Differentially expressed genes in eutopic and ectopic endometrium of women with endometriosis

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Objective: To elucidate the potential mechanisms involved in the physiopathology of endometriosis. We analyzed the differential gene expression profiles of eutopic and ectopic tissues from women with endometriosis.
Design: Prospective laboratory study.
Setting: University hospital.
Patient(s): Seventeen patients in whom endometriosis was diagnosed and 11 healthy fertile women.
Intervention(s): Endometrial biopsy specimens from the endometrium of healthy women without endometriosis and from the eutopic and ectopic endometrium tissues of patients with endometriosis were obtained in the early proliferative phase of the menstrual cycle.
Main Outcome Measure(s): Six paired samples of eutopic and ectopic tissue were analyzed by subtractive hybridization. To evaluate the expression of genes found by rapid subtraction hybridization methods, we measured *CTGF, SPARC, MYC, MMP,* and *IGFBP1* genes by real-time polymerase chain reaction in all samples.
Result(s): This study identified 291 deregulated genes in the endometriotic lesions. Significant expression differences were obtained for *SPARC, MYC,* and *IGFBP1* in the peritoneal lesions and for *MMP3* in the ovarian endometriomas. Additionally, significant differences were obtained for *SPARC* and *IGFBP1* between the peritoneal and ovarian lesions. No significant differences were found for the studied genes between the control and the eutopic endometrium.
Conclusion(s): This study identified 291 genes with differential expression in endometriotic lesions. The deregulation of the *SPARC, MYC, MMP3,* and *IGFBPI* genes may be responsible for the loss of cellular homeostasis in endometriotic lesions. (Fertil Steril® 2010;93:1750–73. ©2010 by American Society for Reproductive Medicine.)

Key Words: Differential gene expression, endometriosis, endometrium, subtractive hybridization

Endometriosis is a benign estrogen-dependent gynecologic disease that affects 10% to 15% of women of reproductive age. It is characterized by endometrium-like tissue outside the uterine cavity (1). The most common site of endometriotic implants is the pelvic cavity, primarily the pelvic peritoneum and the ovary. The etiology of the disease is complex and multifactorial (2).

A better understanding of the mechanisms underlying the complex physiopathology of endometriosis, including a characterization of the gene expression profile and the functions of the genes involved in this phenomenon, will allow for better comprehension of the molecular environment of endometriotic lesions. Several studies regarding differential gene expression patterns, including studies using DNA microarrays, have been performed for endometriosis, with interesting results (3–7).

In addition, the subtraction hybridization methods are designed to identify overexpressed complementary DNAs (cDNAs) in one of the two analyzed groups, detecting any differentially expressed messenger RNA (mRNA) (8). Hu et al. (9) used this method to compare the gene expression profiles of eutopic and ectopic tissue of peritoneal lesions from the same women during the proliferative and secretive phases of the menstrual cycle. Sha et al. (10) compared endometrium cell cultures from women without endometriosis and endometrium from women with endometriosis using combined subtraction hybridization and microarray methods.

In this study, we compared the gene expression profiles of autologous tissues from women with endometriosis in the early proliferative phase of the menstrual cycle using subtractive hybridization. Peritoneal lesions and ovarian endometriomas, which are the most common lesions, were analyzed.

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MATERIALS AND METHODS

This study was approved by the Research Ethics Committee (CEP) of the University Hospital from the School of Medicine of Ribeirão Preto, University of São Paulo (FMRP-USP) (No. 11736/2004). Written informed consent was obtained from each patient.

Samples

A total of 28 patients were selected consecutively with use of the following inclusion criteria: reproductive age (18 to 40 years); early proliferative phase of the menstrual cycle (days 5–8); regular cycles; and no history of hormonal therapy, intrauterine device, or any other birth control for 6 months before the biopsy. Endometriosis stages were settled in agreement with the American Society for Reproductive Medicine classification (11). The stages of the cycles were confirmed by histologic examination and date of last menstruation before the operations. None of the patients had received previous treatment for endometriosis.

The patients were divided into two groups: [1] the control group, in which endometrial biopsy specimens were collected with a Novak curette from 11 women with no diagnosis of endometriosis, fibroids, pelvic adhesive disease, or infertility (all of them had at least one pregnancy) who were referred to our service by the Family Planning Ambulatory unit with tubal ligation indication; and [2] the affected group, comprising 17 patients with a diagnosis of endometriosis (peritoneal lesion or ovarian endometrioma) who were referred to our service by the Endoscopy and Pelvic Pain Ambulatory and Infertility Ambulatory units of the University Hospital for pelvic pain and/or infertility. In the endometriosis group (ovarian), three had never been pregnant and six had at least two pregnancies resulting in live children. In the endometriosis group (peritoneum), five had never been pregnant and three had at least one pregnancy. We did not separate our patients according to ethnicity because Brazilians represent one of the most heterogeneous populations in the world as the result of five centuries of interethnic crosses between people from three continents: the European colonizers, represented mainly by the Portuguese; the African slaves; and the autochthonous Amerindians (12).

Biopsy specimens of eutopic (n = 17) and ectopic (n = 17) endometrium from the same patients were collected by laparoscopy and Novak curette. Among the 17 ectopic endometrium biopsy specimens, 8 were stage II (4 samples), III (2), and IV (2) peritoneal lesions and 9 were stage III (2) and IV (7) ovarian lesions. The diagnosis was confirmed through histopathologic analysis. The samples were placed in a Tissue-Tek O.C.T. Compound cryopreservator (Sakura Finetek USA Inc., Torrance, CA) immediately after collection. They were incubated for 15 minutes in liquid nitrogen and then stored at −80°C.

Ribonucleic Acid Extraction

The total RNA was extracted (50 mg of tissue) with use of TRIzol Reagent (Invitrogen Life Technologies, Paisley, United Kingdom) according to the manufacturer's instructions. The RNA was stored at −80°C for future procedures.

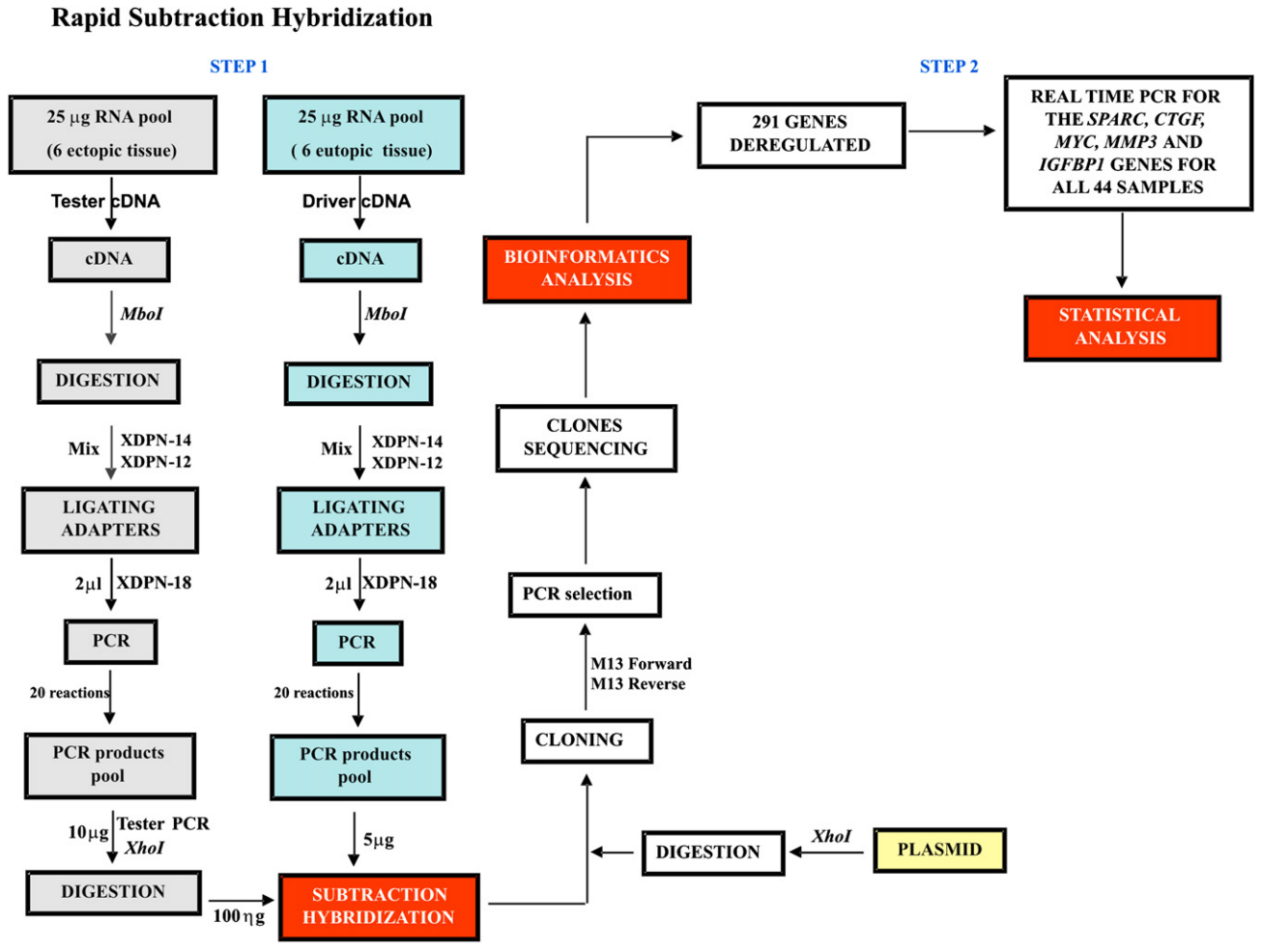
Rapid Subtraction Hybridization of cDNA

Aiming to characterize differential gene expression in endometriosis independent of lesion site, we pooled ovarian endometriomas and peritoneal lesions, which are the most common and representative lesions of the disease. The subtractive hybridization procedures (Fig. 1) followed the method described by Jiang et al. (8). Two subtractive hybridization libraries were made. In the first, the cDNA tester was obtained from the RNA pool of the ectopic tissue (indicating the up-regulated genes in the lesions) and the cDNA driver from the RNA pool of the eutopic tissue; in the second, reverse library, the cDNA tester was obtained from the RNA pool of the eutopic tissue (indicating the down-regulated genes in the lesions) and the cDNA driver from the RNA pool of the ectopic tissue. For library preparation, six paired samples of the affected group were randomly chosen: six ectopic endometrium samples (three ovarian and three peritoneal lesions) and six eutopic endometrium samples from the same patients. The protocol used is described briefly below.

From the 25-μg total RNA pool, the cDNAs were synthesized according to the protocol previously described by Jiang and Fisher (13). The cDNAs were digested with *MboI* (Invitrogen Life Technologies) at 37°C for 1 hour and extracted with phenol-chloroform extraction and ethanol precipitation. The digested cDNAs were mixed with 20 μmol/L of primers XDPN-14 (5′CTGATCACTCGAGA3′) and XDPN-12 (5′GATCTCTCGAGT3′) in 30 μL of 1× T4 DNA Ligase Buffer (Invitrogen Life Technologies), heated at 55°C for 1 minute, and cooled to 14°C within 1 hour. After adding nine units of T4 DNA ligase to the mixtures individually, ligation was carried out overnight at 14°C. The mixtures were diluted to 100 μL with LoTE (3 mmol/L tris[hydroxymethyl]aminomethane [Tris]-HCl, pH 7.5; 0.2 mmol/L ethylenediaminetetraacetic acid, pH 7.5) (Invitrogen Life Technologies), and at least 40 μL of the mixtures was used for polymerase chain reaction (PCR) amplification. The PCR mixtures were set up as follows: 2 μL of the cDNA mixture, 1× Taq Buffer (Invitrogen Life Technologies), 1 U of Taq polymerase (Invitrogen Life Technologies), 200 μmol/L of each deoxyribonucleoside triphosphate (dATP, dTTP, dCTP, dGTP), and 10 μmol/L of primer XDPN-18 (5′CTGATCACTCGAGAGATC3′). The parameters for PCR were one cycle of 5 minutes at 72°C, followed by 25 cycles of 1 minute at 94°C, 1 minute at 55°C, and 1 minute at 72°C, and one cycle of 3 minutes at 72°C. The PCR products were pooled and purified by phenol-chloroform extraction and ethanol precipitation. All procedures were performed for the cDNA obtained from the RNA pool from both the eutopic and the ectopic tissues. Portions (10 μg) of the tester PCR products (eutopic or ectopic) were digested with 20 units of *XhoI* and purified with phenol-chloroform extraction and ethanol precipitation.

FIGURE 1

Flow diagram representing the experimental design for RaSH. *Step 1* shows the experiment carried out for subtractive library 1 (indicating the up-regulated genes in the lesions). This procedure was also carried out for library 2 (indicating the down-regulated genes in the lesions). *Step 2* represents data and procedures performed for both libraries.



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Subtraction hybridization The tester cDNA (100 ng) was mixed with 5 μg of the driver cDNA in 16.6 μL of a hybridization solution (0.5 mol/L NaCl, 50 mmol/L Tris pH 7.5, 0.2% sodium dodecyl sulfate, 40% [vol/vol] formamide) and, after boiling for 5 minutes, incubated at 42°C for 48 hours. The hybridization mixture was phenol-chloroform extracted, ethanol precipitated, and dissolved in 20 μL of LoTE. Part of the mixture (3 μL) was ligated with 1 μg of pZErO-1 (Invitrogen Life Technologies) plasmids, digested with *XhoI* at 16°C for 3 hours, and transformed into One Shot Top 10 Electrocomp *Escherichia coli* (Invitrogen Life Technologies).

Colony screening All bacterial colonies were analyzed by PCR with use of the M13 forward (5′CATTTTGCTGC CGGTC3′) and M13 reverse (5′CAGGAAACAGCTATGA CC3′) primers to verify those that presented an insert. The sequences of these clones were determined with MegaBace 1000 (Amersham Biosciences, Piscataway, NJ) and DYEnamic ET Dye Terminator Sequencing Kit (Amersham Biosciences).

Bioinformatics Analysis

Phred and phrap software were used (14) to select the best quality sequences according to the following pattern: phred cutoff of 0.09, minmatch 10, and minscore 20; sequences with at least 100 base pairs of quality were accepted. The best-quality sequences were submitted to the alignment program BLAST (15). Comparisons against the following databases were performed: human mitochondrial genome (blastn, evalue = $1e^{-05}$), bacteria genome (blastn, evalue = $1e^{-30}$), *Saccharomyces* sequences (blastn, evalue = $1e^{-30}$),

ribosomal proteins (blastx, evalue = $1e^{-30}$), human RefSeq (blastn, evalue = $1e^{-30}$), UniGene sequences (blastn, evalue = $1e^{-25}$), human ESTs (blastn, evalue = $1e^{-20}$), human nr (blastx, evalue = $1e^{-20}$), and nonhuman nr (blastx, evalue = $1e^{-15}$). The sequences classified as RefSeq (reference sequences) were emphasized.

After alignment with the RefSeq database, the sequences that presented at least 90% of the target sequence length at alignment were selected. With use of the adopted criteria, the selected genes were considered homologous to the sequences found in the libraries. Therefore, these genes were categorized by their functions in biologic processes according to the Gene Ontology (GO) terms (http://www.geneontology.org). Additionally, the search program PDQ Wizard v.0.1 Searching PubMed (16), available online (http://www.gti.ed.ac.uk/pdqwizard), was used to find associations between the literature data about endometriosis and the genes obtained in the libraries.

Real-Time PCR

The real-time PCR technique was used to evaluate the expression of genes found by the rapid subtraction hybridization (RaSH) method in all samples individually. Five genes were selected on the basis of the total number of clones and their function related to the etiology of the disease. The selected genes were *SPARC* (10 clones) and *CTGF* (7 clones), found to be up-regulated in the ectopic endometrium, and *IGFBP1* (6 clones), *MMP3* (23 clones), and *MYC* (2 clones), found to be down-regulated in the ectopic endometrium.

Forty-four (10 control endometrium, 17 eutopic, and 17 ectopic tissues) of the collected 45 samples were analyzed with real-time PCR in ABI PRISM 7500 Sequence Detection Systems (Applied Biosystems, Warrington, United Kingdom) to quantify relative expression. One control endometrium sample was chosen randomly to calibrate the assay. The reactions were performed with use of the TaqMan Gene Expression Assays system (TaqMan MGB probes, FAM dye-labeled) from Applied Biosystems. The probes and primers for the *CTGF* (Hs00170014_m1), *IGFBP1* (Hs00236877_m1), *MMP3* (Hs00968308_m1), *SPARC* (Hs00277762_m1), *MYC* (Hs00153408_m1), and *GAPDH* (Hs99999905_m1, endogenous control) genes were obtained with use of the Assay-on-Demand Gene Expression Products (Applied Biosystems).

A real-time PCR was performed in duplicate for each sample according to the following conditions: 10 μL of TaqMan Universal PCR Master Mix (2×) (Applied Biosystems), 1 μL of TaqMan Gene Expression Assay Mix (20×) (Applied Biosystems), and 9 μL of diluted cDNA (1/50 dilution) in a final volume of 20 μL for each reaction. The reaction conditions were 50°C for 2 minutes, then 95°C for 10 minutes, followed by 40 cycles at 95°C for 15 seconds and 60°C for 1 minute. The gene expression level was calculated for each sample according to the $2^{-\Delta\Delta CT}$ (2-Ct) method as previously described in Applied Biosystems User Bulletin No. 2 (PN 4303859). The *GAPDH* gene and calibrator sample were used as normalizers for the $2^{-\Delta\Delta CT}$ calculus.

Statistical Analysis

The gene expression variable was transformed by $\log_{10}$. The logarithmic transformation was necessary because one of the suppositions (linearity) of the linear model analyses was not met (17). The analyses were performed with use of SAS 2003 software (2002–2003; SAS Institute Inc., Cary, NC). Nonpaired *t*-tests were used to compare the gene expression averages obtained between [1] control endometrium from women without endometriosis and eutopic endometrium from women with endometriosis and [2] peritoneal ectopic tissue and ovarian ectopic tissue. Paired *t*-tests were used to compare the gene expression averages obtained between [3] eutopic and ectopic tissue (peritoneal lesions and ovarian endometriomas), [4] eutopic and ectopic tissue from patients with peritoneal lesions, and [5] eutopic and ectopic tissue from patients with ovarian lesions. All tests were performed according to the GLM procedures. Correlation analyses were carried out between the gene expression levels obtained in the ectopic tissues and the lesions staging with the PROC CORR command. Analyses were considered to be statistically significant at $P<.05$.

RESULTS

Complementary DNA Subtractive Hybridization Libraries (RaSH)

After preparation of the libraries, 768 clones were obtained for the reverse library (down-regulated genes), of which 627 were quality sequences categorized as mitochondrial (176), *Saccharomyces* expressed sequence tag (EST) (0), UniGene (4), other sequences (1), ribosomal (16), human EST (8), bacterial (2) (data not shown), and RefSeq (420). For the up-regulated genes library (tester ectopic tissue), 1,056 clones were obtained, of which 712 were of high quality and classified as mitochondrial (39), *Saccharomyces* EST (3), UniGene (21), other sequences (1), ribosomal (136), human EST (18), bacteria (4) (data not shown) and RefSeq (490).

Of the 910 RefSeq, 109 were excluded from the analyses as false-positives (data not shown) because they were present in both libraries. After selection of the RefSeq that presented at least 90% homology with the target sequence in the alignment, there were 345 RefSeqs for the down-regulated genes and 380 for the up-regulated genes in the lesions library. These sequences are summarized in Tables 1 and 2.

Therefore, 191 genes were identified as up-regulated and 100 as down-regulated. Moreover, Tables 1 and 2 highlight the literature references that previously have associated these genes with the disease, as well as the chromosomal regions for these genes and genomic alterations that have been found in these regions in endometriotic lesions by comparative genomic hybridization (CGH). Also, the genes were related to 283 different biologic processes according to gene

TABLE 1

Genes up-regulated in ectopic versus eutopic endometrium of women with endometriosis.

Accession No.	Gene description	Gene symbol	Total No. of clones	Gene locus	Genomic alterations by CGH[a]	Genes associated with endometriosis in the literature (reference number)
NM_015381	Family with sequence similarity 19 (chemokine [C-C motif]-like), member A5	*FAM19A5*	1	22q13.32		
NM_013349	Neuron-derived neurotrophic factor	*NENF*	1	1q32.3	Gain of 1q	
NM_153005	RIO kinase 1 (yeast)	*RIOK1*	1	6p24.3	Gain of 6p	
NM_002510	Glycoprotein (transmembrane) nmb	*GPNMB*	1	7p15		
NM_005973	Papillary renal cell carcinoma (translocation-associated)	*PRCC*	1	1q21.1	Gain of 1q	
NM_000671	Alcohol dehydrogenase 5 (class III), chi polypeptide (ADH5)	*ADH5*	1	4q21–q25		
NM_019054	Family with sequence similarity 35, member A	*FAM35A*	1	10q23.2		
NM_015393	DKFZP564O0823 protein	*DKFZP564O0823*	1	4q13.3–q21.3		
NM_001008215	Hypothetical protein MGC52110	*MGC52110*	1	2q11.2		
NM_006886	ATP synthase, H+ transporting, mitochondrial F1 complex, epsilon subunit	*ATP5E*	1	20q13.32		
NM_001012506	Coiled-coil domain containing 66	*CCDC66*	1	3p14.3		
NM_001011655	Transmembrane protein 44	*TMEM44*	1	3q29		
NM_018255	Elongation protein 2 homolog (*Saccharomyces cerevisiae*)	*ELP2*	1	18q12.2		
NM_014188	SSU72 RNA polymerase II CTD phosphatase homolog (*S. cerevisiae*)	*SSU72*	1	1p36.33		
NM_001037163	Hypothetical protein LOC84792	*MGC12966*	1	7p22.1		
NM_006493	Ceroid-lipofuscinosis, neuronal 5	*CLN5*	1	13q21.1–q32		
NM_005578	LIM domain containing preferred translocation partner in lipoma	*LPP*	1	3q28		
NM_006572	Guanine nucleotide binding protein (G protein), alpha 13	*GNA13*	1	17q24.3	Gain of 17q	
NM_005114	Heparin sulfate (glucosamine) 3-O-sulfotransferase 1	*HS3ST1*	1	4p16		
NM_001039591	Ubiquitin specific peptidase 9, X-linked	*USP9X*	1	Xp11.4		
NM_006196	Poly(rC) binding protein 1	*PCBP1*	1	2p13–p12		

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TABLE 1

Continued.

Accession No.	Gene description	Gene symbol	Total No. of clones	Gene locus	Genomic alterations by CGH[a]	Genes associated with endometriosis in the literature (reference number)
NM_170740	Aldehyde dehydrogenase 5 family, member A1 (succinate–semialdehyde dehydrogenase)	*ALDH5A1*	1	6p22.2–p22.3	Gain of 6p	
NM_012420	Interferon-induced protein with tetratricopeptide repeats 5	*IFIT5*	1	10q23.31		
NM_001032383	Polyglutamine binding protein 1	*PQBP1*	1	Xp11.23		
NM_014924	KIAA0831	*KIAA0831*	1	14q22.3		
NM_002165	Inhibitor of DNA binding 1, dominant negative helix-loop-helix protein	*ID1*	1	20q11		
NM_022484	Transmembrane protein 168	*TMEM168*	1	7q31.32	Gain of 7q	
NM_015932	Proteasome maturation protein	*POMP*	1	13q12.3		
NM_003014	Secreted frizzled-related protein 4	*SFRP4*	1	7p14.1		
NM_020122	Potassium channel modulatory factor 1	*KCMF1*	1	2p11.2		
NM_203330	CD59 molecule, complement regulatory protein	*CD59*	1	11p13		
NM_182810	Activating transcription factor 4 (tax-responsive enhancer element B67)	*ATF4*	1	22q13.1		
NM_001386	Dihydropyrimidinase-like 2	*DPYSL2*	1	8p22–p21		
NM_138786	Transmembrane 4 L six family member 18	*TM4SF18*	1	3q25.1		
NM_021999	Integral membrane protein 2B	*ITM2B*	1	13q14.3		
NM_012072	CD93 molecule	*CD93*	1	20p11.21		
NM_002795	Proteasome (prosome, macropain) subunit, beta type, 3	*PSMB3*	1	17q12	Gain of 17q	
NM_000712	Biliverdin reductase A	*BLVRA*	1	7p14-cen		
NM_001007224	GTPase, IMAP family member 6	*GIMAP6*	1	7q36.1	Gain of 7q	
NM_033429	Calmodulin-like 4	*CALML4*	1	15q23		
NM_015972	Polymerase (RNA) I polypeptide D, 16 kDa	*POLR1D*	1	13q12.2		
NM_003375	Voltage-dependent anion channel 2	*VDAC2*	1	10q22		
NM_015190	DnaJ (Hsp40) homolog, subfamily C, member 9	*DNAJC9*	1	10q22.2		
NM_002970	Spermidine/spermine N1–acetyltransferase	*SAT1*	1	Xp22.1		
NM_001025368	Vascular endothelial growth factor A	*VEGFA*	1	6p12	Gain of 6p	(18)

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TABLE 1

Continued.

Accession No.	Gene description	Gene symbol	Total No. of clones	Gene locus	Genomic alterations by CGH[a]	Genes associated with endometriosis in the literature (reference number)
NM_015483	Kelch repeat and BTB (POZ) domain containing 2	*KBTBD2*	1	7p14.3		
NM_017747	Ankyrin repeat and KH domain containing 1	*ANKHD1*	1	5q31.3		
NM_014267	Chromosome 11 open reading frame 58	*C11orf58*	1	11p15.1		
NM_007236	Calcium-binding protein P22	*CHP*	1	15q13.3		
NM_001031684	Splicing factor, arginine/serine-rich 7, 35 kDa	*SFRS7*	1	2p22.1		
NM_016085	Chromosome 2 open reading frame 28	*C2orf28*	1	2p23.3		
NM_003302	Thyroid hormone receptor interactor 6	*TRIP6*	1	7q22	Gain of 7q	
NM_020121	UDP–glucose ceramide glucosyltransferase-like 2	*UGCGL2*	1	13q32.1		
NM_004568	Serpin peptidase inhibitor, clade B (ovalbumin), member 6	*SERPINB6*	1	6p25	Gain of 6p	
NM_003994	KIT ligand	*KITLG*	1	12q22		(19)
NM_003336	Ubiquitin-conjugating enzyme E2A (RAD6 homolog)	*UBE2A*	1	Xq24–q25		
NM_000466	Peroxisome biogenesis factor 1	*PEX1*	1	7q21.2	Gain of 7q	
NM_002078	Golgi autoantigen, golgin subfamily a, 4	*GOLGA4*	1	3p22–p21.3		
NM_006694	Jumping translocation breakpoint	*JTB*	1	1q21	Gain of 1q	
NM_002399	Meis homeobox	*MEIS2*	1	15q14		
NM_016282	Adenylate kinase 3	*AK3*	1	9p24.1–p24.3		
NM_000019	*Homo sapiens* acetyl–coenzyme A acetyltransferase 1	*ACAT1*	1	11q22.3–q23.1		
NM_005803	Flotillin 1	*FLOT1*	1	6p21.3	Gain of 6p	
NM_002484	Nucleotide-binding protein 1 (MinD homolog, *E. coli*)	*NUBP1*	1	16p13.13		
NM_004938	Death-associated protein kinase 1	*DAPK1*	1	9q34.1		
NM_016299	Heat shock 70 kDa protein 14	*HSPA14*	1	10p13		
NM_001967	Eukaryotic translation initiation factor 4A, isoform 2	*EIF4A2*	1	3q28		
NM_001539	DnaJ (Hsp40) homolog, subfamily A, member 1	*DNAJA1*	1	9p13–p12		
NM_000107	Damage-specific DNA binding protein 2, 48 kDa	*DDB2*	1	11p12–p11		
NM_000184	Hemoglobin, gamma G	*HBG2*	1	11p15.5		
NM_198530	Matrix-remodeling associated 7	*MXRA7*	1	17q25.1–q25.2	Gain of 17q	

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TABLE 1

Continued.

Accession No.	Gene description	Gene symbol	Total No. of clones	Gene locus	Genomic alterations by CGH[a]	Genes associated with endometriosis in the literature (reference number)
NM_001514	General transcription factor IIB	*GTF2B*	1	1p22–p21		
NM_003199	Transcription factor 4	*TCF4*	1	18q21.1		
NM_005066	Splicing factor proline/glutamine-rich (polypyrimidine tract binding protein associated)	*SFPQ*	1	1p34.3		
NM_006636	Methylenetetrahydrofolate dehydrogenase (NADP+ dependent) 2, methenyltetrahydrofolate cyclohydrolase	*MTHFD2*	1	2p13.1		
NM_001001522	Transgelin	*TAGLN*	1	11q23.2		(20)
NM_018222	Parvin, alpha	*PARVA*	1	11p15.3		
NM_000378	Wilms tumor 1	*WT1*	1	11p13		(6)
NM_016107	Zinc finger RNA binding protein	*ZFR*	1	5p13.3		
NM_018025	G patch domain containing 1	*GPATCH1*	1	19q13.11		
NM_006169	Nicotinamide *N*-methyltransferase	*NNMT*	1	11q23.1		
NM_006324	Craniofacial development protein 1	*CFDP1*	1	16q22.2–q22.3		
NM_020154	Chromosome 15 open reading frame 24	*C15orf24*	1	15q14		
NM_019048	Asparagine synthetase domain containing 1	*ASNSD1*	1	2p24.3–q21.3		
XM_943856	PREDICTED: hypothetical protein LOC648185, transcript variant 1	*LOC648186*	1			
NM_020532	Reticulon 4	*RTN4*	1	2p16.3		
NM_001031700	Chromosome 4 open reading frame 18	*C4orf18*	1	4q32.1		
NM_152280	Synaptotagmin XI	*SYT11*	1	1q21.2	Gain of 1q	
NM_001204	Bone morphogenetic protein receptor, type II (serine/threonine kinase)	*BMPR2*	1	2q33–q34		
NM_032784	R-spondin 3 homolog (*Xenopus laevis*)	*RSPO3*	1	6q22.33	Gain of 6q	
NM_001568	Eukaryotic translation initiation factor 3, subunit 6 48 kDa	*EIF3S6*	1	8q22–q23		
NM_014236	Glyceronephosphate *O*-acyltransferase	*GNPAT*	1	1q42	Gain of 1q	
NM_001293	Chloride channel, nucleotide-sensitive, 1A	*CLNS1A*	1	11q13.5–q14		
NM_004162	RAB5A, member RAS oncogene family	*RAB5A*	1	3p24–p22		
NM_002696	Polymerase (RNA) II (DNA directed) polypeptide G	*POLR2G*	1	11q13.1		
NM_053025	Myosin, light chain kinase	*MYLK*	1	3q21		

Meola. Differential gene expression in endometriosis. Fertil Steril 2010.

TABLE 1

Continued.

Accession No.	Gene description	Gene symbol	Total No. of clones	Gene locus	Genomic alterations by CGH[a]	Genes associated with endometriosis in the literature (reference number)
NM_022488	ATG3 autophagy related 3 homolog (*S. cerevisiae*)	*ATG3*	1	3q13.2		
NM_002667	Phospholamban	*PLN*	1	6q22.1	Gain of 6q	
NM_002222	Inositol 1,4,5-triphosphate receptor, type 1	*ITPR1*	1	3p26–p25		
NM_004277	Solute carrier family 25, member 27	*SLC25A27*	1	6p11.2–q12	Gain of 6p	
NM_017599	Vezatin, adherens junctions transmembrane protein	*VEZT*	1	12q22		
NM_001025079	CD47 molecule	*CD47*	1	3q13.1–q13.2		
NM_017680	Asporin	*ASPN*	1	9q22		
NM_002072	Guanine nucleotide binding protein (G protein), q polypeptide	*GNAQ*	1	9q21		
NM_003349	Ubiquitin-conjugating enzyme E2 variant 1	*Kua-UEV*	1	20q13.2		
NM_001037637	Basic transcription factor 3	*BTF3*	1	5q13.2		
NM_005084	Phospholipase A2, group VII (platelet-activating factor acetylhydrolase, plasma)	*PLA2G7*	1	6p21.2–p12	Gain of 6p	
NM_182490	Zinc finger protein 227	*ZNF227*	1	19q13.32		
NM_022059	Chemokine (C-X-C motif) ligand 16	*CXCL16*	1	17p13		
NM_002300	Lactate dehydrogenase B	*LDHB*	1	12p12.2–p12.1		
NM_020313	Cytokine-induced apoptosis inhibitor 1	*CIAPIN1*	1	16q13–q21		
NM_016047	Splicing factor 3B, 14 kDa subunit	*SF3B14*	1	2pter–p25.1		
NM_198178	Microphthalmia-associated transcription factor	*MITF*	1	3p14.2–p14.1		
NM_199189	Matrin 3	*MATR3*	1	5q31.2		
NM_001020658	Pumilio homolog 1 (*Drosophila*)	*PUM1*	1	1p35.2		
NM_153000	Adenomatosis polyposis coli down-regulated 1	*APCDD1*	1	18p11.22		
NM_001015881	TSC22 domain family, member 3	*TSC22D3*	1	Xq22.3		
NM_006003	Ubiquinol-cytochrome c reductase, Rieske iron-sulfur polypeptide 1	*UQCRFS1*	1	19q12–q13.1		
NM_001031713	Chromosome 6 open reading frame 79	*CCDC90A*	1	6p24.3–p23	Gain of 6p	
NM_001839	Calponin 3, acidic	*CNN3*	1	1p22–p21		
NM_001039367	ATPase, H+ transporting, lysosomal 31 kDa, V1 subunit E1	*ATP6V1E1*	1	22q11.1		

Meola. Differential gene expression in endometriosis. Fertil Steril 2010.

TABLE 1

Continued.

Accession No.	Gene description	Gene symbol	Total No. of clones	Gene locus	Genomic alterations by CGH[a]	Genes associated with endometriosis in the literature (reference number)
NM_004899	Brain and reproductive organ–expressed (TNFRSF1A modulator)	*BRE*	2	2p23.2		
NM_001008897	t-Complex 1	*TCP1*	2	6q25.3–q26	Gain of 6q	
NM_015291	DnaJ (Hsp40) homolog, subfamily C, member 16	*DNAJC16*	2	1p36.1		
NM_006197	Pericentriolar material 1	*PCM1*	2	8p22–p21.3		
NM_206839	Mortality factor 4-like 1	*MORF4L1*	2	15q24		
XM_942776	PREDICTED: synaptopodin 2, transcript variant 3	*SYNPO2*	2			
NM_016091	Eukaryotic translation initiation factor 3, subunit 6 interacting protein	*EIF3S6IP*	2	22q		
NM_006628	Cyclic AMP phosphoprotein, 19 kDa	*ARPP-19*	2	15q21.2		
NM_138290	Rap2-binding protein 9	*RPIB9*	2	7q21.12	Gain of 7q	
NM_198467	Round spermatid basic protein 1-like	*RSBN1L*	2	7q11.23	Gain of 7q	
NM_003850	Succinate–CoA ligase, ADP-forming, beta subunit	*SUCLA2*	2	13q12.2–q13.3		
NM_001748	Calpain 2, (m/II) large subunit	*CAPN2*	2	1q41–q42	Gain of 1q	
NM_002264	Karyopherin alpha 1 (importin alpha 5)	*KPNA1*	2	3q21		
NM_002023	Fibromodulin	*FMOD*	2	1q32	Gain of 1q	
NM_018374	Transmembrane protein 106B	*TMEM106B*	2	7p21.3		
NM_013236	Ataxin 10	*ATXN10*	2	22q13.31		
NR_001564	X (inactive)-specific transcript	*XIST*	2	Xq13.2		
NM_004990	Methionine-tRNA synthetase	*MARS*	2	12q13.2		
NM_000454	Superoxide dismutase 1, soluble (amyotrophic lateral sclerosis 1 (adult) (SOD1)	*SOD1*	2	21q22.11		
NM_001001395	LIM domain only 3 (rhombotin-like 2)	*LMO3*	2	12p12.3		
NM_001039618	CREB/ATF bZIP transcription factor	*CREBZF*	2	11q14		
NM_007168	ATP-binding cassette, subfamily A (ABC1), member 8	*ABCA8*	2	17q24	Gain of 17q	
NM_000177	Gelsolin (amyloidosis, Finnish type)	*GSN*	2	9q33		
NM_002738	Protein kinase C, beta 1	*PRKCB1*	2	16p11.2		
NM_013448	Bromodomain adjacent to zinc finger domain, 1A	*BAZ1A*	2	14q12–q13		
NM_173473	Chromosome 10 open reading frame 104	*C10orf104*	2	10q22.1		

Meola. Differential gene expression in endometriosis. Fertil Steril 2010.

TABLE 1

Continued.

Accession No.	Gene description	Gene symbol	Total No. of clones	Gene locus	Genomic alterations by CGH[a]	Genes associated with endometriosis in the literature (reference number)
NM_213674	Tropomyosin 2 (beta)	*TPM2*	2	9p13.2–p13.1		
NM_013254	TANK-binding kinase 1	*TBK1*	2	12q14.1		
NM_005506	Scavenger receptor class B, member 2	*SCARB2*	2	4q21.1		
NM_001031679	Methionine sulfoxide reductase B3	*MSRB3*	2	12q14.3		
NM_199511	Coiled-coil domain containing 80	*CCDC80*	2	3q13.2		
NM_013234	Eukaryotic translation initiation factor 3, subunit 12	*EIF3S12*	2	19q13.2		
NM_020338	Zinc finger, MIZ-type containing 1	*ZMIZ1*	2	10q22.3		
NM_138957	Mitogen-activated protein kinase 1	*MAPK1*	2	22q11.21		
XM_371853	PREDICTED: similar to 60S ribosomal protein L27a	*LOC389435*	2	—		
NM_001615	Actin, gamma 2, smooth muscle, enteric	*ACTG2*	2	2p13.1		
NM_001001894	Tetratricopeptide repeat domain 3	*TTC3*	3	21q22.2		
NM_005724	Tetraspanin 3	*TSPAN3*	3	15q24.3		
NM_206876	Protein phosphatase 1, catalytic subunit, beta isoform	*PPP1CB*	3	2p23		
NM_015375	Receptor interacting protein kinase 5	*RIPK5*	3	1q32.1	Gain of 1q	
NM_000943	Peptidylprolyl isomerase C (cyclophilin C)	*PPIC*	3	5q23.2		
NM_000700	Annexin A1	*ANXA1*	3	9q12–q21.2		
NM_002802	Proteasome (prosome, macropain) 26S subunit, ATPase, 1	*PSMC1*	3	14q32.11		
NM_014585	Solute carrier family 40 (iron-regulated transporter), member 1	*SLC40A1*	3	2q32		
NM_000426	Laminin, alpha 2 (merosin, congenital muscular dystrophy)	*LAMA2*	3	6q22–q23	Gain of 6q	
NM_006513	Seryl-tRNA synthetase	*SARS*	3	1p13.3–p13.1		
NM_014367	Chromosome 3 open reading frame 28	*C3orf28*	3	3q21.1		
NM_003756	Eukaryotic translation initiation factor 3, subunit 3 gamma, 40 kDa	*EIF3S3*	3	8q24.11		
NM_004684	SPARC-like 1 (mast9, hevin)	*SPARCL1*	4	4q22.1		
NM_004064	Cyclin-dependent kinase inhibitor 1B (p27, Kip1)	*CDKN1B*	4	12p13.1–p12		(21)
NM_012134	Leiomodin 1 (smooth muscle)	*LMOD1*	4	1q32	Gain of 1q	
NM_002345	Lumican	*LUM*	4	12q21.3–q22		

Meola. Differential gene expression in endometriosis. Fertil Steril 2010.

TABLE 1

Continued.

Accession No.	Gene description	Gene symbol	Total No. of clones	Gene locus	Genomic alterations by CGH[a]	Genes associated with endometriosis in the literature (reference number)
NM_001780	CD63 molecule	*CD63*	4	12q12–q13		
NM_022138	SPARC-related modular calcium binding 2	*SMOC2*	4	6q27	Gain of 6q	
NM_201442	Complement component 1, s subcomponent	*C1S*	4	12p13		
NM_001743	Calmodulin 2 (phosphorylase kinase, delta)	*CALM2*	4	2p21		
NM_006360	PCI domain containing 1 (herpesvirus entry mediator)	*PCID1*	4	11p13		
NM_003295	Tumor protein, translationally controlled 1	*TPT1*	4	13q12–q14		
NM_002166	Inhibitor of DNA binding 2, dominant negative helix-loop-helix protein	*ID2*	5	2p25		
NM_004048	Beta-2-microglobulin	*B2M*	5	15q21–q22.2		(22)
NM_201348	Proline/arginine-rich end leucine-rich repeat protein	*PRELP*	5	1q32	Gain of 1q	
NM_001018020	Tropomyosin 1 (alpha)	*TPM1*	5	15q22.1		
NM_004537	Nucleosome assembly protein 1-like 1	*NAP1L1*	5	12q21.2		
NM_003333	Ubiquitin A-52 residue ribosomal protein fusion product 1	*UBA52*	6	19p13.1–p12		
NM_014624	S100 calcium binding protein A6	*S100A6*	7	1q21	Gain of 1q	
NM_001901	Connective tissue growth factor	*CTGF*	7	6q23.1	Gain of 6q	(23)
NM_006098	Guanine nucleotide binding protein (G protein), beta polypeptide 2-like 1	*GNB2L1*	9	5q35.3		
NM_003118	Secreted protein, acidic, cysteine-rich (osteonectin)	*SPARC*	10	5q31.3–q32		
NM_033138	Caldesmon 1	*CALD1*	17	7q33	Gain of 7q	
NM_001613	Actin, alpha 2, smooth muscle, aorta	*ACTA2*	29	10q23.3		

Note: ADP = adenosine diphosphate; AMP = adenosine monophosphate; ATP = adenosine triphosphate; ATPase = adenosine triphosphatase; CoA = coenzyme A; GTPase = guanosine triphosphatase; NADP = nicotinamide-adenine dinucleotide phosphate; tRNA = transfer RNA; UDP = uridine diphosphate.

[a] According to Gogusev et al. (24, 25).

Meola. Differential gene expression in endometriosis. Fertil Steril 2010.

TABLE 2

Genes down-regulated in ectopic versus eutopic endometrium of women with endometriosis.

Accession No.	Gene description	Gene symbol	Total No. of clones	Gene locus	Genomic alterations by CGH[a]	Genes associated with endometriosis in the literature (reference number)
NM_032940	Polymerase (RNA) II (DNA directed) polypeptide C, 33 kDa	*POLR2C*	1	16q13–q21		
NM_181472	CKLF-like MARVEL transmembrane domain containing 7	*CMTM7*	1	3p22.3		
NM_002568	Poly(A) binding protein, cytoplasmic 1	*PABPC1*	1	8q22.2–q23		
NM_014300	SEC11-like 1 (*S. cerevisiae*)	*SEC11A*	1	15q25.3		
NM_018509	Leucine-rich repeat containing 59	*LRRC59*	1	17q21.33		
NM_015330	SPECC1-like	*SPECC1L*	1	22q11.23	Loss of 22q	
NM_080821	Chromosome 20 open reading frame 108	*C20orf108*	1	20q13.2		
NM_001014795	Integrin-linked kinase	*ILK*	1	11p15.5–p15.4		
NM_005907	Mannosidase, alpha, class 1A, member 1	*MAN1A1*	1	6q22		
XM_934318	PREDICTED: similar to eukaryotic translation initiation factor 3, subunit 8	*LOC653352*	1			
NM_012426	Splicing factor 3b, subunit 3, 130 kDa	*SF3B3*	1	16q22.1		
NM_001002292	G protein–coupled receptor 177	*GPR177*	1	1p31.3	Loss of 1p	
NM_144570	Chromosome 16 open reading frame 34	*HN1L*	1	16p13.3		
NM_006601	Prostaglandin E synthase 3 (cytosolic)	*PTGES3*	1	12q13.3		
NM_004047	ATPase, H+ transporting, lysosomal 21 kDa, V0 subunit b	*ATP6V0B*	1	1p32.3	Loss of 1p	
NM_000715	Complement component 4 binding protein, alpha	*C4BPA*	1	1q32		
NM_006022	TSC22 domain family, member 1	*TSC22D1*	1	13q14		
NM_207307	Chromosome 3 open reading frame 25	*C3orf25*	1	3q21.3		

Meola. Differential gene expression in endometriosis. Fertil Steril 2010.

TABLE 2

Continued.

Accession No.	Gene description	Gene symbol	Total No. of clones	Gene locus	Genomic alterations by CGH[a]	Genes associated with endometriosis in the literature (reference number)
NM_002423	MMP 7 (matrilysin, uterine)	*MMP7*	1	11q21–q22		(26)
NM_003754	Eukaryotic translation initiation factor 3, subunit 5 epsilon, 47 kDa	*EIF3S5*	1	11p15.4		
NM_001797	Cadherin 11, type 2, OB-cadherin (osteoblast)	*CDH11*	1	16q22.1		
NM_014064	Chromosome 9 open reading frame 32	*C9orf32*	1	9q34.11	Loss of 9q	
NM_014306	Chromosome 22 open reading frame 28	*C22orf28*	1	22q12	Loss of 22q	
NM_001005335	Heterogeneous nuclear ribonucleoprotein L	*HNRPL*	1	19q13.2		
NM_175932	Proteasome (prosome, macropain) 26S subunit, non-ATPase, 13	*PSMD13*	1	11p15.5		
NM_001428	Enolase 1 (alpha)	*ENO1*	1	1p36.3–p36.2	Loss of 1p	
NM_030767	AT-hook transcription factor	*AKNA*	1	9q32	Loss of 9q	
NM_145729	Mitochondrial ribosomal protein L24	*MRPL24*	1	1q21–q22		
NM_003720	Down syndrome critical region gene 2	*DSCR2*	1	21q22.3		
NM_022075	LAG1 homolog, ceramide synthase 2 (S. cerevisiae)	*LASS2*	1	1q21.2		
NM_002950	Ribophorin I	*RPN1*	1	3q21.3		
NM_000088	Collagen, type I, alpha 1	*COL1A1*	1	17q21.33		
NM_003564	Transgelin 2	*TAGLN2*	1	1q21–q25		
NM_001012663	Solute carrier family 3 (activators of dibasic and neutral amino acid transport), member 2	*SLC3A2*	1	11q13		
NM_003580	Neutral sphingomyelinase (N-SMase) activation associated factor	*NSMAF*	1	8q12–q13		

Meola. Differential gene expression in endometriosis. Fertil Steril 2010.

TABLE 2

Continued.

Accession No.	Gene description	Gene symbol	Total No. of clones	Gene locus	Genomic alterations by CGH[a]	Genes associated with endometriosis in the literature (reference number)
NM_022762	Required for meiotic nuclear division 5 homolog B (*S. cerevisiae*)	*RMND5B*	1	5q35.3		
NM_019894	Transmembrane protease, serine 4	*TMPRSS4*	1	11q23.3		
NM_001001998	Exosome component 10	*EXOSC10*	1	1p36.22	Loss of 1p	
NM_000942	Peptidylprolyl isomerase B (cyclophilin B)	*PPIB*	1	15q21–q22		
NM_198334	Glucosidase, alpha; neutral AB	*GANAB*	1	11q12.3		
NM_001017402	Laminin, beta 3	*LAMB3*	1	1q32		
NM_021729	Vacuolar protein sorting 11 homolog (*S. cerevisiae*)	*VPS11*	1	11q23		
NM_015070	Zinc finger CCCH-type containing 13	*ZC3H13*	1	13q14.12		
NM_031457	Membrane-spanning 4-domains, subfamily A, member 8B	*MS4A8B*	1	11q12.2		
NM_002117	Major histocompatibility complex, class I, C	*HLA-C*	1	6p21.3		(27)
NM_000597	Insulin-like growth factor binding protein 2, 36 kDa	*IGFBP2*	1	2q33–q34		(28)
NM_005204	Mitogen-activated protein kinase kinase kinase 8	*MAP3K8*	1	10p11.23		
NM_004078	Cysteine and glycine-rich protein 1	*CSRP1*	1	1q32		
NM_004394	Death-associated protein	*DAP*	1	5p15.2		
NM_173728	Rho guanine nucleotide exchange factor (GEF) 15	*ARHGEF15*	1	17p13.1		
NM_001669	Arylsulfatase D	*ARSD*	2	Xp22.3		
NM_005168	Rho family GTPase 3	*RND3*	2	2q23.3		
NM_000362	TIMP metallopeptidase inhibitor 3 (Sorsby fundus dystrophy, pseudoinflammatory)	*TIMP3*	2	22q12.3	Loss of 22q	

Meola. Differential gene expression in endometriosis. Fertil Steril 2010.

TABLE 2

Continued.

Accession No.	Gene description	Gene symbol	Total No. of clones	Gene locus	Genomic alterations by CGH[a]	Genes associated with endometriosis in the literature (reference number)
NM_006816	Lectin, mannose-binding 2	*LMAN2*	2	5q35.3		
NM_016057	Coatomer protein complex, subunit zeta 1	*COPZ1*	2	12q13.2–q13.3		
NM_001733	Complement component 1, r subcomponent	*C1R*	2	12p13		
NM_152927	Copine I	*CPNE1*	2	20q11.22		
NM_003321	Tu translation elongation factor, mitochondrial	*TUFM*	2	16p11.2		
NM_006801	KDEL (Lys-Asp-Glu-Leu) endoplasmic reticulum protein retention receptor 1	*KDELR1*	2	19q13.3		
NM_004356	CD81 molecule	*CD81*	2	11p15.5		
NM_000358	Transforming growth factor, beta-induced, 68 kDa	*TGFBI*	2	5q31		(29)
NM_003355	Uncoupling protein 2 (mitochondrial, proton carrier)	*UCP2*	2	11q13		
NM_002467	v-myc Myelocytomatosis viral oncogene homolog (avian)	*MYC*	2	8q24.21		(30)
NM_006701	Thioredoxin-like 4[a]	*TXNL4A*	2	18q23		
NM_052886	mal, T-cell differentiation protein 2	*MAL2*	2	8q23		
NM_014764	DAZ associated protein 2	*DAZAP2*	2	12q12		
NM_015343	Dullard homolog (*X. laevis*)	*DULLARD*	2	17p13		
XM_932704	PREDICTED: similar to 60S ribosomal protein L29 (cell surface heparin binding protein HIP)	*LOC643433*	1			
NM_016395	Protein tyrosine phosphatase-like A domain containing 1	*PTPLAD1*	2	15q22.2		
NM_001540	Heat shock 27 kDa protein 1	*HSPB1*	2	7q11.23		(31)
NM_004285	Hexose-6-phosphate dehydrogenase (glucose 1-dehydrogenase)	*H6PD*	1	1p36	Loss of 1p	
NM_001034850	Family with sequence similarity 134, member B	*FAM134B*	1	5p15.1		

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TABLE 2

Continued.

Accession No.	Gene description	Gene symbol	Total No. of clones	Gene locus	Genomic alterations by CGH[a]	Genes associated with endometriosis in the literature (reference number)
NM_005347	Heat shock 70 kDa protein 5 (glucose-regulated protein, 78 kDa)	*HSPA5*	2	9q33–q34.1	Loss of 9q	
NM_014372	Ring finger protein 11	*RNF11*	2	1pter–p22.1	Loss of 1p	
NM_177967	UBA domain containing 2	*UBAC2*	2	13q32.3		
NM_015331	Nicastrin	*NCSTN*	2	1q22–q23		
NM_001349	Aspartyl-tRNA synthetase	*DARS*	2	2q21.3		
NM_001001481	Ubiquitin-conjugating enzyme E2W (putative)	*UBE2W*	2	8q21.11		
NM_002425	Matrix metallopeptidase 10 (stromelysin 2)	*MMP10*	2	11q22.3		
NM_001469	X-ray repair complementing defective repair in Chinese hamster cells 6 (Ku autoantigen, 70 kDa)	*XRCC6*	2	22q13.2–q13.31	Loss of 22q	
NM_007355	Heat shock protein 90 kDa alpha (cytosolic), class B member 1	*HSP90AB1*	3	6p12		
NM_057164	Collagen, type VI, alpha 3	*COL6A3*	3	2q37		
NM_005040	Prolylcarboxypeptidase (angiotensinase C)	*PRCP*	3	11q14		
NM_003128	Spectrin, beta, nonerythrocytic 1	*SPTBN1*	3	2p21		
NM_005727	Tetraspanin 1	*TSPAN1*	3	1p34.1	Loss of 1p	
NM_002797	Proteasome (prosome, macropain) subunit, beta type, 5	*PSMB5*	3	14q11.2		
NM_006732	FBJ murine osteosarcoma viral oncogene homolog B	*FOSB*	2	19q13.32		
NM_020130	Chromosome 8 open reading frame 4	*C8orf4*	3	8p11.2		
NM_001940	Atrophin 1	*ATN1*	3	12p13.31		
NM_014713	Lysosomal-associated protein transmembrane 4 alpha	*LAPTM4A*	3	2p24.1		

Meola. Differential gene expression in endometriosis. Fertil Steril 2010.

TABLE 2

Continued.

Accession No.	Gene description	Gene symbol	Total No. of clones	Gene locus	Genomic alterations by CGH[a]	Genes associated with endometriosis in the literature (reference number)
NM_001101	*Homo sapiens* actin, beta	*ACTB*	4	7p15–p12		
NM_002087	Granulin	*GRN*	3	17q21.32		
NM_014679	Centrosomal protein 57 kDa	*CEP57*	1	11q21		
NM_175744	ras Homolog gene family, member C	*RHOC*	4	1p13.1	Loss of 1p	
NM_005566	Lactate dehydrogenase A	*LDHA*	4	11p15.4		
NM_001002235	Serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 1	*SERPINA1*	6	14q32.1		
NM_000596	Insulin-like growth factor binding protein 1	*IGFBP1*	6	7p13–p12		(32)
NM_001961	Eukaryotic translation elongation factor 2	*EEF2*	7	19pter–q12		
NM_002422	Matrix metallopeptidase 3 (stromelysin 1, progelatinase)	*MMP3*	23	11q22.3		(33)
NM_000518	Hemoglobin, beta	*HBB*	25	11p15.5		
NM_001018049	Progestagen-associated endometrial protein	*PAEP*	126	9q34	Loss of 9q	(34)

Note: ATPase = adenosine triphosphatase; GTPase = guanosine triphosphatase; tRNA = transfer RNA.

[a] According to Gogusev et al. (24, 25).

Meola. Differential gene expression in endometriosis. Fertil Steril 2010.

ontology terms, of which 13 processes seem to be relevant to disease development (Table 3).

Real-Time PCR For the *SPARC, CTGF, MYC, MMP3, and IGFBP1* genes

As shown in Figures 2 and 3, PCR analyses of the five selected genes validated the data obtained by RaSH (indicated by the symbol [3]). These figures also present the other gene expression data from the studied tissues. No significant correlation was detected between expression levels obtained in the ectopic endometrium and the disease staging.

DISCUSSION

With use of the RaSH method, it is possible to analyze genes that are expressed differentially in two samples, including genes that have not been characterized previously and rare transcripts. This method also has simpler hybridization and subtraction steps than other subtractive hybridization methods (8). Hu et al. (9) applied the subtractive hybridization of solid-phase technique using magnetic beads in eutopic and ectopic endometrium of women with peritoneal endometriosis during the proliferative and secretive phases of the menstrual cycle. In our study, the eutopic and ectopic endometrium of women with endometriosis also was analyzed

TABLE 3

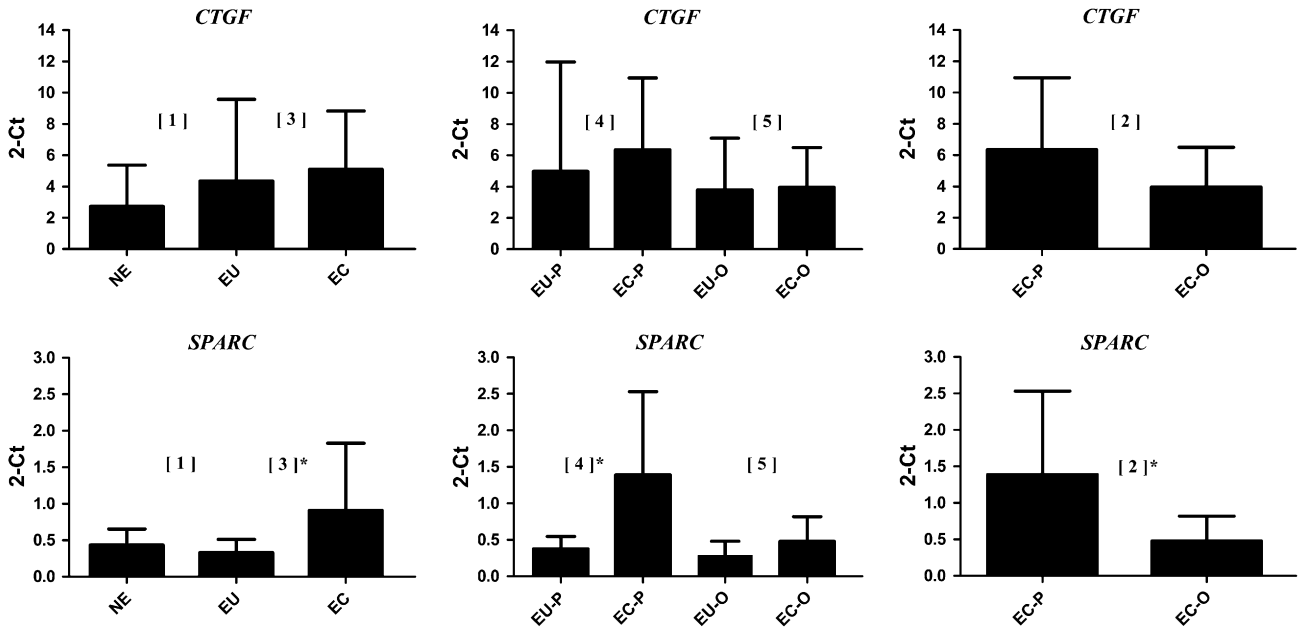
Differentially expressed genes in relevant gene ontology terms.

Gene ontology terms	Genes up-regulated	Genes down-regulated
Angiogenesis	*VEGF A, CTGF*	
Cell adhesion	*CD47, CTGF, KITLG, SCARB2, LPP, PARVA, VEZT, TRIP6, CFDP1, GPNMB, LAMA2, CD93*	*TGFBI, LAMB3, TSPAN1, COL6A3 CDH11, ILK*
Cell motility	*ANXA1, TPM1, CTGF, TSPAN3, GNA13, CALD1 CDKN1B, TRIP6, CXCL16, VEGFA, UBA52, LAMA2*	*HSPB1, TSPAN1*
Cell proliferation	*MITF, CDKN1B, S100A6, TSPAN3, CFDP1, KITLG, ANXA1, VEGFA, GPNMB, BMPR2, PPP1CB, NAP1L1*	*TGFBI, GRN, MYC, TSPAN1, IKL, CD81*
Immune response	*B2M, IFIT5, POMP, CD59, C1S, TBK1*	*HLA-C, C4BPA*
Immune system process	*IFIT5, MITF, B2M, CXXL16, POMP, KITLG, C1S, TBK1, CD59, CD93*	*HLA-C, CD81, C4BPA*
Proteolysis	*CAPN2, C1S, PSMB3, USP9X, UBE2A,UBA52*	*C1R, MMP3, MMP7, MMP10, PRCP, SEC11A, TMPRSS4, PSMB5, NCSTN, RNF11, C4BPA*
Regulation of cell growth	*CTGF, MORF4L1*	*IGFBP1, IGFBP2*
Response to wounding	*CTGF, PLA2G7, GNA13, C1S, ANXA1, CD59, GNAQ*	*SERPINA1, C4BPA*
Tissue remodeling	*SPARC, CTGF*	*CDH11*
Cell communication	*MITF, BRE, CHP, PRKCB1, S100A6, FMOD, ID1, TRIP6, SFRP4, GNA13, NENF, KITLG, ITPR1, CALM2, DAPK1, TBK1, ANXA1, VEGFA, DPYSL2, ALDH5A1, UBA52, BMPR2, GNB2L1, CD47, SPARC, CTGF, CD59, RAB5A, MAPK1, PPIC, RSPO3, GNAQ*	*RHOC, IGFBP1, ARHGEF15, PTGES3, MS4A8B, RND3, NCSTN, MYC, ILK, PTPLAD1, HSPA5, CD81, TIMP3, CEP57, GRN, NSMAF, GPR177*
Cell differentiation	*SFRP4, MITF, BRE, S100A6, CLN5, WT1, CFDP1, GNA13, ITM2B, DAPK1, ANXA1, RTN4, VEGFA, TPT1, UBA52, CTGF, TSC22D3, MAPK1, GNAQ*	*DAP, MYC, HSPA5, HSPB1, PAEP*
Cell death	*MITF, BRE, CFDP1, ITM2B, DAPK1, ANXA1, RTN4, VEGFA, TPT1, TSC22D3, MAPK1*	*DAP, MYC, HSPA5, HSPB1*

Meola. Differential gene expression in endometriosis. Fertil Steril 2010.

FIGURE 2

Real-time PCR data for genes *CTGF* and *SPARC,* which were found to be up-regulated by RaSH in the ectopic endometrium. NE = control endometrium (n = 10), EU = eutopic endometrium (n = 17), EC= ectopic endometrium (n = 17, peritoneal + ovarian), EC-P = ectopic peritoneal (n = 8), EC-O = ectopic ovarian (n = 9), EU-P = eutopic from patients with peritoneal lesion (n = 8), and EU-O = eutopic from patients with ovarian lesion (n = 9). The numbers in parenthesis were used to indicate the comparisons between [1] NE × EU, [2] EC-P × EC-O, [3] EU × EC, [4] EU-P × EC-P, and [5] EU-O × EC-O. *Comparisons that presented significant difference at $P<.05$.



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by RaSH; however, patients in the early proliferative phase of the menstrual cycle with peritoneal and ovarian lesions were selected. Because the gene expression profile is variable across menstrual cycle phases, the selection of a specific cycle phase, in addition to the selection of paired samples, is mandatory to minimize cyclic and individual variability in the women analyzed.

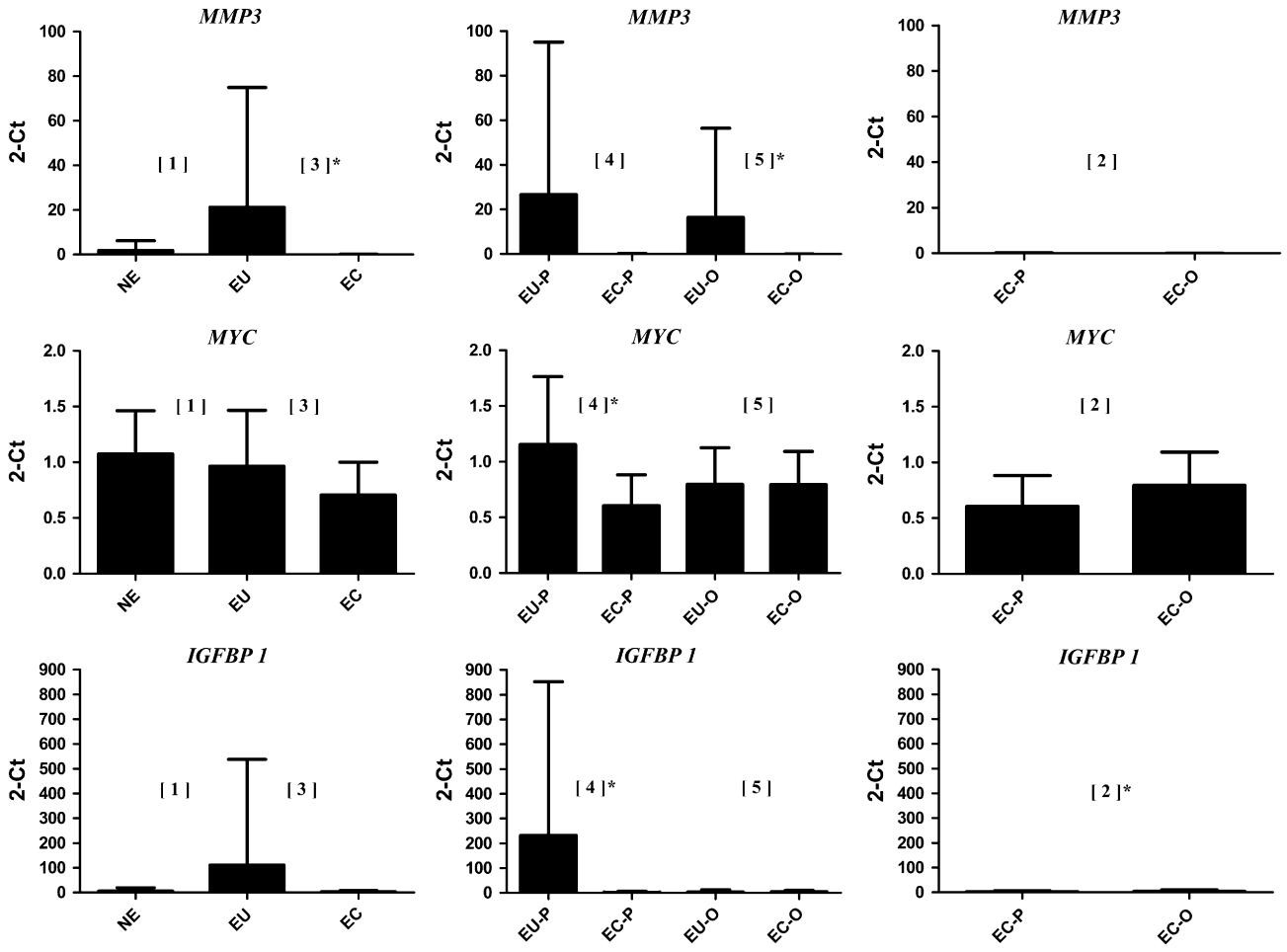
The RaSH method provided 1,339 quality sequences. After rigorous selection and classification, we found that 291 genes were deregulated, with 191 up-regulated and 100 down-regulated in the lesions (Table 1 and 2). Although this technique is efficient for detecting gene expression differences, it has certain limitations. Not all cDNA sequences can be removed completely after subtraction (9). To detect these false-positives, we produced two libraries: the tester was initially evaluated as a tester, then used as a driver. One library indicated the genes more expressed in the lesions (Tester = Ectopic × Driver = Eutopic), whereas the reverse library indicated the genes less expressed in the lesions (Tester = Eutopic × Driver = Ectopic). Such libraries presented 13% of the false-positive sequences among the RefSeq.

In our study, we found 17 genes that have been associated with endometriosis already, such as *MYC, MMP3, IGFBP1, CTGF,* and *PAEP,* and 274 genes (including *SPARC*) that have not yet been studied in relation to the disease and are therefore targets for future analyses (Tables 1 and 2). In addition, we compared the chromosomal regions of the obtained genes with the regions that have been shown previously to have genomic alterations that were detected by CGH in endometriotic lesions. We observed 36 up-regulated genes and 16 down-regulated genes in regions of gain and loss of genomic material, respectively (Tables 1 and 2). These include *PAEP* in the region of loss 9q and *CTGF* in the region of gain 6q. It already has been demonstrated that genomic alterations in patients with endometriosis may be associated with the development of the disease (24, 25). This phenomenon could explain some of our differential expression data.

A number of events are necessary for the establishment of endometriosis, for both implantation and maintenance of the ectopic tissue: menstrual reflux followed by escape from immunologic surveillance, adhesion, invasion, cell proliferation, apoptosis inhibition, angiogenesis, local estrogen production, and response to wounding (2). The deregulation of genes involved in these processes can lead to the establishment and survival of the endometriotic implants. After assembling the genes that were expressed differentially according to their participation in certain biologic processes,

FIGURE 3

Real-time PCR data for genes *MYC*, *MMP3,* and *IGFBP1,* which were found to be down-regulated by RaSH in the ectopic endometrium. NE = control endometrium (n = 10), EU = eutopic endometrium (n = 17), EC = ectopic endometrium (n = 17, peritoneal + ovarian), EC-P = ectopic peritoneal (n = 8), EC-O = ectopic ovarian (n = 9), EU-P = eutopic from patients with peritoneal lesion (n = 8), and EU-O = eutopic from patients with ovarian lesion (n = 9). The numbers in parenthesis were used to indicate the comparisons between [1] NE × EU, [2] EC-P × EC-O, [3] EU × EC, [4] EU-P × EC-P, and [5] EU-O × EC-O. *Comparisons that presented significant difference at $P<.05$.



Meola. Differential gene expression in endometriosis. Fertil Steril 2010.

we found 283 different processes; those most relevant for the disease are presented in Table 3. Such genes are targets for future studies.

To validate the subtractive libraries, the genes *CTGF* and *SPARC*, found to be up-regulated, and the genes *IGFBP1, MMP3,* and *MYC*, found to be down-regulated in the ectopic endometrium, were selected for real-time PCR analysis. In addition to data analyses of the 12 samples used for the preparation of libraries, we increased the sample to include 10 control endometria from women without endometriosis and 17 eutopic tissues and 17 ectopic tissues from women with endometriosis (8 peritoneal and 9 ovarian) to establish a possible involvement of the genes in the disease.

All genes selected confirmed the differences of expression that were detected by the libraries; however, these differences were significant among the eutopic and ectopic groups only for genes *SPARC* and *MMP3*. When the analyses were performed by separating the peritoneal lesion group from the ovarian endometrioma group and comparing them with their autologous tissues, significant differences were obtained for genes *SPARC, MYC,* and *IGFBP1* in the peritoneal lesions and for gene *MMP3* in the peritoneal lesions. *SPARC* and *IGFBP1* also presented significant differences of expression when the peritoneal and ovarian lesions were compared. Most likely, the assembly of the lesions has disguised differences of expression that are specific characteristics of the sites where the lesions appear. Thus, the differences observed

between the eutopic endometrium and the endometriotic lesions from affected patients, as well as among the lesions, may be explained as a direct consequence of the different endocrine environments, such as the peritoneal fluid and the intraovarian microenvironment of the lesions, in relation to the intrauterine environment (35).

SPARC is a matricellular antiadhesive protein that mediates the interactions between the extracellular matrix and the cells (36). This gene is involved in a number of biologic processes including tissue remodeling, cellular adhesion, angiogenesis, proliferation, migration, and tumoral invasion (37). *SPARC* up-regulation has been associated previously with the decrease of E-caderin in melanoma, increasing the tumor invasive potential (38); the increase of cellular motility in breast cancer due to its antiadhesive properties (39); and the stimulation of *VEGF* in endothelial cells leading to an increase of angiogenesis (37). It also has been demonstrated that *SPARC* positively regulates *CTGF*, which responds to *SPARC* by negative feedback (40). *CTGF* is a member of the CCN family (*Cyr61, CTGF, NOV*) that seems to be involved in many cellular functions such as proliferation, adhesion, migration, apoptosis, production of extracellular matrix, embryonic development, tissue differentiation, angiogenesis, and cicatrization in addition to being associated with a number of pathologic conditions such as fibrosis, inflammation, and tumoral growth (41). Absenger et al. (42) detected an increase of *CTFG* expression in the eutopic endometrium from patients with endometriosis in relation to control endometrium using microarrays. Recently, an up-regulation of this gene also was detected in endometriotic lesions induced in animal models (23). Although significant differences between the eutopic and ectopic endometrium have been observed only for *SPARC*, it is likely that the deregulation of these two genes allows for the establishment and survival of the endometriotic implants because of the genes' regulation of the invasion and angiogenesis processes.

Extracellular matrix disarrangement is a normal event that is essential for embryonic development, morphogenesis, reproduction, reabsorption, and tissue remodeling. The matrix metalloproteinases (MMPs) and their inhibitors (tissue inhibitor of metalloproteinases [TIMPs]) are essential participants in this event (43). Although the endometrial expression of MMPs and TIMPs is regulated cyclically (44), abnormal profiles of expression have been detected in ectopic and eutopic tissues from patients with endometriosis, which may be a result of the loss of response to P found in women with this disease. This loss of response can be associated with the failure to suppress MMPs and consequently invasion of the endometrial tissue into extrauterine sites (45). Gilabert-Estellés et al. (46) found higher expression of *MMP3* in eutopic endometrial tissue from women with endometriosis than in the control endometrium. Also, the authors detected a lower proteolytic activity in ovarian endometrioma as a result of *TIMP-1* up-regulation. However, Uzan et al. (33) detected a higher expression of *MMP3* in the endometrium of women without endometriosis compared with the eutopic tissue of women with endometriosis. Moreover, they showed a higher expression of this metalloproteinase in colorectal endometriosis when compared with peritoneal lesions and ovarian endometriomas. In our study, we found a lower expression of *MMP3* in endometriotic lesions, which could indicate less invasive activity of the lesions for being already locally established.

The up-regulation of *MYC* can lead to both cellular proliferation and apoptosis (47). The induction of programmed cell death by this gene is related to cytochrome C release (48). The up-regulation of the antiapoptosis *BCL-2* (B-cell lymphoma/leukemia-2) gene protects the cells against *MYC* action, blocking cytochrome C release (49) but has no effect on the proliferative action of *MYC* (50). *BAX* (BCL2-associated X protein), a proapoptotic member of the bcl-2 family, stimulates cell death by releasing cytochrome C and consequently inducing *MYC* (51). In the eutopic endometrium of women with endometriosis, a decrease in apoptotic cells (52) and *BCL-2* up-regulation and *BAX* down-regulation (53) has been described. Nonetheless, Johnson et al. (30) found *MYC* expression to be higher in the eutopic endometrium of women with endometriosis than in the endometrium of normal women. Although the data regarding the regulation and function of *MYC* are controversial in the literature, it is possible that the low expression of this gene in endometriotic lesions is a result of loss of apoptosis stimulation by an altered *MYC, BCL-2,* and *BAX* pathway in the endometriotic lesions.

Insulin-like growth factor-I (IGF-I) and IGF-II are present in human endometrium and are potent proliferation and growth inducers in endometrial cell cultures (54). These factors are regulated by the IGF-binding proteins family (IGFBPs), where *IGFBP-1* plays a role as *IGF-I* inhibitor (55). The increase of *IGFBP-1* expression in prostate and breast cancer has been associated with tumoral suppression, apoptosis induction, and decrease of cell proliferation (56). A lower expression of this gene was detected in follicular fluid from patients with moderate and severe endometriosis (57), an increased expression was found in the eutopic endometrium of baboons with endometriosis in comparison with the normal endometrium (58), and a decreased expression in endometriotic lesions was found when compared with eutopic tissue from patients with endometriosis (32). Additionally, an IGF-I and IGF-II increase was detected in the peritoneal fluid of patients with endometriosis (59). These literature data support the *IGFBP1* deregulation results found in our study and emphasize the idea that altered expression of the IGF system in endometriosis may be one of the factors responsible for ectopic tissue growth.

Therefore, *SPARC, MYC, MMP3,* and *IGFBPI* deregulation might cause a loss of cellular homeostasis in the endometriotic lesions, contributing to the implantation and survival of ectopic tissue in the extrauterine environment. In the future, it will be necessary to assess the function of the identified genes and characterize their role in the development of the disease.

The eutopic and ectopic endometrium of women with endometriosis shares alterations that are not found in the eutopic endometrium of women without endometriosis, corroborating the idea that this altered endometrium in the peritoneal cavity has the initial potential to develop endometriosis (60). In this study, however, when endometrium from the control group was compared with eutopic endometrium from patients with endometriosis, no significant differences were found in the studied genes. This conflict with the literature may be related to the different phases of the menstrual cycle of the samples studied, the different types of lesions evaluated, and/or the different techniques used in previous studies (47) and our study.

This study provided 291 candidate genes with deregulated gene expression in the early proliferative phase of the menstrual cycle from peritoneal and ovarian endometriotic lesions. Future investigations of these genes may contribute to the elucidation of the physiopathology of endometriosis.

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