanding of pathogenesis, the present study attempted to identify various potential biomarkers. Although certain studies have questioned the unpleasant sensation of endometrial biopsy, the majority of participants are willing to undergo the procedure (34). In the current study, elevated expression levels of ADA2, MMP-11, FOS, SERPINE1, and DUSP1 in women with endometriosis were revealed by mRNA sequencing and RT-qPCR and, thus, these genes were considered as candidate biomarkers. Due to the limited sample size, the sensitivity, specificity, and receiver operating characteristic curve analysis were not calculated for a diagnostic test of endometriosis, which would be vital for a large sample-size study in future.

Despite the novel results, there were limitations of the current study. The primary limitation of the study is the relatively small sample size. In addition, it is difficult to evaluate whether the control endometria are from completely healthy women, as ~6% of endometriotic lesions are only visible under a microscope, and these women macroscopically presented a normal appearance (35). In addition, these preliminary results require validation by downstream experiments.

In conclusion, to the best of our knowledge, the current study presents the first genome-wide gene expression profile of eutopic endometria from women with endometriosis using a high-throughput sequencing technique. Seventy-two DEGs in eutopic endometria from women with endometriosis were compared with normal endometria from control subjects. GO analysis further revealed the important roles of these DEGs in the pathogenesis of endometriosis. Five genes, including MMP-11, DUSP1, FOS, SERPINE1, and ADA2 were further confirmed by RT-qPCR, and the results were consistent with the mRNA sequencing, indicating that these genes may present as novel biomarkers in the endometrium of women with endometriosis. The current study provides a comprehensive, but preliminary insight into elucidating the underlying mechanisms of this complex disorder, which merits further in-depth studies for confirmation.

Acknowledgements

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