Article published online: 2022-09-02

Genes Relating to Biological Processes of Endometriosis: Expression Changes Common to a Mouse Model and Patients

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Key words
endometriosis, mouse syngeneic model, DNA microarray, patients, differentially expressed genes, biological process

received 06.01.2022
accepted 05.07.2022
published online 02.09.2022

Bibliography
Drug Res 2022; 72: 523–533
DOI 10.1055/a-1894-6817
ISSN 2194-9379
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Georg Thieme Verlag, Rüdigerstraße 14,
70469 Stuttgart, Germany

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Supplementary Material is available under https://doi.org/10.1055/a-1894-6817

ABSTRACT

Endometriosis is one of the most common gynecological diseases in women of reproductive age. Retrograde menstruation is considered a major reason for the development of endometriosis. The syngeneic transplantation mouse model is an endometriosis animal model that is considered to mimic retrograde menstruation. However, it remains poorly understood which genetic signatures of endometriosis are reflected in this model. Here, we employed an *in vivo* syngeneic mouse endometriosis model and identified differentially expressed genes (DEGs) between the ectopic and eutopic tissues using microarray analysis. Three gene expression profile datasets, GSE5108, GSE7305, and GSE11691, were downloaded from the Gene Expression Omnibus database and DEGs between ectopic and eutopic tissues from the same patients were identified. Gene ontology analysis of the DEGs revealed that biological processes including cell adhesion, the inflammatory response, the response to mechanical stimulus, cell proliferation, and extracellular matrix organization were enriched in both the model and patients. Of the 195 DEGs common to the model and patients, 154 showed the same expression pattern, and 28 of these 154 DEGs came up when PubMed was searched for each gene along with the terms "endometriosis" and "development". This is the first comparison of the DEGs of the mouse syngeneic endometriosis model and those of patients, and we identified the biological processes common to the model and patients at the transcriptional level. This model may be useful to evaluate the efficacy of drugs which target these biological processes.

Introduction

Endometriosis is one of the most common gynecological diseases in women of reproductive age, and it is diagnosed in about 5 % to 10 % of women during their reproductive years, which is approximately 176 million women in the world [1]. Endometriosis is defined as the presence of endometrial-like lesions outside the uterus, primarily in the peritoneum, ovaries, bowel, uterosacral ligaments, and fallopian tubes, which has a great impact on quality of life [2]. The combined oral contraceptive pill and progestogens are widely used as therapies for endometriosis [3]. Although they are effective for some symptoms of endometriosis such as pain, they are not a complete therapy; some patients show recurrence of the disease after withdrawal of the therapy and one-third of patients are non-responders due to progesterone resistance [4]. Thus, new therapeutic options which have a mechanism of action that is different from that of hormonal drugs and which act on endometriotic lesions are desirable for the treatment of endometriosis.

To achieve this goal, the extrapolation of information from animal models to humans is essential; however, extrapolation is complicated because rodents do not develop endometriosis spontaneously [5]. Among the several rodent models available, the syngeneic mouse model is often used because it is considered to mimic

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retrograde menstruation [6], which is one of the main causes of the development of endometriosis [7]. However, few studies have comprehensively compared the biological processes of endometriosis in patients and in the model, and the usefulness of this animal model in the interpretation of the pathophysiology of endometriosis in humans is not yet fully understood.

In recent years, transcriptome analysis has been one of the technologies most utilized to study human diseases at the gene expression level, and it has contributed to the development of data integration approaches to discover molecular biomarkers in human pathologies and targets for new drugs [8]. Therefore, in the present study, we employed a syngeneic mouse endometriosis model and used transcriptome analysis to investigate the differentially expressed genes and the biological processes common to the model and endometriosis patients.

Materials and Methods

Animals

Seven-week-old female BALB/cCrSlc mice (n = 65) were purchased from Japan SLC Inc. (Hamamatsu, Japan). The mice were housed under conditions of controlled temperature (20–26 °C), humidity (35–75 %), and lighting (12-h light/dark cycle) with water and food ad libitum. The study was conducted in compliance with the Internal Regulations on Animal Experiments at Nippon Shinyaku Co., Ltd. (Kyoto, Japan), which are based on the Law for the Humane Treatment and Management of Animals (Law No. 105, October 1, 1973).

Ovariectomy and mouse model of endometriosis

Eight-week-old mice were anesthetized with Isoflurane Inhalation Solution [Pfizer] (Mylan Inc., Canonsburg, Pennsylvania, USA). The mice were ovariectomized through bilateral paravertebral incisions, and the muscular and skin incisions were closed with 6–0 black silk suture. Butorphanol tartrate (1 mg/kg; Fujifilm Wako Pure Chemical Co., Osaka, Japan) and ampicillin sodium (100 mg/kg; Viccillin; Meiji Seika Pharma Co., Ltd., Tokyo, Japan) were administered subcutaneously. At the end of the procedure, estradiol valerate in sesame oil (2 µg/animal) was administered intramuscularly every week to all mice. The day of ovariectomy was designated as day 0. On day 7, the mice were divided into three groups by their body weight: 10 mice in the sham group, 14 mice in the donor group, and 28 mice in the recipient group. To construct the syngeneic mouse endometriosis model, uterine tissues from the donor mice were harvested and minced into small cell aggregates in Medium 199 with Hanks' Balanced Salts (Thermo Fisher Scientific, Inc., Waltham, Massachusetts, USA) supplemented with penicillin-streptomycin mixed solution (Nacalai Tesque Inc., Kyoto, Japan), then equal volumes of uterine cell suspension were transferred into the peritoneal cavities of the recipient mice at a ratio of one donor to two recipients. For the sham group, the same volume of Medium 199 with Hanks' Balanced Salts was injected into the peritoneal cavities of the mice. To reduce the local surgical response to trauma, we incised the upper right side of mice and transferred the uterine cell suspension into their lower left peritoneal cavities through the indwelling needle. The wounds of the mice were closed with 6–0 black silk suture and bupivacaine hydrochloride hydrate (2.5 mg/kg; Marcaine Injection; Aspen Japan Co., Ltd., Tokyo, Japan) and ampicillin sodium (100 mg/kg) were administered subcutaneously. On day 35, the recipient mice were euthanized and all ectopic cysts and uterine tissues were carefully and exclusively removed from each mouse with a small scissors and forceps, infused with RNAlater solution (Thermo Fisher Scientific, Inc.) and stored at −80 °C for analysis of gene expression.

Microarray analysis

Total RNA were isolated from the mouse ectopic cystic tissue and eutopic uterus using an RNeasy Lipid Tissue Mini Kit (Qiagen Inc., Hilden, Germany) (n = 5 animals per group). The quality and concentration of the RNA was checked using an Agilent 2100 bioanalyzer. The RNA Integrity Number (RIN) was used to evaluate RNA integrity and all samples used for the microarray analysis had RIN ≥ 7.0. Purified RNA was labeled by using the GeneChip WT Plus Reagent Kit (Thermo Fisher Scientific, Inc.), then hybridized to a Clariom S Mouse Array (Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. Experiments from RNA isolation to microarray analysis were conducted at Filgen, Inc. (Nagoya, Japan). Briefly, CEL files were processed using Affymetrix Expression Console software (Thermo Fisher Scientific, Inc.) and subjected to normalization using the Signal Space Transformation-Robust Multiarray Analysis (SST-RMA) method for the following analysis. The number of probes detected was 22,206 and genes whose expression changed at least two-fold with $p < 0.05$ (Student's t-test) in the ectopic cystic tissue compared to the eutopic tissue in the syngeneic endometriosis mouse model or in the eutopic tissue in the model compared to the sham group were considered to be differentially expressed. Gene ontology (GO) analysis was conducted on the significantly differentially expressed genes (DEGs) using the Database for Annotation, Visualization and Integrated Discovery [9] (DAVID; Laboratory of Human Retrovirology and Immunoinformatics). GO terms for biological processes with $p < 0.05$ (Fisher's exact test with the Benjamini-Hochberg multiple-testing correction) were considered significant. The datasets are available from the National Center for Biotechnology Information/Gene Expression Omnibus, and can be accessed with GSE190209.

Endometriosis patient data collection

The BaseSpace Correlation Engine (Illumina, Inc., San Diego, California, USA) bioinformatics database was used to investigate the microarray gene expression profiles of the endometriosis patients, in which data were reanalyzed as determined by NextBio analysis [10]. We found three datasets (GSE5108 [11], GSE7305 [12] and GSE11691 [13]) in which the gene expression in ectopic tissue is compared to that in eutopic tissue from the same patients.

Analysis of DEGs from patient datasets

The files from the three datasets were individually processed and normalized according to the BaseSpace Correlation Engine platform, and genes whose expression changed in ectopic tissue at least two-fold compared to eutopic tissue with $p < 0.05$ were considered to be the DEGs of each dataset. The genes which showed the same expression pattern (up-regulated or down-regulated) in at least two datasets were defined as the DEGs of the endometriosis patients. GO analysis was conducted on the DEGs of patients

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using DAVID. GO terms for biological processes with $p < 0.05$ (Fisher's exact test with the Benjamini-Hochberg multiple-testing correction) were considered significant.

Comparison of data between the syngeneic mouse endometriosis model and patients

The data for the GO analysis of the syngeneic mouse endometriosis model were combined with those of the patients, then GO terms common to them were identified using TIBCO Spotfire data analysis software (TIBCO Software Inc., Palo Alto, California, USA). The DEGs common to the model and patients were identified using the BaseSpace Correlation Engine. To investigate the relationship between each common DEG and endometriosis, PubMed (National Center for Biotechnology Information) was searched for each common DEG along with the terms "endometriosis" or "endometriosis" and "development". Studies on genes which were not shown to be associated with endometriosis in patients (e. g., studies in animal models only or on endometriosis-associated ovarian carcinoma) were excluded.

Results

DEGs in the syngeneic mouse endometriosis model

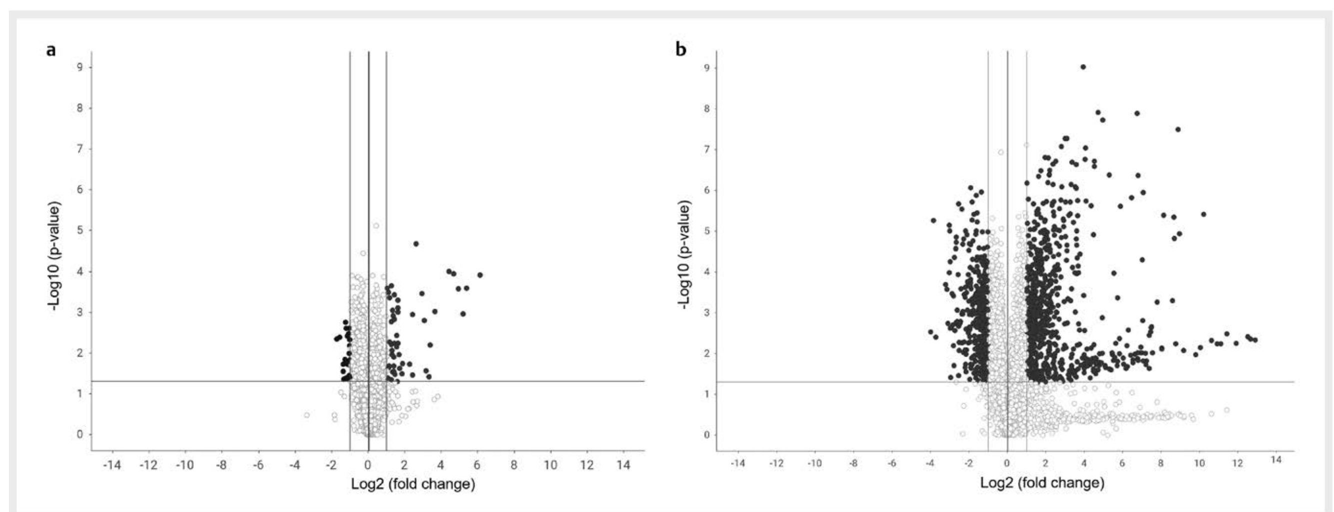
We used DNA microarray analysis to identify the changes in gene expression in the syngeneic mouse endometriosis model. Seventy-seven out of 22,206 genes were differentially expressed in the eutopic uterus of the model compared to that of sham-operated mice, comprising 54 up-regulated and 23 down-regulated genes, hereinafter referred to as the DEGs in the eutopic uterus (▶ **Fig. 1a**). We then investigated the DEGs in the ectopic cystic tissue of the model mice compared to those in their eutopic uteri. We identified 1,154 out of 22,206 genes as DEGs, comprising 742 up-regulated and 412 down-regulated genes, and these are hereinafter referred to as the DEGs in ectopic tissue (▶ **Fig. 1b**). These results show that the expression of some genes was different between the eutopic and ectopic tissues of the model mice.

DEGs in the endometriosis patients of three datasets from NCBI GEO

We identified DEGs in the endometriosis patients using three datasets from NCBI GEO in which the gene expression between eutopic and ectopic lesions from the endometriosis patients was compared using microarray analysis. We identified 2633 genes in GSE5108, 3787 in GSE7305, and 494 in GSE11691. Of these, 950 genes showed the same expression pattern in at least two datasets and were defined as the DEGs common to the patients. They comprised 530 up-regulated and 420 down-regulated genes (▶ **Fig. 2**).

GO analysis of DEGs in the mouse model and endometriosis patients

To find biological processes associated with the DEGs, we used gene ontology (GO) analysis. We found that DEGs in the eutopic uterus of the model mice represented the enrichment of two biological processes, the response to lipopolysaccharide and neutrophil chemotaxis (▶ **Table 1**). The DEGs in the ectopic tissue of the model mice represented the enrichment of 75 biological processes, including muscle contraction, cell adhesion, response to hypoxia, and the inflammatory response (**Supplementary Table 1**). The DEGs in the patients represented the enrichment of 28 biological processes, including extracellular matrix organization, cell adhesion, and the inflammatory response (**Supplementary Table 2**). We then matched GO terms which were enriched both in the ectopic tissue of the model mice and in the patients, and found that 12 biological processes were common to them (▶ **Table 2** and ▶ **Fig. 3**), including cell adhesion, the inflammatory response, the response to mechanical stimulus, cell proliferation and extracellular matrix organization. This result suggests that these biological processes are important in both the model and patients.



▶ **Fig. 1** Results of DNA microarray analysis in the mouse endometriosis model. The volcano plots represent the DEGs between (**a**) the eutopic uterus in the sham mice and in the syngeneic endometriosis mouse model or (**b**) the eutopic uterus and ectopic tissue in the model. DEGs satisfy the criteria $\log_2$(fold change) > 1 or < −1 and $p < 0.05$ (Student's t-test). Significantly differentially expressed genes are shown as black dots. DEGs, differentially expressed genes.

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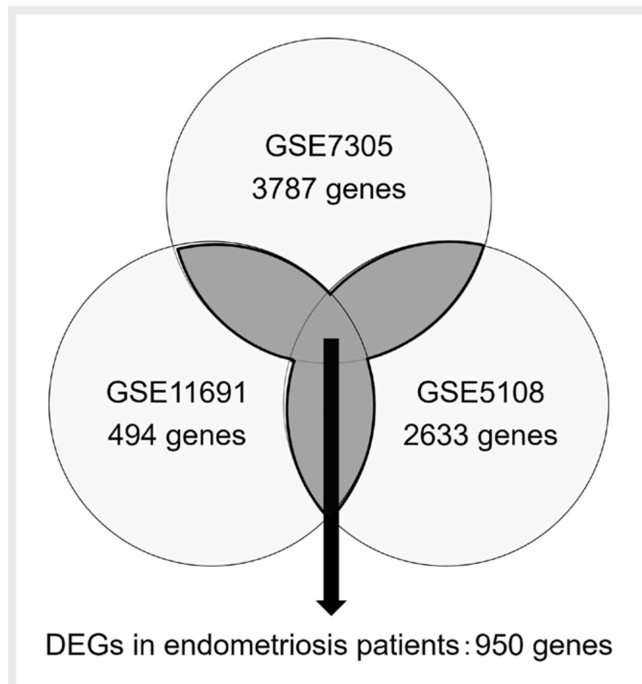


▶ **Fig. 2** Identification of DEGs in endometriosis patients. Datasets (GSE5108, GSE7305 and GSE11691) from the NCBI GEO database in which the gene expression of ectopic and ectopic tissue is compared were used for analysis. The DEGs of each dataset were displayed in Venn diagrams and the overlapping DEGs, that is, DEGs which showed the same expression pattern (up-regulated or down-regulated) in at least two datasets, were defined as common DEGs in the endometriosis patients.

▶ **Table 1** The significantly enriched biological processes associated with DEGs in the eutopic uterus of the mouse model

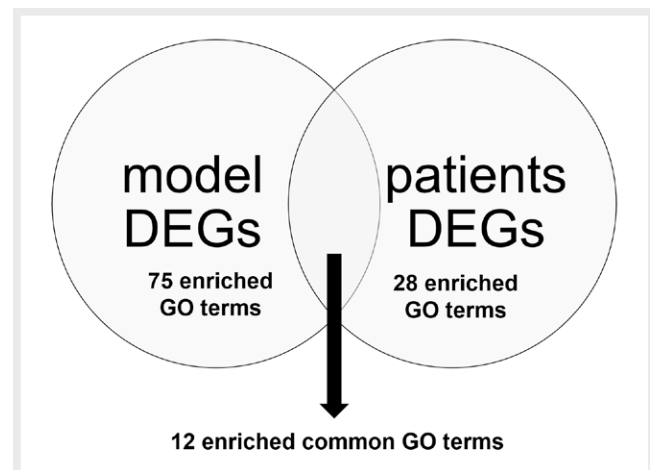
GO Term		Count	p-value
GO:0032496	response to lipopolysaccharide	7	0.03
GO:0030593	neutrophil chemotaxis	5	0.03

DEGs common to the syngeneic mouse endometriosis model and endometriosis patients

To identify gene-expression changes common to the model and the patients, we compared the DEGs between them. We found that they shared 195 DEGs, of which 154 showed the same expression pattern (that is, 115 genes were up-regulated and 39 were down-regulated in both the model and the patients; ▶ **Table 3** and ▶ **Fig. 4**). We defined these 154 genes as the DEGs common to the model and the patients. We then explored the gene annotations of the common DEGs, and found that some of them were annotated by GO terms which were enriched in both the model and patients (▶ **Table 4**).

The roles of DEGs common to the syngeneic mouse endometriosis model and endometriosis patients in endometriosis

To investigate possible roles played by the DEGs common to the model and the patients, we searched for a relationship between



▶ **Fig. 3** Identification of biological processes common to the syngeneic mouse endometriosis model and endometriosis patients. Gene ontology (GO) analysis was conducted using DEGs in the ectopic tissue of the model mice and the patients, and biological processes that were enriched in both were identified.

▶ **Table 2** GO terms common to the syngeneic mouse endometriosis model and endometriosis patients

GO term		Mouse model		Endometriosis patients	
		Gene Count	p-value	Gene Count	p-value
GO:0007155	cell adhesion	74	3.2.E-11	60	7.0.E-08
GO:0006954	inflammatory response	51	6.7.E-07	46	1.1.E-04
GO:0009612	response to mechanical stimulus	17	9.9.E-05	12	3.0.E-02
GO:0008285	negative regulation of cell proliferation	47	3.6.E-04	38	3.8.E-02
GO:0030198	extracellular matrix organization	22	3.8.E-04	37	5.1.E-08
GO:0043627	response to estrogen	17	7.7.E-04	12	5.0.E-02
GO:0001525	angiogenesis	33	1.0.E-03	29	3.1.E-03
GO:0045766	positive regulation of angiogenesis	21	1.8.E-03	20	2.2.E-03
GO:0007568	aging	24	1.1.E-02	21	3.8.E-02
GO:0006955	immune response	32	1.4.E-02	45	2.2.E-03
GO:0070098	chemokine-mediated signaling pathway	12	1.8.E-02	13	3.5.E-02
GO:0048247	lymphocyte chemotaxis	9	2.6.E-02	8	5.0.E-02

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▶ **Table 3** DEGs common to the syngeneic mouse endometriosis model and endometriosis patients

Gene	Description	Fold changein model	Fold change in patients (average of 3 datasets)
up-regulated genes			
Hp	Haptoglobin	468.70	9.18
Cfd	complement factor D (adipsin)	405.34	6.15
Fabp4	fatty acid binding protein 4, adipocyte	280.26	30.81
Hspb6	heat shock protein, alpha-crystallin-related, B6	144.70	2.31
Serpina3n	serine (or cysteine) peptidase inhibitor, clade A, member 3 N	58.06	5.74
Cryab	crystallin, alpha B	42.87	3.25
Hsd11b1	hydroxysteroid 11-beta dehydrogenase 1	41.74	20.65
Gpnmb	glycoprotein (transmembrane) nmb	39.08	3.53
Ldb3	LIM domain binding 3	33.46	3.86
Cpxm2	carboxypeptidase X 2 (M14 family)	31.13	16.65
Rgs16	regulator of G-protein signaling 16	30.34	2.62
Serpine2	serine (or cysteine) peptidase inhibitor, clade E, member 2	22.80	18.67
Thbs2	thrombospondin 2	20.39	4.11
Lrrc2	leucine rich repeat containing 2	17.94	4.20
Filip1l	filamin A interacting protein 1-like	17.62	3.99
Col12a1	collagen, type XII, alpha 1	16.63	5.26
Fmod	Fibromodulin	15.29	2.75
Thbs4	thrombospondin 4	12.82	3.89
Mgp	matrix Gla protein	12.49	4.65
Timp1	tissue inhibitor of metalloproteinase 1	12.08	5.25
Thbs1	thrombospondin 1	11.39	6.86
C1qtnf7	C1q and tumor necrosis factor related protein 7	10.29	2.27
Itm2a	integral membrane protein 2 A	9.54	7.11
Sfrp2	secreted frizzled-related protein 2	8.96	21.75
Il7r	interleukin 7 receptor	8.48	5.82
Slit3	slit homolog 3 (Drosophila)	8.08	2.86
Itgbl1	integrin, beta-like 1	7.92	4.05
Angptl1	angiopoietin-like 1	7.46	13.75
Sulf1	sulfatase 1	7.43	3.22
Bgn	Biglycan	6.91	3.43
Ghr	growth hormone receptor	6.84	2.79
Inhba	inhibin beta-A	6.45	8.09
Cd163	CD163 antigen	6.37	5.35
Chl1	cell adhesion molecule with homology to L1CAM	5.96	36.95
Pdgfrl	platelet-derived growth factor receptor-like	5.72	3.80
Fhl5	four and a half LIM domains 5	5.64	2.58
Olfml1	olfactomedin-like 1	5.54	2.55
Nupr1	nuclear protein 1	5.43	2.37
Rcan2	regulator of calcineurin 2	5.20	8.91
Frzb	frizzled-related protein	5.04	5.21
Scn7a	sodium channel, voltage-gated, type VII, alpha	4.81	37.20
Lyz2	lysozyme 2	4.75	4.23
Vgll3	vestigial like 3 (Drosophila)	4.62	3.04
Lhfp	lipoma HMGIC fusion partner	4.53	3.59
Lbh	limb-bud and heart	4.52	2.50
Wisp2	WNT1 inducible signaling pathway protein 2	4.52	13.38
Gfpt2	glutamine fructose-6-phosphate transaminase 2	4.37	2.24
Msr1	macrophage scavenger receptor 1	4.36	3.90
Ctss	cathepsin S	4.01	2.59

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▶ **Table 3** Continued.

Gene	Description	Fold changein model	Fold change in patients (average of 3 datasets)
C4a	complement component 4 A (Rodgers blood group)	3.97	7.01
Rgs5	regulator of G-protein signaling 5	3.85	3.40
Dpysl3	dihydropyrimidinase-like 3	3.84	8.99
Prelp	proline arginine-rich end leucine-rich repeat	3.80	7.90
Itgb2	integrin beta 2	3.65	2.42
Aspn	aspirin	3.60	4.09
Meox2	mesenchyme homeobox 2	3.55	3.09
Cbs	cystathionine beta-synthase	3.53	2.58
Nrp2	neuropilin 2	3.47	8.76
Ccdc80	coiled-coil domain containing 80	3.43	8.69
S100a6	S100 calcium binding protein A6 (calcyclin)	3.42	2.22
Folr2	folate receptor 2 (fetal)	3.42	2.20
Kcnma1	potassium large conductance calcium-activated channel, subfamily M, alpha member 1	3.42	2.55
Pdlim5	PDZ and LIM domain 5	3.36	2.71
Podn	Podocan	3.34	4.29
Plxdc2	plexin domain containing 2	3.32	2.78
Steap4	STEAP family member 4	3.32	4.67
Ltbp2	latent transforming growth factor beta binding protein 2	3.08	6.01
Spsb1	splA/ryanodine receptor domain and SOCS box containing 1	3.06	2.45
Eltd1	EGF, latrophilin seven transmembrane domain containing 1	2.99	2.30
Sytl2	synaptotagmin-like 2	2.96	5.78
Gpx3	glutathione peroxidase 3	2.91	10.59
Hmox1	heme oxygenase (decycling) 1	2.90	4.67
Chrdl1	chordin-like 1	2.88	5.43
Ncf4	neutrophil cytosolic factor 4	2.87	3.66
Loxl1	lysyl oxidase-like 1	2.85	2.76
Rarres1	retinoic acid receptor responder (tazarotene induced) 1	2.78	7.20
Rerg	RAS-like, estrogen-regulated, growth-inhibitor	2.75	5.45
Sep4	septin 4	2.75	3.94
Pdgfd	platelet-derived growth factor, D polypeptide	2.71	5.77
Col14a1	collagen, type XIV, alpha 1	2.69	3.54
Nfasc	Neurofascin	2.68	14.96
Tspan7	tetraspanin 7	2.67	2.67
Colec12	collectin sub-family member 12	2.66	3.25
Igsf6	immunoglobulin superfamily, member 6	2.65	2.96
Cdh5	cadherin 5	2.64	2.47
Plvap	plasmalemma vesicle associated protein	2.57	2.96
Clu	Clusterin	2.55	8.12
Fry	furry homolog (Drosophila)	2.55	3.56
Chi3l1	chitinase 3-like 1	2.55	9.68
Fcgr3	Fc receptor, IgG, low affinity III	2.54	5.88
Itga7	integrin alpha 7	2.53	3.01
Man1c1	mannosidase, alpha, class 1 C, member 1	2.52	3.40
Dkk3	dickkopf homolog 3 (Xenopus laevis)	2.51	3.51
Tril	TLR4 interactor with leucine-rich repeats	2.50	3.49
Pros1	protein S (alpha)	2.48	6.98
Fcgr2b	Fc receptor, IgG, low affinity IIb	2.44	3.29
Jam2	junction adhesion molecule 2	2.44	2.92
Ccr1	chemokine (C-C motif) receptor 1	2.42	2.48

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▶ **Table 3** Continued.

Gene	Description	Fold changein model	Fold change in patients (average of 3 datasets)
Grk5	G protein-coupled receptor kinase 5	2.26	2.93
Pde1a	phosphodiesterase 1 A, calmodulin-dependent	2.26	3.38
Npl	N-acetylneuraminate pyruvate lyase	2.25	4.02
Ptprb	protein tyrosine phosphatase, receptor type, B	2.25	2.54
Serping1	serine (or cysteine) peptidase inhibitor, clade G, member 1	2.20	5.47
Gpr116	G protein-coupled receptor 116	2.14	3.21
Nr4a1	nuclear receptor subfamily 4, group A, member 1	2.13	2.31
Fst	Follistatin	2.11	6.28
Cpa3	carboxypeptidase A3, mast cell	2.08	2.87
Aox1	aldehyde oxidase 1	2.08	17.10
Gnb4	guanine nucleotide binding protein (G protein), beta 4	2.08	2.36
Cd22	CD22 antigen	2.07	3.19
Nuak1	NUAK family, SNF1-like kinase, 1	2.05	3.74
Gpc6	glypican 6	2.03	3.29
9430020K01Rik	RIKEN cDNA 9430020K01 gene	2.02	3.09
C7	complement component 7	2.02	73.71
Laptm5	lysosomal-associated protein transmembrane 5	2.01	2.94
down-regulated genes			
Hsd11b2	hydroxysteroid 11-beta dehydrogenase 2	−8.06	−5.61
Mogat1	monoacylglycerol O-acyltransferase 1	−7.94	−4.09
Kcnip4	Kv channel interacting protein 4	−6.37	−4.81
Gcnt3	glucosaminyl (N-acetyl) transferase 3, mucin type	−5.21	−2.26
Car12	carbonic anyhydrase 12	−5.21	−6.39
Slc15a2	solute carrier family 15 (H+/peptide transporter), member 2	−4.27	−4.48
Pgbd5	piggyBac transposable element derived 5	−3.38	−4.45
Crabp2	cellular retinoic acid binding protein II	−3.28	−6.77
Mme	membrane metallo endopeptidase	−3.28	−4.71
Ckb	creatine kinase, brain	−3.27	−3.01
Krt8	keratin 8	−3.23	−3.75
Krt19	keratin 19	−3.19	−3.69
Tfcp2l1	transcription factor CP2-like 1	−3.13	−3.44
Tspan13	tetraspanin 13	−3.01	−3.43
Galnt4	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyl-transferase 4	−2.93	−11.19
Agr2	anterior gradient 2 (Xenopus laevis)	−2.85	−11.16
Fam174b	family with sequence similarity 174, member B	−2.71	−2.27
Galnt3	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyl-transferase 3	−2.71	−2.45
Rorb	RAR-related orphan receptor beta	−2.66	−7.46
Tspan1	tetraspanin 1	−2.53	−4.88
Gpsm2	G-protein signalling modulator 2 (AGS3-like, C. elegans)	−2.51	−2.90
Aldh1a2	aldehyde dehydrogenase family 1, subfamily A2	−2.49	−9.64
Prr15	proline rich 15	−2.44	−7.56
Rasef	RAS and EF hand domain containing	−2.36	−2.79
Esr1	estrogen receptor 1 (alpha)	−2.34	−7.53
Rev3l	REV3-like, catalytic subunit of DNA polymerase zeta RAD54 like (S. cerevisiae)	−2.34	−3.11
Ptn	Pleiotrophin	−2.31	−3.03
Tmem30b	transmembrane protein 30B	−2.29	−4.84
Cd24a	CD24a antigen	−2.26	−22.91
Qpct	glutaminyl-peptide cyclotransferase (glutaminyl cyclase)	−2.25	−4.16
Cndp2	CNDP dipeptidase 2 (metallopeptidase M20 family)	−2.20	−3.14

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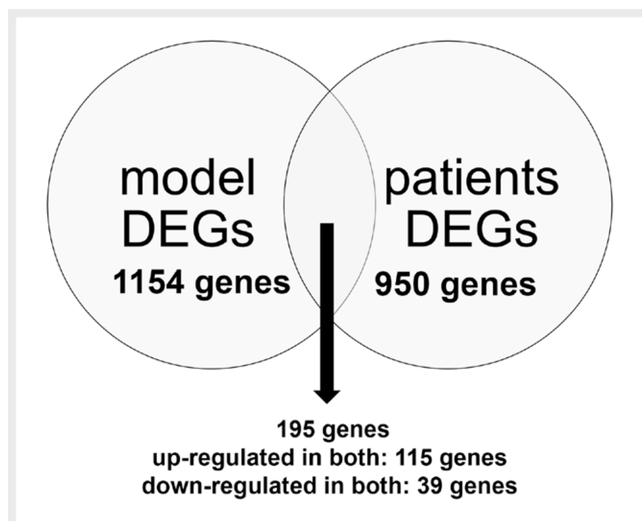
▶ Table 3 Continued.

Gene	Description	Fold changein model	Fold change in patients (average of 3 datasets)
Wfdc2	WAP four-disulfide core domain 2	−2.20	−10.44
Stxbp6	syntaxin binding protein 6 (amisyn)	−2.16	−9.21
Rab25	RAB25, member RAS oncogene family	−2.15	−5.87
Llgl2	lethal giant larvae homolog 2 (Drosophila)	−2.14	−2.27
Npr2	natriuretic peptide receptor 2	−2.14	−2.80
Ppap2c	phosphatidic acid phosphatase type 2 C	−2.08	−4.03
Irf6	interferon regulatory factor 6	−2.04	−5.03
Gjb6	gap junction protein, beta 6	−2.00	−5.75



▶ Fig. 4 Identification of DEGs common to the syngeneic mouse endometriosis model and endometriosis patients. DEGs in the ectopic tissue of the model mice were compared to those in the patients. The DEGs of each dataset were displayed in Venn diagrams and the overlapping DEGs identified by selecting genes which showed the same expression pattern (up-regulated or down-regulated).

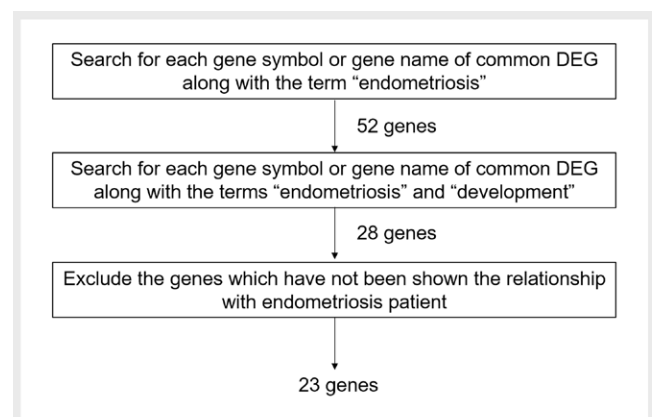
▶ Table 4 GO terms which were enriched in DEGs common to the syngeneic mouse endometriosis model and endometriosis patients

GO term	genes	
cell adhesion	17	Gpnmb, Thbs2, Col12a1, Thbs4, Thbs1, Sulf1, Chl1, Wisp2, Itgb2, Col14a1, Nfasc, Cdh5, Itga7, Cd22, Nuak1, 9430020K01Rik, Cd24a
inflammatory response	6	Thbs1, Cd163, C4a, Chi3l1, Tril, Ccr1
response to mechanical stimulus	2	Thbs1, Chi3l1
negative regulation of cell proliferation	13	Serpine2, Sfrp2, Slit3, Inhba, Frzb, Wisp2, Podn, Hmox1, Rerg, Cdh5, Aldh1a2, Irf6, Gjb6
extracellular matrix organization	1	Ccdc80
response to estrogen	5	Kcnma1, Hmox1, Krt19, Esr1, Cd24a
Angiogenesis	5	Meox2, Nrp2, Ccdc80, Hmox1, Ptprb
positive regulation of angiogenesis	5	Thbs1, Sfrp2, Itgb2, Hmox1, Chi3l1
Aging	5	Cryab, Timp1, Itgb2, Serping1, Gjb6
immune response	7	Thbs1, Ctss, Colec12, Fcgr2b, Ccr1, C7, Cd24a
chemokine-mediated signaling pathway	1	Ccr1

the common DEGs and endometriosis by using PubMed. When we searched for each gene along with the term “endometriosis”, 52 of 154 genes came up (**Supplementary Table 3** and ▶ **Fig. 5**). When we searched for each gene along with the terms “endometriosis” and “development”, 23 genes came up that had some association with endometriosis in patients (▶ **Table 5**).

Discussion

In the present study, we found that biological processes including cell adhesion, the inflammatory response, the response to mechanical stimulus, cell proliferation, extracellular matrix organization (ECM), and the estrogen response were enriched in both the model and patients. We found that thrombospondin 1 (Thbs1), tissue inhibitor of metalloproteinase 1 (Timp1), and cell adhesion molecule with homology to L1CAM (Chl1) were up-regulated in both the model and patients. These genes are known to play a role in cell adhesion and/or ECM organization, biological processes important for the attachment and invasion of ectopic cells in tissues [14–16].



▶ Fig. 5 Flowchart for Pubmed search. PubMed (National Center for Biotechnology Information) was searched for each common DEG along with the term “endometriosis” or the terms “endometriosis” and “development”

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▶ **Table 5** The DEGs common to the model and patients along with the terms "endometriosis" and "development" found by searching PubMed

Gene	Number of publications	Reference lists
up-regulated genes		
Hp	2	Piva M et al., Glycoconj J. 2002 Jan;19(1):33–41. Sharpe-Timms KL et al., Hum Reprod. 2000 Oct;15(10):2180–5.
Hsd11b1	1	Zhen Lin et al., J Food Biochem. 2021 May;45(5):e13717.
Timp1	6	Luddi A et al., Int J Mol Sci. 2020 Apr 18;21(8):2840.Szymanowski K et al.,Ann Agric Environ Med. 2016 Dec 23;23(4):649–653. Stilley JA et al., Biol Reprod. 2010 Aug 1;83(2):185–94. Collette T et al.,Hum Reprod. 2006 Dec;21(12):3059–67. Li Y et al., Zhonghua Fu Chan Ke Za Zhi. 2006 Jan;41(1):30–3. Collette T et al., Hum Reprod. 2004 Jun;19(6):1257–64.
Thbs1	3	Liu Y et al., Am J Reprod Immunol. 2020 Jun;83(6):e13236. Gilabert-Estellés J et al., Hum Reprod. 2007 Aug;22(8):2120–7. Tan XJ et al., Fertil Steril. 2002 Jul;78(1):148–53.
Slit3	1	Greaves E et al., Endocrinology. 2014 Oct;155(10):4015–26.
Inhba	1	Lin J et al., Mol Hum Reprod. 2011 Oct;17(10):605–11.
Cd163	3	Kusunoki M et al., Med Mol Morphol. 2021 Jun;54(2):122–132. Krasnyi AM et al., Biomed Khim. 2019 Aug;65(5):432–436. Itoh F et al., Fertil Steril. 2013 May;99(6):1705–13.
Chl1	2	Jiang L et al., Int J Immunopathol Pharmacol. 2020 Jan-Dec;34:2058738420976309. Zhang C et al., Eur J Obstet Gynecol Reprod Biol. 2019 May;236:177–182.
Prelp	1	Araujo FM et al., Braz J Med Biol Res. 2017 Jul 3;50(7):e5782.
Itgb2	1	Sundqvist J et al., Hum Reprod. 2012 Sep;27(9):2737–46.
S100a6	1	Peng Y et al., Gynecol Endocrinol. 2018 Sep;34(9):815–820.
Gpx3	1	Mirza Z et al., Diagnostics (Basel) . 2020 Jun 19;10(6):416.
Hmox1	2	Van LA et al., Fertil Steril. 2002 Mar;77(3):561–70. Imanaka S et al., Arch Med Res. 2021 Aug;52(6):641–647.
Fcgr3	1	Mei J et al., Autophagy. 2018;14(8):1376–1397.
Ccr1	3	Li T et al., Biomed Pharmacother. 2020 Sep;129:110476. Trummer D et al., Acta Obstet Gynecol Scand. 2017 Jun;96(6):694–701. Kyama CM et al., Curr Med Chem. 2008;15(10):1006–17.
Nr4a1	1	Qingdong Z et al., Cell Physiol Biochem. 2018;45(3):1172–1190.
Fst	2	Kimber-Trojnar Ż et al., J Clin Med. 2021 Jun 23;10(13):2762. Luisi S et al., Womens Health (Lond). 2015 Aug;11(5):603–10.
down-regulated genes		
Crabp2	1	Sokalska A et al., J Clin Endocrinol Metab. 2013 Mar;98(3):E463–71.
Krt19	1	Konrad L et al., Reprod Sci. 2019 Jan;26(1):49–59.
Aldh1a2	1	Jiang Y et al., J Endocrinol. 2018 Mar;236(3):R169-R188.
Esr1	18	Wang J etal., Clin Lab. 2020 Aug 1;66(8). Huang ZX et al., J Cell Mol Med. 2020 Sep;24(18):10693–10704. Gibson DA et al., J Endocrinol. 2020 Sep;246(3):R75-R93. Chantalat E et al., Int J Mol Sci. 2020 Apr 17;21(8):2815. Tang ZR et al., Cells. 2019 Sep 21;8(10):1123. Yilmaz BD et al., Hum Reprod Update. 2019 Jul 1;25(4):473–485. Osiński M et al., Ginekol Pol. 2018;89(3):125–134. Sapkota Y et al., Nat Commun. 2017 May 24;8:15539. Hamilton KJ et al., Curr Top Dev Biol. 2017;125:109–146. Xiong W et al., Reproduction. 2015 Dec;150(6):507–16 Zhang Q et al., Gynecol Obstet Invest. 2015;80(3):187–92. Huang PC et al., Environ Sci Pollut Res Int. 2014 Dec;21(24):13964–73. Wang W et al., Reprod Biomed Online. 2013 Jan;26(1):93–8 Li Y et al., Gene. 2012 Oct 15;508(1):41–8. Veillat V et al., Am J Pathol. 2012 Sep;181(3):917–27. Matsuzaka Y et al., Environ Health Prev Med. 2012 Sep;17(5):423–8. Athanasios F et al., Arch Gynecol Obstet. 2012 Apr;285(4):1001–7. Smuc T et al., Mol Cell Endocrinol. 2009 Mar 25;301(1–2):59–64.
Cd24a	1	Sundqvist J et al., Hum Reprod. 2012 Sep;27(9):2737–46.
Wfdc2	1	Chen T et al., J Clin Lab Anal. 2021 Sep;35(9):e23947.

Thus, these genes might be critical for the development of endometriosis via cell attachment and invasion in both model and patients. The inflammatory and immune responses are also critical to the development of endometriosis. Single-cell analysis has shown that T cells in endometriosis are less activated, cytotoxic T cell populations and the proportion of natural killer cells in endometriosis lesions are decreased, and the ratio of monocytes to macrophages is increased in endometriosis cysts whose main population highly expresses CD206 and CD163, which have been described as M2 macrophage markers [17]. In the present study, the gene expression of haptoglobin and CD163 was upregulated in both the model and patients. Haptoglobin is an acidic glycoprotein and ligand of CD163, which is a surface hemoglobin-haptoglobin scavenger receptor, and is related to the development of endometriosis [18]. These results suggest that M2 macrophages might be critical for the development of endometriosis in both model and patients. Furthermore, endometriosis is considered to be an estrogen-dependent disease. Previous studies have shown that the aberrant expression of hormone receptors in endometriosis lesions, including high estrogen receptor 2 (Esr2) to Esr1 ratios, is related progesterone

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resistance [19]. In our study, the gene expression of Esr1 was decreased in both the model and patients, suggesting that the estrogen response is also important in the pathogenesis of this model, despite the fact that the rodent model does not exhibit menstruation. Thus, this model partly reflects the pathophysiology of endometriosis that occurs in humans as mentioned above, and it might be useful for evaluating the efficacy of new therapeutic agents targeting biological processes that include cell adhesion and ECM remodeling, inflammatory and immune responses, cell proliferation, angiogenesis, and the estrogen response.

We found for the first time that gene expression in the eutopic uterus was changed in the model, and the biological processes associated with the genes whose expression was changed were response to lipopolysaccharide and neutrophil chemotaxis. Previous work has shown that the expression of lipopolysaccharide in the endometrium of endometriosis patients is increased compared to that in healthy controls [20]. These findings suggest that the model reflects the environment not only in ectopic lesions but also in the eutopic endometrium of endometriosis patients.

In addition to this model, immunocompromised models, in which human endometrial tissue is injected into mice, are useful for examining the multiple cellular pathways associated with the development of human endometriosis. However, immunocompromised models may not mimic the inflammatory or immune response of endometriosis patients because of the lack of a fully competent immune system in such mice [21]. The surgical immunocompetent model reflects the inflammation response, cell proliferation and the estrogen response of patients, yet it may not mimic early events in the development of endometriosis such as retrograde menstruation due to the surgical induction of ectopic growth [21]. There is reported to be no change in the levels of cytokeratin or E-cadherin in the epithelial cells of ectopic endometrium, or in the excessive collagen deposition or alpha-SMA positive myofibroblasts in the ectopic endometrium of the surgical mouse endometriosis model [22]. In the present study, the expression of genes related to the inflammatory or immune response and ECM remodeling was changed in the syngeneic mouse endometriosis model, indicating that this model may be distinct from other models.

A limitation of our study is that we did microarray analysis of whole tissues at a specific time point. The model was found not to reflect some biological process in humans, such as endopeptidase activity and platelet degranulation, at least under the present experimental conditions. However, since the level of gene expression would be expected to change with time after construction of the model, or according to the estrous cycle or the component cells, spatiotemporal single-cell RNA sequencing should be more effective for future study. To obtain data on gene expression in endometriosis patients, we used the gene expression data of endometriosis patients from three datasets in which the gene expression in ectopic tissue is compared to that in eutopic tissue, and reanalyzed them in order to unify the analysis method between the patient datasets. However, similar data would have been reported consecutively, so we should also analyze those new data to increase the sample size. Furthermore, in the future we should confirm the relationship between disease severity and the gene expression of key molecules which seem to be important for the development of the disease. Additionally, it is not clear whether the DEGs common to the model and patients are the cause or the result of the pathogenesis of endometriosis. To resolve this issue, experiments using a suppressor or initiator for each gene are necessary. On the basis of the DEGs identified in this study, further work would be expected to clarify molecular mechanisms underlying the pathogenesis of endometriosis, which may lead to the identification of new biomarkers and/or treatment targets for this disease.

Acknowledgements

The study was supported in part by Nippon Shinyaku Co., Ltd. We thank Dr. Gerald E. Smyth for English-language editing of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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