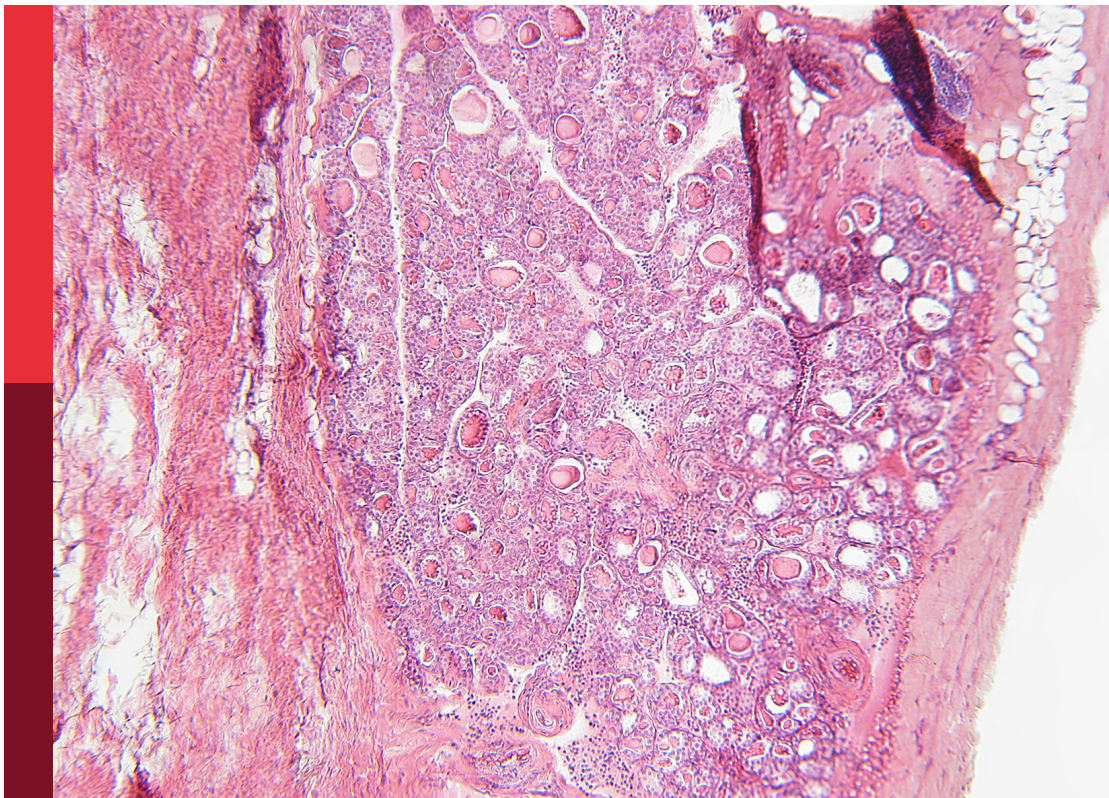
frontiers | Research Topics

Endometriosis: Pathogenesis, diagnosis and treatment, volume II

Edited by
Simone Ferrero, Abdel Halim Harrath and Mainak Dutta

Published in
Frontiers in Endocrinology
Frontiers in Physiology



ISSN 1664-8714
ISBN 978-2-8325-4894-3
DOI 10.3389/978-2-8325-4894-3

About Frontiers

Frontiers is more than just an open access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers journal series

The Frontiers journal series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the *Frontiers journal series* operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews. Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the *Frontiers journals series*: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area.

Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers editorial office: frontiersin.org/about/contact

Endometriosis: Pathogenesis, diagnosis and treatment, volume II

Topic editors

Simone Ferrero — University of Genoa, Italy

Abdel Halim Harrath — King Saud University, Saudi Arabia

Mainak Dutta — Birla Institute of Technology and Science, United Arab Emirates

Citation

Ferrero, S., Harrath, A. H., Dutta, M., eds. (2024). *Endometriosis: Pathogenesis, diagnosis and treatment, volume II*. Lausanne: Frontiers Media SA.

doi: 10.3389/978-2-8325-4894-3

Table of contents

frontiers | Frontiers in Endocrinology

TYPE Editorial
PUBLISHED 18 December 2023
DOI 10.3389/fendo.2023.1334596

OPEN ACCESS

EDITED AND REVIEWED BY
Richard Ivell,
University of Nottingham, United Kingdom

*CORRESPONDENCE
Simone Ferrero
simone.ferrero@unige.it

RECEIVED 07 November 2023
ACCEPTED 24 November 2023
PUBLISHED 18 December 2023

CITATION
Ferrero S and Dutta M (2023) Editorial: Endometriosis: pathogenesis, diagnosis and treatment, volume II.
Front. Endocrinol. 14:1334596.
doi: 10.3389/fendo.2023.1334596

Editorial: Endometriosis: pathogenesis, diagnosis and treatment, volume II

Simone Ferrero[1,2]* and Mainak Dutta[3]

[1]DINOGMI, University of Genova, Genova, Italy, [2]IRCCS Ospedale Policlinico San Martino, Genova, Italy, [3]Department of Biotechnology, Birla Institute of Technology and Science (BITS) Pilani-Dubai Campus, Academic City, Dubai, United Arab Emirates

KEYWORDS

endometriosis, estrogen, pathogenesis, hypoxia, genetics, DNA methylation, microbiome, diagnosis

Editorial on the Research Topic
Endometriosis: pathogenesis, diagnosis and treatment, volume II

Endometriosis is a chronic, multifactorial, estrogen-dependent inflammatory disease characterised by the presence of endometriotic tissue outside the uterus, and it affects 6-10% of reproductive-age women (1). It causes pain symptoms (dysmenorrhea, deep dyspareunia, chronic pelvic pain) and infertility, thus significantly impairing quality of life. The gold standard for diagnosis of endometriosis is laparoscopy, followed by histological confirmation of the biopsy sample. Although non-invasive techniques, including ultrasonography and magnetic resonance imaging, may allow the detection of endometriosis (2, 3), diagnosing this condition is still challenging, and a non-invasive biomarker is not available (2). In addition, the etiology of the disease remains unclear.

Several studies included in this Research Topic tried to elucidate mechanisms involved in the pathogenesis of endometriosis. Identifying molecular pathways may also help develop diagnostic tools and identify potential pharmacological targets.

Endometriosis is typical of the reproductive age, and it usually regresses after menopause; however, it may recur if hormone replacement therapy is administered. Estrogens have a pivotal role in the pathogenesis of endometriosis because of their proliferative and inflammatory properties. Several studies included in this Research Topic investigated the role of estrogens in the pathogenesis of endometriosis. A review by Mercorio et al. described the endocrine microenvironment of endometriotic lesions. The review highlighted that the enzymatic pathways leading to locally increased synthesis of estrogens in endometriosis involve aromatase, 17β-hydroxysteroid dehydrogenase type 1, type 2 and type 5, steroid sulfatase, and estrogen sulfotransferase. A retrospective study by Emond et al. evaluated whether estradiol and its biologically active metabolites are differentially associated with endometriosis. The study included 209 women with endometriosis and 115 without endometriosis. Higher 2OH-3MeO-estrone was linked to an increased risk of endometriosis. Patients with ovarian endometriosis had enhanced 2-hydroxylation with higher 2MeO-estrone and 2OH-estrone levels. Abdominal, pelvic and back pain symptoms were also linked to higher 2OH-3MeO-estrone levels. Another review by Yuan et al. assessed the impact of several factors on estrogen-mediated epithelial-mesenchymal transition in the

emergence of several diseases in the female reproductive tract, primarily endometriosis. Inhibition of estrogen biosynthesis may be a future target of endometriosis treatment (3).

According to the "retrograde menstruation theory", when endometrial cells fall from the uterine cavity into the peritoneal cavity, they face severe hypoxic stress. A review by Zhou et al. evaluated the role of hypoxia-induced unfolded protein response in regulating the development of endometriotic lesions.

El Idrissi et al. investigated protein networks most strongly associated with the symptomatology of endometriosis. The authors found that biological pathways involving interleukin and/or cytokine signalling were linked to endometriosis-related symptoms.

It is well-known that genetic factors contribute to the pathogenesis of endometriosis. In this Research Topic, Bae et al. studies pathways underlying the pathogenesis of endometriosis by investigating differentially expressed genes in patients with endometriosis and healthy controls. One hundred eighteen differentially expressed genes were identified (79 upregulated and 39 downregulated) by integrating publicly available datasets. The authors validated the identified genes via immunohistochemical analysis of tissues obtained from patients with endometriosis and controls. They found that TLR4/NF-κB and Wnt/frizzled signalling pathways, as well as estrogen receptors, may be involved in the progression of endometriosis.

DNA methylation is essential in the regulation of gene expression. Changes in DNA methylation have been associated with altered gene expression in the endometrium, and they may contribute to the pathogenesis of endometriosis. A study by Lei et al. investigated candidate genes of endometriosis through integrated analysis of genome-wide gene expression and DNA methylation profiles. Eutopic and ectopic endometrial tissues were obtained from patients with ovarian endometriomas. Genome-wide methylation profiling identified 17551 differentially methylated loci, with 9777 hypermethylated and 7774 hypomethylated loci.

The microbiome may have a role in the development of endometriosis. A review by Uzuner et al. included in this Research Topic evaluated the role of the microbiome in the formation and progression of endometriosis via inflammatory pathways. The dysbiosis seen in endometriosis is thought to be both causative and a consequence of the pathogenesis. Gut, peritoneal fluid and female reproductive tract microbiota have been studied to understand if there are any microbiome signatures specific to endometriosis that can be used as a non-invasive test of the disease. Theoretically, manipulating the microbiome may also help treat endometriosis.

Endometriosis is associated with a decrease in ovarian reserve (4), and surgical treatment of endometriomas may further decrease ovarian function (5). A retrospective study by Liu et al. investigated the association between different ovarian reserves and reproductive and perinatal outcomes in patients with endometriosis. The study revealed that although patients with endometriosis with normal ovarian reserve and high ovarian reserve had increased reproductive outcomes, patients with endometriosis with diminished ovarian reserve still had an acceptable live birth rate and a similar cumulative live birth rate with available oocytes. Moreover, patients with normal ovarian reserve and high ovarian reserve might not exhibit a decreased risk of abnormal perinatal outcomes, except for gestational diabetes mellitus.

We want to express our profound gratitude to all the authors who contributed to this Research Topic by publishing their best works.

Author contributions

SF: Writing – original draft. MD: Writing – review & editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Publisher's note

References

1. Ferrero S, Arena E, Morando A, Remorgida V. Prevalence of newly diagnosed endometriosis in women attending the general practitioner. *Int J Gynaecol Obstet* (2010) 110(3):203–7. doi: 10.1016/j.ijgo.2010.03.039

2. Nisenblat V, Bossuyt PM, Shaikh R, Farquhar C, Jordan V, Scheffers CS, et al. Blood biomarkers for the non-invasive diagnosis of endometriosis. *Cochrane Database Syst Rev* (2016) 2016(5):CD012179. doi: 10.1002/14651858.CD012179

3. Barra F, Romano A, Grandi G, Facchinetti F, Ferrero S. Future directions in endometriosis treatment: discovery and development of novel inhibitors of estrogen biosynthesis. *Expert Opin Investig Drugs* (2019) 28(6):501–4. doi: 10.1080/13543784.2019.1618269

4. Tian Z, Zhang Y, Zhang C, Wang Y, Zhu HL. Antral follicle count is reduced in the presence of endometriosis: a systematic review and meta-analysis. *Reprod BioMed Online* (2021) 42(1):237–47. doi: 10.1016/j.rbmo.2020.09.014

5. Zhang Y, Zhang S, Zhao Z, Wang C, Xu S, Wang F. Impact of cystectomy versus ablation for endometrioma on ovarian reserve: a systematic review and meta-analysis. *Fertil steril* (2022) 118(6):1172–82. doi: 10.1016/j.fertnstert.2022.08.860

frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 02 September 2022
DOI 10.3389/fendo.2022.869053

OPEN ACCESS

EDITED BY
Abdel Halim Harrath,
King Saud University, Saudi Arabia

REVIEWED BY
Julia Vallve,
University of California, San Francisco, United States
Sadia Afrin Bristy,
Khulna University, Bangladesh
Md Habibur Rahman,
Islamic University, Kushtia, Bangladesh

*CORRESPONDENCE
Benjamin C. Guinhouya
benjamin.guinhouya@univ-lille.fr

SPECIALTY SECTION
This article was submitted to Reproduction, a section of the journal Frontiers in Endocrinology

RECEIVED 09 February 2022
ACCEPTED 17 August 2022
PUBLISHED 02 September 2022

CITATION
El Idrissi F, Fruchart M, Belarbi K, Lamer A, Dubois-Deruy E, Lemdani M, N'Guessan AL, Guinhouya BC and Zitouni D (2022) Exploration of the core protein network under endometriosis symptomatology using a computational approach. *Front. Endocrinol.* 13:869053. doi: 10.3389/fendo.2022.869053

Exploration of the core protein network under endometriosis symptomatology using a computational approach

Fatima El Idrissi[1,2], Mathilde Fruchart[1,3], Karim Belarbi[2,4], Antoine Lamer[1,3], Emilie Dubois-Deruy[5], Mohamed Lemdani[2,3], Assi L. N'Guessan[6], Benjamin C. Guinhouya[1,3]* and Djamel Zitouni[2,3]

[1]Univ. Lille, UFR 3S, Faculté Ingénierie et Management de la Santé, Lille, France, [2]Univ. Lille, UFR 3S, Faculté de Pharmacie, Lille, France, [3]Univ. Lille, CHU Lille, ULR 2694 - METRICS, Lille, France, [4]Univ. Lille, Inserm, CHU-Lille, Lille Neuroscience & Cognition, Lille, France, [5]Univ. Lille, Inserm, CHU Lille, Institut Pasteur de Lille, U1167 - RID-AGE - Facteurs de risque et déterminants moléculaires des maladies liées au vieillissement, Lille, France, [6]Univ. Lille, UMR CNRS 8524, Laboratoire Paul Painlevé, Villeneuve d'Ascq, Cedex, France

Background: Endometriosis is defined by implantation and invasive growth of endometrial tissue in extra-uterine locations causing heterogeneous symptoms, and a unique clinical picture for each patient. Understanding the complex biological mechanisms underlying these symptoms and the protein networks involved may be useful for early diagnosis and identification of pharmacological targets.

Methods: In the present study, we combined three approaches (i) a text-mining analysis to perform a systematic search of proteins over existing literature, (ii) a functional enrichment analysis to identify the biological pathways in which proteins are most involved, and (iii) a protein–protein interaction (PPI) network to identify which proteins modulate the most strongly the symptomatology of endometriosis.

Results: Two hundred seventy-eight proteins associated with endometriosis symptomatology in the scientific literature were extracted. Thirty-five proteins were selected according to degree and betweenness scores criteria. The most enriched biological pathways associated with these symptoms were (i) Interleukin-4 and Interleukin-13 signaling ($p = 1.11 \times 10^{-16}$), (ii) Signaling by Interleukins ($p = 1.11 \times 10^{-16}$), (iii) Cytokine signaling in Immune system ($p = 1.11 \times 10^{-16}$), and (iv) Interleukin-10 signaling ($p = 5.66 \times 10^{-15}$).

Conclusion: Our study identified some key proteins with the ability to modulate endometriosis symptomatology. Our findings indicate that both pro- and anti-inflammatory biological pathways may play important roles in the symptomatology of endometriosis. This approach represents a genuine systemic method that may complement traditional experimental studies. The

current data can be used to identify promising biomarkers for early diagnosis and potential therapeutic targets.

KEYWORDS

endometrium, cell signaling, female infertility, systems biology, text-mining

Introduction

Endometriosis is a gynecological inflammatory disease affecting women of reproductive age (1, 2). Approximately 200 million women worldwide, 10% to 15% of women of reproductive age and 2.5% of postmenopausal women are affected by endometriosis (3, 4). Three main forms of endometriosis are described: (i) ovarian endometriosis, (ii) superficial peritoneal endometriosis and (iii) deep infiltrating endometriosis (DIE) (1, 5), the latter being recognized as the most severe form (6, 7). Endometriosis is defined by implantation and invasive growth of endometrial tissue in extra-uterine locations, causing chronic pelvic pain, dyspareunia, dysmenorrhea, menorrhagia, bowel symptoms, and infertility (1, 6, 8). Endometriosis symptoms are associated with substantial reductions in quality of life (9, 10). Living with severe cyclic or continuous pelvic pain can lead to stress, anxiety, depression and absenteeism from work (11, 12).

Endometriosis is a multifactorial disease, with complex pathophysiological mechanisms, of which genetic and environmental components are still poorly evaluated (13, 14). The etiology of endometriosis is not completely understood. Several hypotheses have been put forward concerning the histological origins of endometriosis; the most accepted theory being Sampson's theory of retrograde menstruation, which involved fragments of menstrual endometrium being disseminated through the fallopian tubes (7, 9, 13). However, this phenomenon is observed in nearly 90% of women, suggesting that immune and hormonal dysfunctions may add to the observed fragmentation, yielding the adhesion, survival and proliferation of the lesions (1, 2, 10). Several mechanisms such as exacerbated production of growth and pro-inflammatory factors, an increase in estradiol expression combined with progesterone resistance, and an overexpression of reactive oxygen species might be involved in the development of endometriosis (10, 14, 15). Distinct immunological abnormalities involving angiogenesis, vasculogenesis and inflammation have been well described. These processes involve molecules including the *VEGF* factor that triggers angiogenesis, *tumor necrosis factor* (*TNF*)-α, which plays an essential role in increasing proliferative potential and acts primarily as a precursor to initiating an inflammatory response by activating a cascade of other cytokines, such as *IL-1, IL-6* and *CXCL8* (8, 16–18). The ineffectiveness of using anti-inflammatory agents to treat endometriosis shows that the disease is more related to the loss of balance between pro- and anti-inflammatory molecules (4). It appears clearly today that to understand the complexity of this disease, it is necessary to study the processes involved as a whole, as well as potential interactions between their components.

There is currently no treatment for endometriosis. On the other hand, the time between the development of the first lesions and the diagnosis is estimated between 7 and 10 years (2, 10). Thus, two major challenges must be met: identification of early diagnostic biomarkers, and that of potential therapeutic targets. An increasing number of studies are based on the search for biomarkers involved in endometriosis in order to develop less invasive diagnostic methods (i.e., urine tests, blood tests) (19, 20). The biological complexity under endometriosis has been previously addressed using computational biology approaches on the basis endometriosis-related alternations (e.g. immune cell infiltration) (21) or the development and progression of endometriosis (22). However, to the best of our knowledge, no study had used endometriosis-related symptoms to build up its underlying biomolecular processes.

Finding useful information in the genetic data generated for endometriosis is very challenging and computational biology can be helpful. Our study used tools of computational biology to identify biological processes and protein networks underlying the symptomatology of endometriosis. We combined text-mining, functional enrichment and protein-protein interaction analyses to suggest some biomarkers or therapeutic targets deserving further exploration.

Materials and methods

Protein collection with text-mining

The Medline database was used as a data source to perform a systematic search of genes associated with endometriosis symptomatology. The PubMed® search query ["endometriosis" AND ("dysmenorrhea" OR "metrorrhagia" OR "dyspareunia" OR "dyschesia" OR "symptoms")] was used

to retrieve the PMIDs related both to endometriosis and at least one of its symptoms. Articles, from the inception of PubMed until December 2020, which considered a human model, dealt with of reproductive age (i.e. women between 13-44 years old), and published in English, were included. A text-mining of genes related to all types of endometriosis was carried out using the Pubtator resource, which has been developed as an extension of the NCBI, to provide access to biomedical and genomic information (https://www.ncbi.nlm.nih.gov/research/pubtator/) (23, 24). Then, the genes identified with Pubtator in the title, abstract and full text of the PMIDs list was retrieved using the panda library in Python language (www.python.org/).

Text-mining was performed using the GNormPlus Pipeline, which includes two modules: gene mention recognition and gene name normalization. This pipeline has an accuracy of 87.1% (25). We then used 309 UniProtKB Retrieve/ID mapper (https://www.uniprot.org/) to retrieve the UniProtKB protein identifiers associated to these Gene ID (26, 27). Uniprot provides a comprehensive collection of all known, manually annotated protein sequence data.

Gene set enrichment

The essential part of this analysis consisted in translating the genetic signatures into information that can help to understand the underlying biological mechanisms. The annotations determine which proteins are significantly enriched in an entry list compared to a reference list. Gene Ontology (GO) enrichment of the collected proteins was first performed using the GeneCodis (https://genecodis.genyo.es/), with annotation from GO Cellular Component, GO Molecular Function and GO Biological Process categories (28, 29). The most enriched annotations were then visualized using the ggplot2 package in R language (www.r-project.org). Functional enrichment analysis of the proteins was subsequently performed and visualized using the Reactome Pathway Database (https://reactome.org) (30, 31). The functional pathways were sorted in ascending order according to their p-value, and proteins involved in the 10 most significantly enriched functional pathways (i.e. with the lowest p-values) were selected for subsequent analysis.

Protein-protein functional interaction

The STRING protein query database was used to build a protein-protein functional interaction network in Cytoscape 3.7.2 (32). STRING is known as the primary source to depict and visualize the interaction among various proteins (32). The minimum combined score was set at 0.9 to retain only highest-confidence functional and physical interactions. In the network the nodes correspond to proteins and the edges to the interactions between each protein. We then used the CentiScaPe Cytoscape plug-in to calculate the node degree and betweenness centrality of each protein. The nodes (proteins) that had a degree centrality and a betweenness centrality greater than or equal to the mean were identified as key proteins more likely to modulate symptoms of endometriosis.

Results

Protein collection

The workflow of the study is described in Figure 1. The number of articles published on endometriosis symptomatology has been growing exponentially in recent years (Supplementary Figure 1). Our PubMed database queries yielded 2,177 articles published on the topic from 1990 to 2020. The PMIDs of these articles were downloaded and processed using Pubtator. A total of 309 genes were initially obtained, and then converted into unique protein identifiers, which were translated into 278 reviewed proteins, which in turn were linked for further analysis.

Enrichment analysis

The top eight enriched terms of the Cellular component, Molecular Function and Biological Process are presented in Figure 2. Cellular Components showed an enrichment of the proteins expressed in *extracellular region, extracellular space, cytoplasm, plasma membrane, membrane, cytosol, nucleus* and *extracellular exosome*. Molecular Function annotations showed that the proteins involved in the top eight terms were expressed in binding proteins (including *protein binding, identical protein binding, signaling receptor binding, enzyme binding, metal ion binding*), *cytokine activity, G protein-coupled receptor activity* and *hormone activity*. Biological Process annotations revealed that the most highly enriched terms were *signal transduction, cytokine-mediated signaling pathway, positive regulation of gene expression, inflammatory response, positive regulation of cell population proliferation, G protein-coupled receptor signaling pathway, immune response* and *negative regulation of apoptotic process*. The two most enriched Biological Process terms were signal transduction ($p = 7.40 \times 10^{-62}$) and cytokine-mediated signaling pathway ($p = 1.33 \times 10^{-56}$), which were closely related to the pathology of endometriosis.

All the proteins mined were analyzed in the Reactome Database in order to visualize biological processes associated with endometriosis' symptomatology. The 28 global pathways analyses are shown in Figure 3. The 10 most enriched pathways were selected: (1) Interleukin-4 and Interleukin-13 signaling ($p = 1.11 \times 10^{-16}$), (2) signaling by Interleukins ($p = 1.11 \times 10^{-16}$), (3) Cytokine signaling in Immune system ($p = 1.11 \times 10^{-16}$), (4) Interleukin-10 signaling ($p = 5.66 \times 10^{-15}$), (5) Signal transduction ($p = 6.93 \times 10^{-12}$), (6) Extra-nuclear estrogen

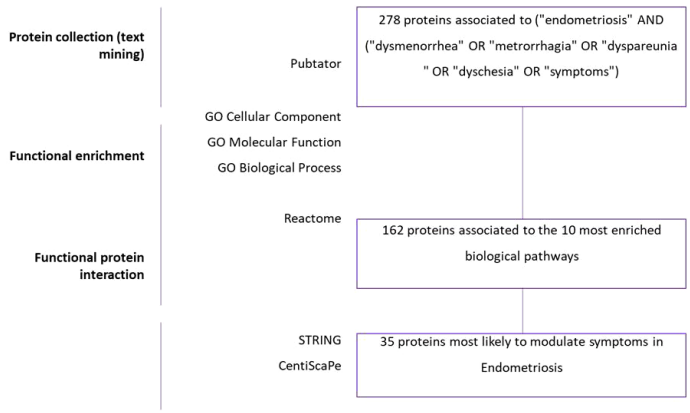


FIGURE 1
Summary of data mining results. Text-mining: Three hundred and nine genes were found by using Pubtator and total of 278 proteins ID were reviewed on Uniprot. Gene Ontology: Biological process, Cellular component, Molecular function analyses were performed in GeneCodis. Gene set enrichment: Pathway analysis was performed in GeneCodis to enrich 278 genes. Then, 162 significant genes were derived by protein-protein interaction analysis using STRING and Cytoscape. Thirty-five significant genes were selected for the final analysis with degree and betweenness criteria using Centiscape and Cytoscape.

signaling ($p = 3.37 \times 10^{-9}$), (7) Signaling by GPCR ($p = 4.34 \times 10^{-9}$), (8) GPCR ligand binding ($p = 4.43 \times 10^{-9}$), (9) Immune System ($p = 4.56 \times 10^{-9}$), (10) Interleukin-1 processing ($p = 1.50 \times 10^{-7}$) (Table 1). We extracted all the proteins involved in the 10 biological pathways mentioned above and removed the duplicates. One hundred sixty-two unique proteins from the 10 most enriched pathways were retained for protein-protein interaction analysis.

Protein-protein functional interaction

To be retained, proteins had to exhibit a higher than average degree (>5.27) and betweennes (166.57), to be both first neighbors of the given node and the shortest path linking two nodes. A total of 35 nodes (proteins) with 150 edges (interactions) were: *Mitogen-Activated Protein Kinase 1, 3 and 14; Interleukin 1β, 2, 4, 6, 10, 13 and 17A; C3; Protein Kinase C Delta; Neurotrophic Receptor Tyrosine Kinase 1; Recombinant Insulin; Adrenoceptor Beta 2; Transcription factor p65; Transforming Growth Factor Beta 1; C-X-C Motif Chemokine Ligand 8; Tumor necrosis factor; Nuclear Factor Kappa B Subunit 1; Caspase-3 precursor; Tumor protein 53; Matrix metallopeptidase 9; Vascular endothelial growth factor A; Metallopeptidase Inhibitor 1; Human Corticotrophin Releasing Hormone Receptor 2; Androgen receptor; Prostaglandin E Receptor 1; Arginine Vasopressin; Proopiomelanocortin; KRAS Proto-Oncogene; Protein kinase B; C-C Motif Chemokine Ligand*

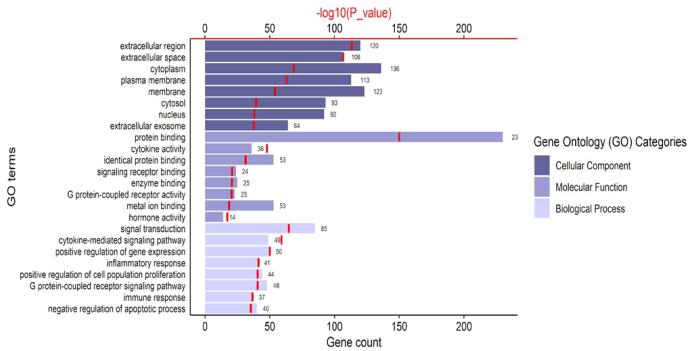


FIGURE 2
The top 8 significant Gene Ontology terms of common genes. The bar charts represent the counts of genes classified in the Cellular Components, Molecular Functions, Biological Pathways, respectively. The red line chart represents the significance of enrichment terms (-log10 (p_value)).

10.3389/fendo.2022.869053

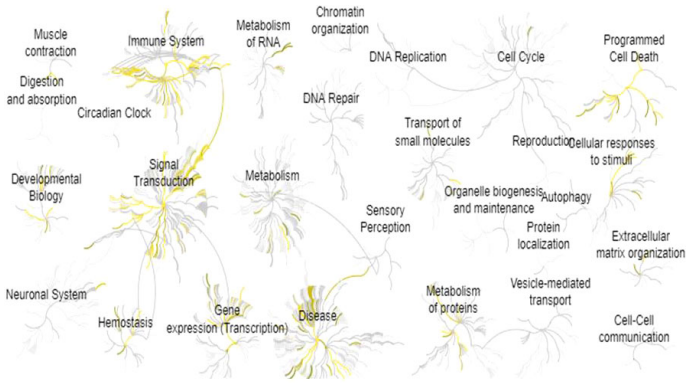


FIGURE 3
Pathway enrichment analysis for all the 278 proteins identified by text-mining. This analysis was performed by using Reactome Pathway Database. Yellow means pathways that are significantly overrepresented.

11; Catenin Beta 1; Protein Tyrosine Kinase 2 (Table 2). Finally, prevailing protein-protein interactions network was visualized with STRING (Figure 4). These proteins were considered to be the most modulated in the symptomatology of endometriosis and thus could explain the underlying biological mechanisms of endometriosis.

Discussion

This study provided a text-mining approach that tapped data from bioinformatics banks, with the aim of investigating the protein network related to the symptomatology of endometriosis. A holistic approach was favored to understand the associated complex biological mechanisms behind this symptomatology. Expressed protein may support the advent of some symptoms, which should help health professionals and clinicians in their investigations. As an attempt to address the knowledge gaps surrounding this disease, a special feature of our approach relies on the interest in all the genes collected in the literature as being related to the symptomatology of endometriosis. These genes are often detected based on the results of isolated experimentations.

Thirty-five key proteins were identified in the current study as potential modulators of the symptomatology of endometriosis. Inflammation in endometriosis, widely supported by existing literature, is reflected by an overexpression of inflammatory cytokines, inhibition of endothelial function and hormonal dysregulations (10, 33). A pro-inflammatory cytokine such as *Interleukin-1 β* (*IL-1β*) may enhance the proliferation of endometriotic cells. *IL-1β* can also trigger the production of *IL-6* and *IL-8* (other pro-inflammatory cytokines), which are involved in more proliferation and the decrease of apoptotic rate (14). Inflammation state not only leads to dysmenorrhea, dyspareunia and infertility (34) but also cause oxidative stress connected to poor-quality embryos and

TABLE 1 Summary of the 10 most enriched biological pathways, grouping 162 unique proteins associated to endometriosis symptomatology using Reactome Pathway Database.

Pathway name	Count	Total genes in genome	Entities p-value
Interleukin-4 and Interleukin-13 signaling	30	111	1.11×10^{-16}
Signaling by Interleukins	59	457	1.11×10^{-16}
Cytokine signaling in Immune system	70	804	1.11×10^{-16}
Interleukin-10 signaling	17	45	5.66×10^{-15}
Signal Transduction	117	2574	6.93×10^{-12}
Extra-nuclear estrogen signaling	15	80	3.37×10^{-9}
Signaling by GPCR	46	706	4.34×10^{-9}
GPCR ligand binding	36	469	4.43×10^{-9}
Immune System	99	2249	4.56×10^{-9}
Interleukin-1 processing	6	9	1.50×10^{-7}

Count: enriched protein number in the pathway.

TABLE 2 Proteins with higher than average betweenness and degree in the protein-protein interaction network.

Protein Name	UniProtKB ID	Betweenness (average 166.57)	Degree (average 5.27)
POMC	P01189	2562.6	25
CXCL8	P10145	1927.9	27
MAPK1	P28482	1879.0	28
AKT1	P31749	1531.5	21
CCL11	P51671	1332.1	8
IL6	P05231	1289.2	27
IL2	P60568	1275.9	19
MAPK3	P27361	1258.2	26
INS	P01308	1251.5	11
VEGFA	P15692	1183.3	20
C3	P01024	1177.1	20
NFKB1	P19838	1151.5	21
MAPK14	Q16539	875.4	17
KRAS	P01116	870.6	16
CTNNB1	P35222	853.3	13
TNF	P01375	600.7	24
IL4	P05112	582.8	17
PTGER1	P34995	583.3	8
IL10	P22301	533.6	21
RELA	Q04206	493.7	21
IL1B	P01584	460.8	22
IL13	P35225	456.7	16
NTRK1	P04629	440.4	8
TP53	P04637	425.4	17
ADRB2	P07550	420.2	12
CRHR2	Q13324	379.1	12
CASP3	P42574	374.4	10
AVP	P01185	352.2	16
MMP9	P14780	345.0	12
TGFB1	P01137	282.8	10
AR	P10275	282.1	8
PRKCD	Q05655	269.8	13
IL17A	Q16552	256.8	9
PTK2	Q05397	253.7	11
TIMP1	P01033	222.1	10

immature oocytes (14). The over-activation of macrophages and mast cells in endometriosis produces *IL-1β*, *TNF-α*, *IL-6*, and *IL-10* (35) found in our analysis. Even if the involvement of the *IL-10* pathway in endometriosis is still poorly understood, the *IL-10 signaling pathway* has an ability to block cytokines and chemokines from macrophages being the root of inflammatory processes (36, 37). Moreover, anti-inflammatory *signaling pathways IL-4 and IL-13* are involved in the cellular immune response, particularly T helper type 2 (38). *IL-4* and *IL-13* type I and II receptor signaling pathways are linked to Signal Transducer and Activator of Transcription 6 (*STAT6*) expression (39), responsible for mediating *IL-4* and *IL-13* immune signaling, increase proliferation, adhesion and viability of endometriosis lesions (38). *Suppressor Of Cytokine Signaling protein 1* (*SOCS1*) inhibits *STAT6* expression and has a protective effect by activating cell apoptosis (39). *SOCS1* dysregulation may exacerbate the *IL-4* and *IL-13* properties associated with endometriosis.

Hence, the findings from the current study pinpoint that both pro-inflammatory and anti-inflammatory pathways are involved in the symptomatology of endometriosis. This was assumed to play a paradoxical role in acute and chronic phases of the disease due to pathological immune imbalance (38). Interestingly, a study on the mouse model has shown that pre-existing peritoneal inflammation did not contribute to the development of endometriosis and could even reduce it

(33). This supports the need to provide understanding of the precise role of inflammation in this disease.

Some previously identified targets in the literature (i.e. *IL-1, IL-6, IL-4, and VEGF*) were emphasized by our analysis. The *IL-1 pathway* is a pro-inflammatory cytokine that activated *NF-kB* inflammatory pathways (34). Dysregulation of cytokines and *NF-kB* factor induces both an inflammatory process and an immune system dysfunction involved in endometriosis (40). The *NF-kB* biological pathway has several subunits such as the *p65* involved in the regulation of cell survival. The pathway expressed by *p65* also plays a role in the inflammatory response and contributes to angiogenesis and metastasis survival (41–43). *NF-kB1* subunit promotes the expression of inflammatory cytokines (44, 45).

Similar to the existing literature, the implication of *TNF-α* is again emphasized in this study on Figure 4, portraying the most important protein interactions. *TNF-α*, which plays an essential role in increasing proliferative potential, acts primarily as a precursor in initiating an inflammatory response by activating a cascade of other cytokines, such as *IL-1, IL-6* and the vascular endothelial growth factor (*VEGF*) (7, 46). Abnormal *VEGF* levels may impair the process of angiogenesis, which is favorable to embryonic implantation, thus justifying the high miscarriage rate (47, 48). The current study also highlighted some lesser-known targets (i.e. *PGE1, AVP*) (49–51), which may require deeper investigations. Excessive expression of *Prostaglandin E receptor 1* (*PGE1*) is linked to an inflammatory reaction as for tumor protein 53 (*p53*). Unlike our finding, no link between endometriosis and arginine vasopressin (*AVP*) or Caspase-3 has been reported in the literature, and this may require more exploration (46–48, 52). Furthermore, the *KRAS* protein, stressed in the protein network (Figure 4) is a factor that is overexpressed in skeletal muscle and myocardium, uterus, adrenal cortex and some bone marrow stem cells (53). A mutation of *KRAS* can cause hyperplasia. This may explain the occurrence of endometrial hyperplasia in endometriosis (49, 54). *TGF-β1* also plays a role in muscle diseases by inhibiting myogenesis and regeneration (55). This involves the development of fibrosis or atrophy of the muscle skeleton (56, 57). Finally, our study pointed out *Neurotrophic Receptor Tyrosine Kinase 1* (*NTRK1*) protein, which may be involved in neuropathic pain. Nerve growth factor (*NGF*) is the main ligand of *NTRK1* (26). It was shown that *NGF* is directly associated with pelvic pain and more specifically with dysmenorrhea, dyspareunia, painful bladder syndrome and irritable bowel syndrome (58, 59). These pains reach the nervous system by provoking a nociception. Such a damage to the nervous system would lead to neuropathic or neuroinflammatory pain (59, 60). In summary, this apparent crosstalk between immune cells, nerves, and central pain pathways is providing an opportunity to develop more targeted therapies against endometriosis and its symptoms (20).

Although the proteins identified should be taken with caution, given the heterogeneity of the studied tissues, the different forms of the disease and techniques used, the current study provides an overall picture of the proteins involved in the symptomatology of endometriosis. In future studies, it would be

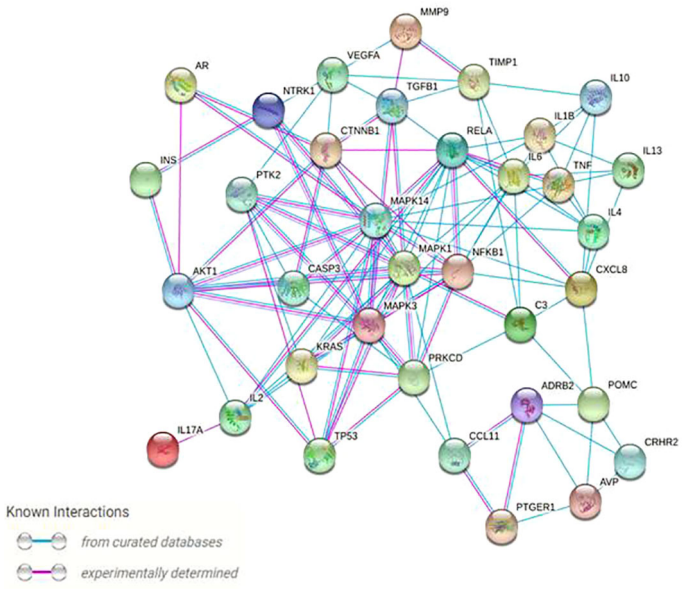


FIGURE 4
Protein–protein high (confidence score 0.9) physical and functional interactions network of the 35 targeted proteins generated by the String and Centiscape softwares. Network nodes represent proteins; blue edges represent known interactions from curated databases, and pink edges represent experimentally determined interactions.

interesting to examine the involvement of these proteins given the stage of endometriosis and the phases of the menstrual cycle. It is noteworthy that protein interactions that are not already known in the STRING database may lead to discarding key targets involved in the symptomatology of endometriosis. Some proteins have been deeply investigated in the literature while others are rarely studied proteins, and thus may not be detected during text-mining. Also, the network of proteins obtained does not allow weighing to them according to the importance of their involvement in the disease. Thus this approach proves to be complementary to other studies exploring the completeness of the genome (e.g. Genome-wide association studies, GWAS) or using the Gene Expression Omnibus database (GEO) to study differentially expressed genes (61–63). Our study still remains an exploratory analysis that was based on the general symptomatology of endometriosis. We do not get any information on either the different forms of endometriosis or the different stages of the disease. Potentially, this approach can be replicated at a higher level of detail to allow comparing the biological implications of eutopic and ectopic endometrium. In the same way, the query can be refined to select articles from different stages of the disease (early or advanced stage) or from different phases of the menstrual cycle. Hence, the comparison of the signaling pathways related to the early stage of endometriosis (with a search for genes in the articles involved in the early stage of the disease) and the more advanced stage warrants attention. Finally, because Pubtator tools may not have perfect discriminative ability in distinguishing between genes and alleles, some overlapping cannot be completed ruled out.

Taken together, our data highlight that pathways associated to endometriosis symptomatology have sometimes paradoxical roles, certainly resulting in a loss of balance, and these may be time-dependent. Then, developing strategies to enhance their protective effects or to combat their pathological responses at specific stages of the disease could prove therapeutic potential for endometriosis. In conclusion, our study identifies 35 interrelated key proteins with the highest ability to control pathways associated to endometriosis symptomatology. While some proteins such as *IL-1β, IL-6, IL-4, and VEGF* are largely evaluated, our data are suggestive of further investigation on proteins such as *PGE1* and *AVP*. Our study prioritizes potential biomarkers and key targets, and further assessing them in endometriosis could help for the development of diagnostic tools and therapeutic strategies for endometriosis.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

FE and MF designed the study, contributed to the methodology, data curation and execution of the study, analyzed the data and wrote the manuscript. KB and ED-D contributed to the methodology and reviewed the manuscript. AL, ML, and AN reviewed the manuscript. BG and DZ designed the study and revised the manuscript providing their expertise. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.869053/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Cumulative number of publications related to endometriosis symptomatology by year (1990 to 2020). Bar chart represents articles meeting the inclusion criteria (i.e human model, women in reproductive age, published in English, which addressed endometriosis and at least one associated clinical sign).

References

1. Bulun, SE, Yilmaz, BD, Sison, C, Miyazaki, K, Bernardi, L, Liu, S, et al. Endometriosis. *Endocr Rev* (2019) 40(4):1048–79. doi: 10.1210/er.2018-00242

2. Smolarz, B, Szyłło, K, and Romanowicz, H. Endometriosis: Epidemiology, classification, pathogenesis, treatment and genetics (Review of literature). *Int J Mol Sci* (2021) 22(19):10554. doi: 10.3390/ijms221910554

3. Prodromidou, A, Machairas, N, Paspala, A, Hasemaki, N, and Sotiropoulos, GC. Diagnosis, surgical treatment and postoperative outcomes of hepatic endometriosis: A systematic review. *Ann Hepatol* (2020) 19(1):17–23. doi: 10.1016/j.aohep.2019.08.006

4. Zhu, J, Xue, X, He, Z, Zhang, J, and Sun, H. Using network pharmacology and molecular docking to explore the underlying anti-inflammatory mechanism of wuyao-danshen to treat endometriosis. *Ann Transl Med* (2022) 10(4):198. doi: 10.21037/atm-22-419

5. Carpinello, OJ, Sundheimer, LW, Alford, CE, Taylor, RN, and DeCherney, AH. Endometriosis. In: Feingold, KR, Anawalt, B, Boyce, A, Chrousos, G, de Herder, WW, Dungan, K, et al, editors. *Endotext*. South Dartmouth, MA: MDText.com, Inc (2000).

6. Chapron, C, Marcellin, L, Borghese, B, and Santulli, P. Rethinking mechanisms, diagnosis and management of endometriosis. *Nat Rev Endocrinol* (2019) 15(11):666–82. doi: 10.1038/s41574-019-0245-z

7. Koninckx, PR, Ussia, A, Adamyan, L, Tahlak, M, Keckstein, J, Wattiez, A, et al. The epidemiology of endometriosis is poorly known as the pathophysiology and diagnosis are unclear. *Best Pract Res Clin Obstet Gynaecol* (2021) 71:14–26. doi: 10.1016/j.bpobgyn.2020.08.005

8. Klemmt, PAB, and Starzinski-Powitz, A. Molecular and cellular pathogenesis of endometriosis. *Curr Womens Health Rev* (2018) 14(2):106–16. doi: 10.2174/1573404813666170306163448

9. Coxon, L, Horne, AW, and Vincent, K. Pathophysiology of endometriosis-associated pain: A review of pelvic and central nervous system mechanisms. *Best Pract Res Clin Obstet Gynaecol* (2018) 51:53–67. doi: 10.1016/j.bpobgyn.2018.01.014

10. García-Gómez, E, Vázquez-Martínez, ER, Reyes-Mayoral, C, Cruz-Orozco, OP, Camacho-Arroyo, I, and Cerbón, M. Regulation of inflammation pathways and inflammasome by sex steroid hormones in endometriosis. *Front Endocrinol* (2020) 10:935. doi: 10.3389/fendo.2019.00935

11. Soliman, AM, Coyne, KS, Gries, KS, Castelli-Haley, J, Snabes, MC, and Surrey, ES. The effect of endometriosis symptoms on absenteeism and presenteeism in the workplace and at home. *J Manag Care Spec Pharm* (2017) 23(7):745–54. doi: 10.18553/jmcp.2017.23.7.745

12. Fiala, L, Lenz, J, and Bob, P. Effect of psychosocial trauma and stress on sexual dysfunction in women with endometriosis. *Med (Baltimore)* (2021) 100(31): e26836. doi: 10.1097/MD.0000000000026836

13. Burney, RO, and Giudice, LC. Pathogenesis and pathophysiology of endometriosis. *Fertil Steril* (2012) 98(3):511–9. doi: 10.1016/j.fertnstert.2012.06.029

14. Samimi, M, Pourhanifeh, MH, Mehdizadehkashi, A, Eftekhar, T, and Asemi, Z. The role of inflammation, oxidative stress, angiogenesis, and apoptosis in the pathophysiology of endometriosis: Basic science and new insights based on gene expression. *J Cell Physiol* (2019) 234(11):19384–92. doi: 10.1002/jcp.28666

15. Vercellini, P, Viganò, P, Somigliana, E, and Fedele, L. Endometriosis: pathogenesis and treatment. *Nat Rev Endocrinol* (2014) 10(5):261–75. doi: 10.1038/nrendo.2013.255

16. Heydari, S, Kashani, L, and Noruzinia, M. Dysregulation of angiogenesis and inflammatory genes in endometrial mesenchymal stem cells and their contribution to endometriosis. *Iran J Allergy Asthma Immunol* (2021) 20(6):740. doi: 10.18502/ijaai.v20i6.8025

17. Zarezadeh Mehrabadi, A, Aghamohamadi, N, Khoshmirsafa, M, Aghamajidi, A, Pilehforoshha, M, Massoumi, R, et al. The roles of interleukin-1 receptor accessory protein in certain inflammatory conditions. *Immunology* (2022) 166(1):38–46. doi: 10.1111/imm.13462

18. Zhang, T, De Carolis, C, Man, GCW, and Wang, CC. The link between immunity, autoimmunity and endometriosis: a literature update. *Autoimmun Rev* (2018) 17(10):945–55. doi: 10.1016/j.autrev.2018.03.017

19. Méar, L, Com, E, Fathallah, K, Guillot, L, Lavigne, R, Guével, B, et al. The eutopic endometrium proteome in endometriosis reveals candidate markers and molecular mechanisms of physiopathology. *Diagn (Basel)* (2022) 12(2):419. doi: 10.3390/diagnostics12020419

20. Saunders, PTK, and Horne, AW. Endometriosis: Etiology, pathobiology, and therapeutic prospects. *Cell* (2021) 184(11):2807–24. doi: 10.1016/j.cell.2021.04.041

21. Chen, S, Chai, X, and Wu, X. Bioinformatical analysis of the key differentially expressed genes and associations with immune cell infiltration in development of endometriosis. *BMC Genom Data* (2022) 23:20. doi: 10.1186/s12863-022-01036-y

22. Peng, Y, Peng, C, Fang, Z, and Chen, G. Bioinformatics analysis identifies molecular markers regulating development and progression of endometriosis and potential therapeutic drugs. *Front Genet* (2021) 12:622683. doi: 10.3389/fgene.2021.622683

23. Wei, C-H, Allot, A, Leaman, R, and Lu, Z. PubTator central: automated concept annotation for biomedical full text articles. *Nucleic Acids Res* (2019) 47 (W1):W587–W93. doi: 10.1093/nar/gkz389

24. PubTator. *NLM/NCBI BioNLP research group. discover biomedical entities in more than 30 million biomedical publications* (2013). Available at: https://www.ncbi.nlm.nih.gov/research/pubtator/.

25. Wei, C-H, Kao, H-Y, and Lu, Z. GNormPlus: An integrative approach for tagging genes, gene families, and protein domains. *BioMed Res Int* (2015) 2015:918710. doi: 10.1155/2015/918710

26. UniProt. The UniProt consortium. UniProt: the universal protein knowledgebase. *Nucleic Acids Res* (2017) 45(D1):D158–69. doi: 10.1093/nar/gkw1099

27. UniProt. (2002). Available at: https://www.uniprot.org/.

28. García-Moreno, A, López-Domínguez, R, Ramirez-Mena, A, Pascual-Montano, A, Aparicio-Puerta, E, and Hackenberg, M. GeneCodis 4: Expanding the modular enrichment analysis to regulatory elements. *bioRXiv* (2021), 1–9. doi: 10.1101/2021.04.15.439962

29. GeneCodis. *Gene annotations co-occurence discovery* (2007). Available at: https://genecodis.genyo.es/.

30. Fabregat, A, Jupe, S, Matthews, L, Sidiropoulos, K, Gillespie, M, Garapati, P, et al. The reactome pathway knowledgebase. *Nucleic Acids Res* (2018) 46(D1): D649–D55. doi: 10.1093/nar/gkx1132

31. reactome. *Find reactions, proteins and pathways* (2003). Available at: https://reactome.org/.

32. Doncheva, NT, Morris, JH, Gorodkin, J, and Jensen, LJ. Cytoscape StringApp: Network analysis and visualization of proteomics data. *J Proteome Res* (2019) 18(2):623–32. doi: 10.1021/acs.jproteome.8b00702

33. Jiang, L, Yan, Y, Liu, Z, and Wang, Y. Inflammation and endometriosis. *Front Biosci (Landmark Ed)* (2016) 21(5):941–8. doi: 10.2741/4431

34. Malvezzi, H, Hernandes, C, Piccinato, CA, and Podgaec, S. Interleukin in endometriosis-associated infertility-pelvic pain: systematic review and meta-analysis. *Reproduction* (2019) 158(1):1–12. doi: 10.1530/REP-18-0618

35. Wei, Y, Liang, Y, Lin, H, Dai, Y, and Yao, SJ. Autonomic nervous system and inflammation interaction in endometriosis-associated pain. *Neuroinflammation* (2020) 17(1):80. doi: 10.1186/s12974-020-01752-1

36. Moore, KW, de Waal Malefyt, R, Coffman, RL, and O'Garra, A. Interleukin-10 and the interleukin-10 receptor. *Annu Rev Immunol* (2001) 19:683–765. doi: 10.1146/annurev.immunol.19.1.683

37. Hogg, C, Panir, K, Dhami, P, Rosser, M, Mack, M, Soong, D, et al. Macrophages inhibit and enhance endometriosis depending on their origin. *Proc Natl Acad Sci U S A* (2021) 118(6):e2013776118. doi: 10.1073/pnas.2013776118

38. Zhou, W-J, Yang, H-L, Shao, J, Mei, J, Chang, K-K, Zhu, R, et al. Anti-inflammatory cytokines in endometriosis. *Cell Mol Life Sci* (2019) 76(11):2111–32. doi: 10.1007/s00018-019-03056-x

39. Hershey, GKK. IL-13 receptors and signaling pathways: An evolving web. *J Allergy Clin Immunol* (2003) 111(4):677–90. doi: 10.1067/mai.2003.1333

40. Adewuyi, EO, Sapkota, Y, Auta, A, Yoshihara, K, Nyegaard, M, Griffiths, LR, et al. Shared molecular genetic mechanisms underlie endometriosis and migraine comorbidity. *Genes (Basel)* (2020) 11(3):268. doi: 10.3390/genes11030268

41. Bozkurt, M, Şahin, L, and Ulaş, M. Hysteroscopic polypectomy decreases NF-κB1 expression in the mid-secretory endometrium of women with endometrial polyp. *Eur J Obstet Gynecol Reprod Biol* (2015) 189:96–100. doi: 10.1016/j.ejogrb.2015.03.032

42. Kim, SH, Ihm, HJ, Oh, YS, Chae, HD, Kim, CH, and Kang, BM. Increased nuclear expression of nuclear factor kappa-b p65 subunit in the eutopic endometrium and ovarian endometrioma of women with advanced stage endometriosis. *Am J Reprod Immunol* (2013) 70(6):497–508. doi: 10.1111/aji.12161

43. Wang, J, Zhou, M, Zhang, Q-G, Xu, J, Lin, T, Zhou, R-F, et al. Prognostic value of expression of nuclear factor kappa-B/p65 in non-GCB DLBCL patients. *Oncotarget* (2016) 8(6):9708–16. doi: 10.18632/oncotarget.14182

44. Lorenzini, T, Fliegauf, M, Klammer, N, Frede, N, Proietti, M, Bulashevska, A, et al. Characterization of the clinical and immunologic phenotype and management of 157 individuals with 56 distinct heterozygous NFKB1 mutations. *J Allergy Clin Immunol* (2020) 46(4):901–11. doi: 10.1016/j.jaci.2019.11.051

45. Wang, Y, Wu, B, Zhang, M, Miao, H, and Sun, J. Significant association between rs28362491 polymorphism in NF-κB1 gene and coronary artery disease: a meta-analysis. *BMC Cardiovasc Disord* (2020) 20(1):278. doi: 10.1186/s12872-020-01568-0

46. Sarker, A, Ginn, R, Nikfarjam, A, O'Connor, K, Smith, K, Jayaraman, S, et al. Utilizing social media data for pharmacovigilance: A review. *J BioMed Inform* (2015) 54:202–12. doi: 10.1016/j.jbi.2015.02.004

47. Chen, H, Deng, X, Yang, Y, Shen, Y, Chao, L, Wen, Y, et al. Expression of GRIM-19 in missed abortion and possible pathogenesis. *Fertil Steril* (2015) 103 (1):138–46.e3. doi: 10.1016/j.fertnstert.2014.10.012

48. Harmsen, MJ, Wong, CFC, Mijatovic, V, Griffioen, AW, Groenman, F, Hehenkamp, WJK, et al. Role of angiogenesis in adenomyosis-associated abnormal uterine bleeding and subfertility: a systematic review. *Hum Reprod Update* (2019) 25(5):647–71. doi: 10.1093/humupd/dmz024

49. Bouaziz, J, Mashiach, R, Cohen, S, Kedem, A, Baron, A, Zajicek, M, et al. How artificial intelligence can improve our understanding of the genes associated with endometriosis: Natural language processing of the PubMed database. *BioMed Res Int* (2018) 2018:1–7. doi: 10.1155/2018/6217812

50. Liu, F, Lv, X, Yu, H, Xu, P, Ma, R, and Zou, K. In search of key genes associated with endometriosis using bioinformatics approach. *Eur J Obstet Gynecol Reprod Biol* (2015) 194:119–24. doi: 10.1016/j.ejogrb.2015.08.028

51. Liu, J-L, and Zhao, M. Prioritization of susceptibility genes for ectopic pregnancy by gene network analysis. *Int J Mol Sci* (2016) 17(2):191. doi: 10.3390/ijms17020191

52. Sarkar, S, Hobson, AR, Hughes, A, Growcott, J, Woolf, CJ, Thompson, DG, et al. The prostaglandin E2 receptor-1 (EP-1) mediates acid-induced visceral pain hypersensitivity in humans. *Gastroenterology* (2003) 124(1):18–25. doi: 10.1053/gast.2003.50022

53. Timar, J, and Kashofer, K. Molecular epidemiology and diagnostics of KRAS mutations in human cancer. *Cancer Metastasis Rev* (2020) 39(4):1029–38. doi: 10.1007/s10555-020-09915-5

54. Sideris, M, Emin, EI, Abdullah, Z, Hanrahan, J, Stefatou, KM, Sevas, V, et al. The role of KRAS in endometrial cancer: A mini-review. *Anticancer Res* (2019) 39 (2):533–9. doi: 10.21873/anticanres.13145

55. Yang, Y, Zhang, N, Lan, F, Van Crombruggen, K, Fang, L, Hu, G, et al. Transforming growth factor-beta 1 pathways in inflammatory airway diseases. *Allergy* (2014) 69(6):699–707. doi: 10.1111/all.12403

56. Abrigo, J, Simon, F, Cabrera, D, Cordova, G, Trollet, C, and Cabello-Verrugio, C. Central role of transforming growth factor type beta 1 in skeletal muscle dysfunctions: An update on therapeutic strategies. *Curr Protein Pept Sci* (2018) 19(12):1189–200. doi: 10.2174/1389203718666171117101916

57. Loverro, G, Maiorano, E, Napoli, A, Selvaggi, L, Marra, E, and Perlino, E. Transforming growth factor-beta 1 and insulin-like growth factor-1 expression in ovarian endometriotic cysts: a preliminary study. *Int J Mol Med* (2001) 7(4):423–9. doi: 10.3892/ijmm.7.4.423

58. Kajitani, T, Maruyama, T, Asada, H, Uchida, H, Oda, H, Uchida, S, et al. Possible involvement of nerve growth factor in dysmenorrhea and dyspareunia associated with endometriosis. *Endocr J* (2013) 60(10):1155–64. doi: 10.1507/endocrj.EJ13-0027

59. Zheng, P, Zhang, W, Leng, J, and Lang, J. Research on central sensitization of endometriosis-associated pain: a systematic review of the literature. *J Pain Res* (2019) 12:1447–56. doi: 10.2147/JPR.S197667

60. Orr, NL, Wahl, KJ, Lisonek, M, Joannou, A, Noga, H, Albert, A, et al. Central sensitization inventory in endometriosis. *Pain* (2022) 163(2):e234–e45. doi: 10.1097/j.pain.0000000000002351

61. Nyholt, DR, Low, S-K, Anderson, CA, Painter, JN, Uno, S, Morris, AP, et al. Genome-wide association meta-analysis identifies new endometriosis risk loci. *Nat Genet* (2012) 44(12):1355–9. doi: 10.1038/ng.2445

62. Rahman, MH, Peng, S, Hu, X, Chen, C, Rahman, MR, Uddin, S, et al. A network-based bioinformatics approach to identify molecular biomarkers for type 2 diabetes that are linked to the progression of neurological diseases. *Int J Environ Res Public Health* (2020) 17(3):1035. doi: 10.3390/ijerph17031035

63. Rahman, MH, Rana, HK, Peng, S, Hu, X, Chen, C, Quinn, JMW, et al. Bioinformatics and machine learning methodologies to identify the effects of central nervous system disorders on glioblastoma progression. *Brief Bioinform* (2021) 22(5):bbaa365. doi: 10.1093/bib/bbaa365

frontiers | Frontiers in Endocrinology

TYPE Systematic Review
PUBLISHED 20 September 2022
DOI 10.3389/fendo.2022.950866

OPEN ACCESS

EDITED BY
Mainak Dutta,
Birla Institute of Technology and Science, United Arab Emirates

REVIEWED BY
Philippa Saunders,
University of Edinburgh, United Kingdom
Arnab Banerjee,
Birla Institute of Technology and Science, India
Koninckx R. Philippe,
KU Leuven, Belgium

*CORRESPONDENCE
Nicola Pluchino
Nicola.Pluchino@hcuge.ch

SPECIALTY SECTION
This article was submitted to Reproduction, a section of the journal Frontiers in Endocrinology

RECEIVED 23 May 2022
ACCEPTED 24 August 2022
PUBLISHED 20 September 2022

CITATION
Mercorio A, Giampaolino P, Romano A, Dällenbach P and Pluchino N (2022) Is intracrinology of endometriosis relevant in clinical practice? A systematic review on estrogen metabolism.
Front. Endocrinol. 13:950866.
doi: 10.3389/fendo.2022.950866

Is intracrinology of endometriosis relevant in clinical practice? A systematic review on estrogen metabolism

Antonio Mercorio[1], Pierluigi Giampaolino[2], Andrea Romano[3], Patrick Dällenbach[1] and Nicola Pluchino[1]*

[1]Department of Pediatrics Gynecology and Obstetrics, Division of Gynecology, Geneva University Hospitals, Geneva, Switzerland, [2]Department of Public Health, School of Medicine, University of Naples Federico II, Naples, Italy, [3]Obstetrics and Gynaecology Department, GROW - School for Oncology and Reproduction, Maastricht University, Maastricht, Netherlands

Endometriosis is a chronic, multifactorial, estrogen-dependent disease. The abnormal endocrine microenvironment of endometriosis lesions is considered a main feature and multiple enzymatic pathways leading to local increased synthesis of estrogens have been identified. However, the relevance of intracrinology in clinical practice is still lacking. Medline, Embase, Scopus database were systematically searched for studies reporting on local estrogens metabolism of endometriotic lesions. The main enzymatic pathways involved in the intracrinology of endometriosis such as aromatase (CYP19A1), 17β-hydroxysteroid dehydrogenase (HSD17B) type 1, type 2 and type 5, steroid sulfatase (STS), estrogen sulfotransferase (SULT1E1) were assessed with a critical perspective on their role in disease endocrine phenotyping, drug resistance and as therapeutic targets. Overall, studies heterogeneity and missing clinical data affect the interpretation of the clinical role of these enzymes. Although the use of some drugs such as aromatase inhibitors has been proposed in clinical practice for two decades, their potential clinical value is still under investigation as well as their modality of administration. A closer look at new, more realistic drug targets is provided and discussed. Altered expression of these key enzymes in the lesions have far reaching implication in the development of new drugs aimed at decreasing local estrogenic activity with a minimal effect on gonadal function; however, given the complexity of the evaluation of the expression of the enzymes, multiple aspects still remains to be clarified.

Systematic Review Registration: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42022311329, identifier CRD42022311329.

KEYWORDS

endometriosis, estrogen, metabolism, pelvic pain, endometrium

Introduction

Endometriosis is described by the presence of endometrial-like tissue outside the uterus. It is defined as an estrogen-dependent, inflammatory, and chronic disease, often associated with pelvic pain and subfertility/infertility. The etiology remains uncertain and seems to be related to a complex interplay between family of genes associated with the immune system functioning, sex steroid hormone pathways (neuro)inflammation and environmental risk factors (1).

The estrogen-dependent nature of endometriosis is well known and clinically highlighted by the relapse of the disease in premenopausal women post discontinuation of anti-gonadotropic therapy, as well as in postmenopausal women following the administration of estrogen replacement therapy (2–4) Estrogens show direct cellular antiapoptotic and proliferative effects on the endometriosis lesion as well as promote the pro-inflammatory microenvironment, leading to the chronic nature of the disease (2).. Recent genomics, transcriptomics, and proteomics data, show that endometriosis tissue has a different hormonal environment (5). Increased estrogenic responsiveness, based on the aberrant number and ratio of ERalpha/ER beta (6), abnormal estrogen signaling, progesterone resistance have provided evidence that estrogen effects in normal endometrium do not fully replicate their action on endometriosis (7). Moreover, several studies have shown that endometriotic lesion represents a microenvironment where a multistep enzymatic process leads to an altered metabolism of DHEA or other androgen precursors into bioactive estrogens (Table 1). This mechanism which was called "intracrinology" was primarily defined by Fernand Labrie (8) as the combination of steroids that exhibit their action in the same cell without any pericellular space secretion. More recently, the term has been extended to the tissue microenvironment as the capacity to regulate tissue steroid concentrations from circulating precursors (9, 10).

The ability of peripheral tissues to use blood precursors and generate steroids has been quite well characterized for tissue remodeling and critical phases such as implantation, early pregnancy development and menstruation (11, 12).The evidence that intracrinology might play a role in the aberrant endocrine environment of endometriosis (13) have far-reaching implications for disease physiopathology, molecular characterization, and drug targeting. Although a large body of evidence in the last ten years supports the view that that endometriosis is a heterogeneous disease, molecular and endocrine clustering processes are just beginning to be understood. The macroscopic appearance of the disease poorly correlates with painful symptoms. Hormonal signatures have the potential to optimize disease characterization and management in the clinical setting. The present article aims to review the available literature investigating the association of intracrinology of endometriosis with clinical features of the disease. Current and ongoing clinical studies targeting intracrinology will be critically reviewed.

Abbreviations: AIs, Aromatase inhibitors; HSD17B, 17β-hydroxysteroid dehydrogenase; STS, Steroid sulfatase; SULT1E1, Estrogen sulfotransferase; DHEA, Dehydroepiandrosterone; DNG, Dienogest; T, testosterone; E1, estrone; E2, estrdiol; A4, Androstenedione; COX-2, cyclooxygenase-2; PGE2, Prostaglandin E2; COCs, combined oral contraceptives; GnRHa, gonadotropin-releasing hormone agonist; NETA, norethisterone acetate; GnRHa, gonadotropin-releasing hormone agonist; IVR, Intra vaginal ring; AKR1C3, 17β-hydroxysteroid dehydrogenase type 5.

Materials and methods

Search strategy

Following protocol registration with PROSPERO (Registration number CRD42022311329) a systematic review was conducted according to Preferred Reporting Items for Systematic Reviews (PRISMA) guidelines. No approval from the Institutional Research Ethics Committee was sought owing to the nature of this work. The following search query was submitted throug Medline, Embase and Scoups database: ("oestrogen"[All Fields] OR ''estrogens"[MeSH Terms] AND (''metabolism"[All Fields] OR ''metabolism"[MeSH Terms] OR ''metabolic networks and pathways"[MeSH Terms] AND ("endometriosis"[MeSH Terms] OR ("endometriosis"[All Fields]). In addition, Embase and Medline were searched with broader terms: endometriosis AND estrogen metabolism AND steroid sulfatase; endometriosis AND estrogen metabolism AND 17beta hydroxysteroid dehydrogenase; endometriosis AND estrogen metabolism AND estrogen sulfotransferase.

Study selection

We limited the search to publications in English and excluded article published earlier than 1995. Potentially eligible studies were retrieved in full text for the assessment of their eligibility. Study selection was conducted independently by two reviewers (AM and NP) with disagreement resolved by consensus. We included all studies that satisfied the following eligibility criteria, i) They were human based studies. Studies on animal models and *in vitro* studies investigating human tissue derived cell lines were excluded, ii) They presented original research data, iii) Participants had endometriosis confirmed by histology. In order to highlight the association of the enzymes involved in the intracrinology of endometriosis and the clinical features of the disease, we decided to focus preferentially on their protein expressions levels which reflects gene function more directly than mRNA and is more directly related to phenotype; however, due to the paucity of data present in literature this focused analysis was possible only for the "aromatase" enzyme.

Data extraction and analysis

A standardized critical appraisal and data extraction tool was generated using criteria from CASP (Critical Appraisal Skills Programme) and statements, PRISMA, STARD (Standards for Reporting Diagnostic accuracy studies) and STROBE (Strengthening The Reporting of Observational Studies in Epidemiology) as appropriate. Two reviewers independently appraised the articles and extracted data (AM and NP).

Risk of bias

To reduce selection bias, the abstracts and full text papers were evaluated by masking the authors as far as possible and by basing decisions regarding relevance and eligibility on the independent appraisal by two reviewers. Bias in studies included was assessed independently by the two reviewers.

Results

Study selection

Total articles retrieved after duplicated removal were 1023. Analysis of the titles and abstract led to the removal of 949 papers, including repeated hits and articles based on disease other than endometriosis or full text in English not available. Out of the 74 articles remained for full-text analysis, 28 were excluded because they did not provide original results (i.e. reviews) or they referred to preclinical data. References in the selected articles were controlled for missing inclusions and six articles were included manually (Figure 1). In the end a total of 40 articles were included in this systematic review.

Characteristics of the included studies

In the following sections, studies evaluating the expressions of the enzymes controlling the synthesis/inactivation of estrogens in endometriosis are examined with respect to clinical stage of disease, severity of symptoms and disease localization. Analysis of targeted enzyme therapy and current hormonal treatments effect on intracrinology regulation are provided. Four are the principal pathways that have received more attention and that likely contribute more than others to aberrant endocrine regulation: aromatase,17β-hydroxysteroid dehydrogenase, sulfatase and sulfotransferase (Figure 2).

Aromatase

Aromatase has been extensively investigated in endometriosis. However, findings are still conflicting. Aromatase is encoded by the CYP19A1 gene, a member of the cytochrome P450 superfamily, which consists of monooxygenases that catalyze several reactions involved in steroidogenesis. It is physiologically expressed in growing ovarian follicles by granulosa cells, promoting the conversion of androstenedione (A4) and testosterone (T) to estrone (E1) and estradiol (E2), respectively. In the last ten years, it has been proposed that endometriotic tissue features different aromatase expression compared to normal endometrium, which is responsible for the local production of estradiol and in turn stimulates the production of the cyclooxygenase type 2 (COX-2) enzyme, resulting in elevated levels of prostaglandin E2 (PGE2), which is a potent stimulator of aromatase activity in endometriosis (14). Nevertheless, it should be taken into account that immunohistochemistry is a semi-quantitative method which does not consent the actual "quantification" and cannot determine the activity of an enzyme but only detect its presence. This positive feedback produces a local, continuous stream of estrogen and PGE2 in endometriotic tissue, supporting a loop between a hyperestrogenic environment, inflammation and cell proliferation (15, 16). *In vitro* and *in vivo* studies clearly show the expression of aromatase in stromal and epithelial cells within endometriotic lesions. *In vitro*, mechanistic models support its crucial role. However, the full picture of endometriotic aromatase in the clinical setting has not been fully clarified.

Aromatase expression in human tissue

To date, most investigations have been performed at the mRNA level or using Western blotting. There is a paucity of data utilizing tissue staining techniques, and the results are conflicting. Although mRNA expression is informative of protein expression, mRNA copies do not necessarily reflect the level of functional protein: posttranscriptional and posttranslational regulation induces functionally important changes that cannot be seen at the mRNA level (17). Therefore, we focus mainly on protein expression, which reflects gene function more directly than mRNA and is more directly related to phenotype (Table 2). Based on our research criteria, only six studies evaluated aromatase expression at the protein level using immunohistochemistry. The level of staining differs among studies in relation to the type of endometriosis, number of positive patients and correlation with clinical symptoms.

In two cohorts of mixed lesions (ovary, peritoneal and deep implants), positive immunohistochemical expression of

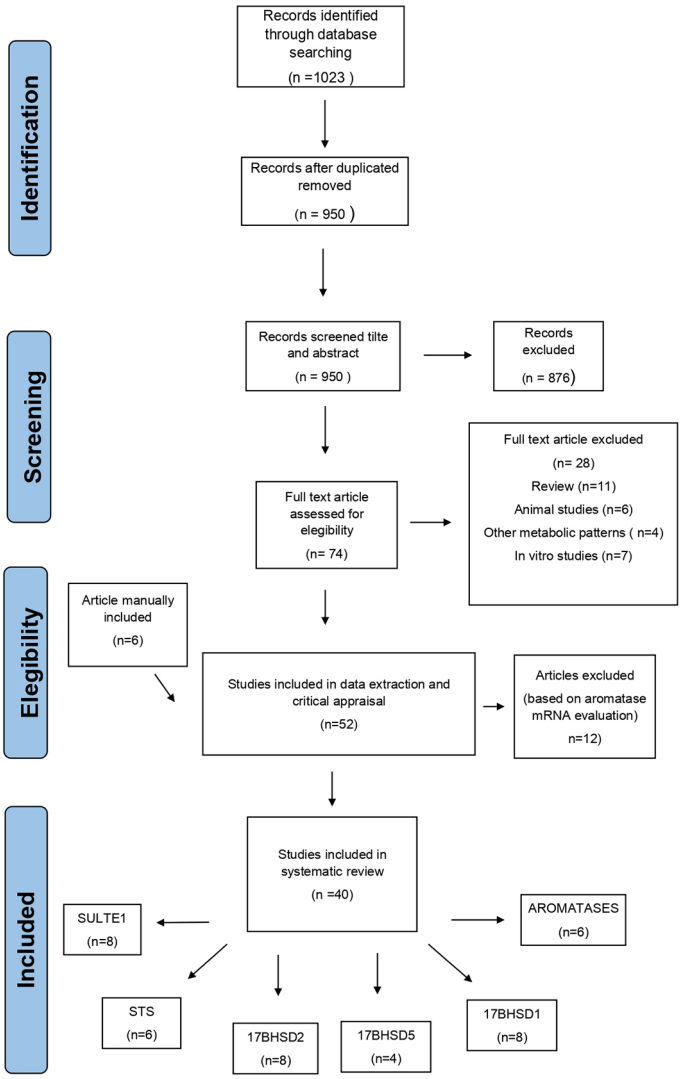


FIGURE 1
Flow diagram of the systematic search strategy on published studies reporting about the expression of the enzymes involved in the intracrinology of endometriosis.

aromatase was found in only 38/62 women (61.3% of patients with histologically confirmed endometriosis) (21) and in 32/35 patients with mild to moderate endometriosis according to the revised American Fertility Society (AFS) classification (18). These patients showed multiple endometriomas, frequently bilateral, and more moderate-to-severe chronic pelvic pain. Eutopic endometrium often stained aromatase in endometriosis patients (18, 21), being detectable by IHC in 66/92 patients but also in the endometrium of 13/14 (95%) patients with severe dysmenorrhea but free from endometriosis. Interesting in asymptomatic women (control group) aromatase expression was not detected (18). This finding suggests that functional endometrial changes leading to an increase in aromatase expression may therefore precede the development of endometriosis and have a positive correlation with dysmenorrhea severity.

In a recent study, patients experiencing moderate to severe deep dyspareunia showed higher epithelial aromatase in deep lesions involving the pouch of the Douglas than patients experiencing absence to mild dyspareunia (8). However, in 209 samples of mixed-type endometriosis (peritoneal, ovarian and rectovaginal lesions), aromatase expression was not detected in either ectopic or eutopic endometrial tissue, although there was no mention of pain symptoms (23) or even stage of disease (20).

Unfortunately, conflicting data and methodological concerns affect the interpretation of the role of this enzyme in endometriotic implants, and consistent proof of aromatase expression is still lacking. Some of the variations in the

10.3389/fendo.2022.950866

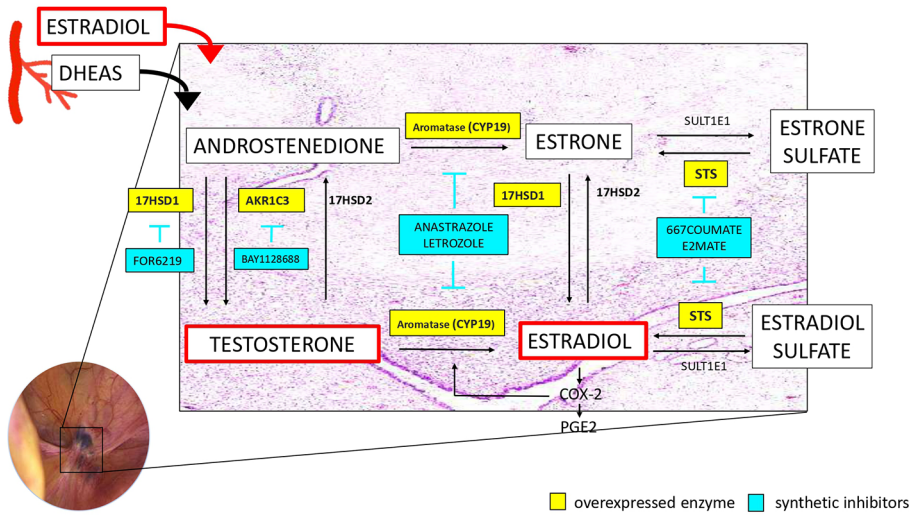


FIGURE 2
Schematic representation of the intracrine estrogen metabolism with the additional data on the overexpressed enzymes and their synthetic inhibitors.

reported patterns of expression may relate to the use of different antibodies, analysis of eutopic endometrium at different stages of the cycle, the type of lesion or the inflammatory status. Moreover, fragments of ovarian tissue may be included in the histological sections of specimens of ovarian endometriosis, affecting the accuracy of the evaluation.

Aromatase inhibitors (AIs)

Despite the paucity of human data, aromatase blockade has been proposed as a treatment for endometriosis since its first successful use in a postmenopausal woman with aggressive and refractory endometriosis (24). Thereafter, different studies, published over the last twenty years, have evaluated the efficacy of AI treatment alone or combined with other hormonal therapies. Selective third-generation AIs, letrozole and anastrozole, have been found to have better efficacy and tolerability than earlier agents (25). Seven clinical trials have compared the use of these drugs with other treatments for endometriosis (Table 3). Aromatase inhibitors, whenever given in combination with progestins, GnRHa or administered alone, showed greater results in terms of pain reduction than conventional hormonal treatments. Anastrozole (1 mg/day), administered in conjunction with goserelin for 6 months in patients with severe baseline endometriosis (rASRM score >40),

TABLE 1 Estrogen concentrations in women with and without endometriosis.

Sample Type	Hormonal status	E2 (pg/ml)		E2/E1 ratio	
		Median	25/75%	Median	25/75%
Healthy endometrium	Proliferative	532	334.7/736.0	8.34	7.33/9.27
	Secretory	66	52.5/100.8 ##	0.69	0.50/1.40###
Endometrium (Patients affected by endometriosis)	Proliferative	649.3	404.0/1168.7	8.64	4.72/13.11
	Secretory	68.5	25.0/157.3##	0.80	0.69/0.99###
Superficial endometriosis	Proliferative	238.0	78.9/397.0†	5.50	0.82/7.21
	Secretory	176.0	49.4/355.0	3.95	1.36/9.47
Ovarian endometriosis	Proliferative	3430.0	1809.7/21600.0*	4.35	1.69/5.53
	Secretory	305.0	199.0/758.0##	1.34	1.04/3.28#
Deep infiltrating endometriosis	Proliferative	112.0	53.9/162.0	1.06	0.60/2.60 *
	Secretory	25.0	25.0/117.3	2.00	1.44/4.77
Peritoneal fluid (22)	Proliferative	200			
	Secretory	800			

*P < 0.05 vs. eutopic endometrium within cycle phase. †P < 0.05 vs. proliferative phase. #, ##, ###, P < 0.05, < 0.01, and < 0.001, respectively, vs. serum within the cycle phase. From Huhtinen K et al. Endometrial and endometriotic concentrations of estrone and estradiol are determined by local metabolism rather than circulating levels. J Clin Endocrinol Metab. 2012 Nov;97 (11):4228-35.

TABLE 2 Expression (protein levels), enzymatic activity of aromatase in endometriotic lesions and correlation with disease characteristics, eutopic and healty endometrium.

Authors	Patients with endometriosis (n)	Method (Antibody)	Expressionor activity	Lesion localization	Severity of symptoms	Stage of disease	Eutopic endometrial tissue	Control
Hudelist et al. 2007 (18)	35	IHC (Harada)	32/35	* Mixed. * No Correlation	No correlation	* Moderate and severe (ASRM) * No correlation	Positive	Negative
Acien et al. 2007 (19)	62	IHC (DPC Biermann)	38/62	* Mixed. * Positive correlation with ovarian lesions (especially bilateral)	Positive correlation with moderate to severe chronic pelvic pain	* Moderate and severe (ASRM) * No correlation	Negative	Negative
Colette et al. 2009 (20)	127	IHC (Acris. Serotec)	No expression	* Mixed	–	–	Negative	Negative
Maia et al. 2012 (21)	92	IHC (Serotec)	66/92	* Endometrial biopsy	Positive correlation with dysmenorrhea (even in patients free from endometriosis)	* ASRM I to IV * Positive correlation with stage of disease	Positive	Negative
Pluchino et al 2020 (6)	83	IRS (Harada)	–	* Deep endometriosis involving the pouch of the Douglas	Positive correlation with moderate to severe deep dyspareunia	* AFS III–IV	–	–

AFS, American Fertility Society; ASRM, American Society for Reproductive Medicine; HPLC high performance liquid chromatography; IHC, immunohistochemistry; IRS, immunoreactive score.

showed greater improvement in pain Total Pelvic Symptom Score (TPSS) than patients using goserelin alone (ΔTPSS-P baseline -24 months PMT 5.0 vs. 3.3 p<0.0001) (26). The efficacy of AIs combined with norethisterone acetate (NETA) (letrozole 2.5 mg/day plus norethisterone acetate 2.5 mg/day) versus NETA alone (2.5 mg/day) has been specifically evaluated in rectovaginal endometriosis (27). Significantly lower dyspareunia and chronic pelvic pain were reported in the group receiving the double drug regimen after 3 months. Letrozole was also investigated as single molecule in a randomized clinical trial based demonstrating the lowest mean chronic pelvic pain at 5 months compared to danazol (600 mg/day) and placebo. No data, however, are available regarding the stage, type, and localization of disease (28). Four additional studies investigating more than 200 patients showed comparable results in terms of pain reduction between AIs vs. GnRHa or AI plus NETA vs. NETA alone or AI plus GnRH (29–32), making less clear the clinical added value of AI over conventional, first-line, treatments. The onset of recurrence of symptoms during follow-up was always comparable among all studies. Adding AI to current hormonal treatment does not offer advantages over the single drug, as control group. Only one study, showed a longer interval before recurrence of symptoms during the 24-month follow-up: recurrence was registered in only 3/40 (8%) of patients in the combined arm (AI + goserelin) compared to 14/40 (35%) in the goserelin-only arm (26). Regarding side effects, AIs in association with progestins did not seem to have a more detrimental impact than progestin alone in two trials (30, 32). However, in Ferrero et al., a high incidence of adverse events in patients taking letrozole plus norethisterone acetate was registered (two patients interrupted the treatment because of severe joint pain, others had severe migraine attacks, myalgia and breakthrough bleeding) (27). As a consequence, "global degree satisfaction" was not different among groups despite a better pain reduction in patients receiving AI plus progestins. When AI was associated with GnRHa, a higher rate of side effects was found, and Ferrero et al. were forced to end the study preterm due to the high incidence of adverse effects (arthralgia, decreased libido, hot flashes) (30). This supports the hypothesis that cotreatment with progestins is more accepted than cotreatment with gonadotropin-releasing hormone analogs. In terms of bone mineral density, no significant change were reported when AIs were combined with NETA (27). In contrast, when associated with GnRH, AIs resulted in a significant decrease in the mineral bone density at 6 months (26, 30); however, none of these patients fell into the category of osteopenia and, furthermore, this reduction was not confirmed at 2 years of treatment withdrawal (26).

Three trials evaluated whether AIs were superior to other hormonal treatments for the regression of endometriosis lesions (30–32). The combination of letrozole plus NETA showed a

TABLE 3 Clinical trials comparing AIs (alone or combined) with other treatments in women with endometriosis.

Authors	Type of study	Treatments	Length of treatment/ Follow Up (months)	Stageof disease	Type of lesion	Recurrence	Evaluation of pain	Adverseeffects	Lesion reduction (US)	Bone loss (BMD)
Soysal et al. 2004 (26)	Patients: 40 vs 40 Double arm blind RND.	Anastrazole 1mg/day + Goserelin 3.6mg/4w vs Placebo + Goserelin 3.6mg/4w	6/24	Severe endometriosis (ASRM score >40)	–	Later and less recurrence rate of symptoms	Greater reduction for the whole follow up (TPSS)	No difference at 24 weeks "menopausal quality of life score"	–	Greater bone loss (24 weeks) No difference (24 months)
Ferrero et al. 2009 (27)	Patients: 41 vs 41 Double arm OL non RND	Letrozole 2.5mg/d + NETA 2.5mg/d Vs NETA	6/12	–	Rectovaginal nodules	Comparable recurrence	Greater reduction for chronic pelvic pain and dyspareunia (VAS)	More frequent and no difference in global treatment satisfaction	–	No difference
Roghaei et al. 2010 (28)	Patients: 38 vs 37vs 31 Triple arm OL RND	Letrozole 2.5mg Vs Danazol 600mg Vs Placebo	6/6	–	–	–	Greater reduction of chronic pelvic pain and dyspareunia at 5 months (VAS)	Less frequent and severe	–	–
Alborzi et al. 2011 (29)	Patients: 47vs 40vs 57 Triple arm OL RND	Letrozole 2.5mg Vs Triptorelin 3.75mg/4w Vs No medication	2/12	AFS I - IV	–	Comparable recurrence	Comparable reduction (VAS)	More functional cysts formation	–	–
Ferrero et al. 2011 (30)	Patients 17 vs18 Double arm OL RND	Letrozole 2.5mg/d + NETA 2.5mg Vs Letrozole 2.5mg/d + Triptorelin 11.25mg/3m	6/6	–	Rectovaginal nodules	–	Comparable reduction (VAS)	More adverse effect and more patients left the study in Letrozole + triptorelin group	Greater reduction In Letrozole + Triptorelin group	Greater bone loss in Letrozolo+ Triptorelin group
Ferrero et al 2013 (31)	Patients 18 vs10 vs 8 vs 26 vs 30	NETA 2.5mg/d Vs	12/12	–	Rectovaginal nodules	–	–	Comparable	Significant and comparable reduction for all treatments	–

(Continued)

10.3389/fendo.2022.950866

TABLE 3 Continued

Authors	Type of study	Treatments	Length of treatment/ Follow Up (months)	Stageof disease	Type of lesion	Recurrence	Evaluation of pain	Adverseeffects	Lesion reduction (US)	Bone loss (BMD)
	Multiple arm OL not RND	Triptorelin 11.25/3m + tibolone 2.5mg/d Vs Letrozole 2.5mg/d + NETA 2.5mg/d Vs desogestrel 0.075mg/d Vs EE0.02mg + Desogestrel 0.15 mg								
Ferrero et al 2014 (32)	Patients: 20vs20 Double arm OL non RND	Letrozole 2.5mg/d + NETA 2.5mg/d Vs NETA 2.5mg/d	6/12	–	Ovarian endometrioma	Comparable recurrence	Comparable reduction (VAS)	Comparable with no difference in global treatment satisfaction	Significant and comparable reduction (6 months) return to baseline dimensions (6months after treatment)	–

AFS,American Fertility Society; ASRM, American Society for Reproductive Medicine; TPSS, Total pelvic symptom score;VAS, visual analogue scale; NETA, norethisterone acetate; RND randomized; OL, open label; US, ultrasound; BMD, bone mass density.

10.3389/fendo.2022.950866

greater reduction in endometrioma at 6 months of treatment than the administration of Neta alone (-74.4± 4.2% vs. -46.8± 3.8%). Regrettably, no patients reported a complete regression of cysts, and endometriomas usually regrew after treatment discontinuation (32). Two studies confirm the reduction of the size of rectovaginal lesions (30, 31). In a multiple arms trial, letrozole 2.5 mg/d + NETA 2.5 mg/d was compared with other first-line regimens and no difference of volume reduction was recorded (31). When letrozole is administered in combination with triptorelin demonstrated a significant reduction in the volume of the endometriotic nodules after 6 months (16.1 cm3 ± 10.0% vs. 10.2 cm3 ± 6.3%; p = 0.048) in comparison to NETA alone (30).

The heterogeneity of the current literature limits the evaluation of the potential advantages provided by the use of AIs for the treatment of endometriosis as well as the assessment of side effects: severity and type of the disease are often underreported, and follow-up of pain symptoms is not long enough to make consistent conclusions regarding their implementation in clinical practice. Based on current recommendations, AIs should be now offered only in case of surgical and other hormonal therapies failure (33). To minimize the impact on systemic estrogen levels, administration of an aromatase inhibitor (AI) *via* an intravaginal ring (IVR) has been proposed. This approach offers the advantage of providing sustained and controlled drug release and requires a lower dose to achieve equivalent pharmacodynamic efficacy compared to oral administration (it avoids the first-pass effect of the liver) (34). Phase I and Phase II studies on an IVR containing a combination of anastrazole and levonorgestrel gave reassuring results in terms of safety and tolerability at different doses as well as contraceptive efficacy (35, 36). However, the clinical effect of this combination is still unknown. A pilot study demonstrated modest efficacy following administration of vaginal anastrozole (0.25 mg/d) for 6 months offered to 10 women suffering from rectovaginal endometriosis: although a small, statistically significant improvement in dysmenorrhea was observed, chronic pelvic pain, dyspareunia and rectovaginal lesion size remained unchanged. The inefficacy of the therapy could be attributed to inadequate dose exposure given the absence of a reduction in circulating E2 (37). Endometriosis-targeted inhibition of local aromatase, despite its promising potential, has recently been discredited, using a new chick embryo allantoic membrane (CAM) model incorporating xenografted human endometriosis cysts: in this recent study was shown that topical treatment with anastrozole reduced the size of the lesions, corroborating the presence of aromatase activity in endometriotic tissue. However, when systemic estrogens reached the grafted endometriotic tissue, the effect of the local inhibition of aromatase by anastrozole was blunted. This finding supports the speculation that endometriosis aromatase cannot be a drug target without the inhibition of systemic estrogen synthesis (38).

17β-Hydroxysteroid dehydrogenase (HSD17B)

17β-Hydroxysteroid dehydrogenase (HSD17B) isozymes are a group of alcohol oxidoreductases, of which several isoforms are expressed in human tissues. Of particular interest is the activity of HSD17B1, which is responsible for the production of testosterone (T) and 17 beta estradiol (E2) from weak androstenedione (A4) and estrone (E1), respectively, and HSD17B2, which, in contrast, catalyzes the opposite reaction, metabolizing E2 to the less active E1. These pathways are believed to be involved in the abnormal ratio E2/E1 in the ectopic endometrium. Interesting data on the activity of these enzymes in endometriosis comes from Delvoux et al: in this study, endometriotic tissues of 14 women affected by moderate to severe endometriosis (endometrioma, deep infiltrative and superficial peritoneal endometriosis) showed a marked increase in HSD17B1 activity, leading to higher estradiol (E2) production than normal endometrial tissue (23). Conversely, the activity of the enzymes responsible for the oxidation of 17-estradiol into the less active estrone was significantly lower. Therefore, the net balance between oxidization and reduction favored the production of 17-beta E2 in ectopic tissues compared with endometrial tissues of healthy patients.

HSD17B1 expression in human tissue

Despite this premise, discordant results arise from studies evaluating HSD17B1 expression (Table 4). At the protein level, there was no evidence of HSD17B1 hyperexpression in endometriosis tissue (41, 45). In 14 samples of rectovaginal endometriosis, HSD17B1 protein levels were found to be even lower than those in normal endometrium. This result diverges from HSD17B1 mRNA level, which was higher than in controls, raising the hypothesis that the expression of the HSD17B1 gene may therefore not necessarily reflect changes at the functional protein level (41). Delvoux et al. and found a greater than 6000-fold increase in HSD17B1 mRNA expression in endometriosis (Stade IV rASRM, mixed lesions) compared to eutopic tissue (46). In addition, inhibition of HSD17B1 by a specific inhibitor (3-[15b-estronyl]-N-(5-methyl-thiazol-2-yl)-propionamide) was achieved, decreasing the production of 17-estradiol by at least 85% in 70% of patient biopsies tested ex-vivo. Three additional studies (a total of 101 patients were evaluated), upregulation of the HSD17B1 enzyme was confirmed only in ovarian endometriosis (42–44). Interestingly, data from two studies (39, 45) are in contrast with the abovementioned results and no difference of HSD17B1mRNA between endometriosis (each type) and controls (eutopic endometrium in patients without endometriosis) were detected.

TABLE 4 17BHSD type 1, type 2 mRNA and protein expression in endometriotic tissue.

Authors	Patient with endometriosis	Method	Lesion localization	Stage of disease	17 BHSD type 1 expression (compared to healthy endometrial tissue)		17 BHSD type 2 expression (compared to healthy endometrial tissue)	
					mRNA	Protein (Ab)	mRNA	Protein (Ab)
Zeitoun et al.1998 (39)	14	Northern Blot IHC	Extraovarian endometriotic implants	–	≈	-	↓	↓ (C2-12)
Matsuzaki et al. 2006 (40)	16	Q-PCR	Rectovaginal	–	-	-	–	–
Dassen et al.2007 (41)	14	Q-PCR IHC	Rectovaginal	–	↑	↓ (Pineda)	–	–
Smuc et al. 2007 (42)	16	Q-PCR	Endometrioma	Moderate and severe (ASRM)	↑	-	≈	-
Smuc et al. 2009 (43)	24	Q-PCR	Endometrioma	–	↑	-	≈	-
Huhtinen et al. 2012 (44)	60	Q-PCR	Peritoneal Ovarian Deep endometriosis	–	↑ in endometrioma	-	↓	-
Colette et al. 2013 (45)	79	Q-PCR IHC	Peritoneal, ovarian, rectovaginal	–	≈	≈ (Novocastra)	↓ in endometrioma. (Protein and rectovaginal Tech group) lesion	≈
Delvoux et al. 2014 (46)	29	Q-PCR HPLC	Peritoneal, ovarian, rectovaginal	Moderate and severe (ASRM)	↑ Inhibitor tested lead to decreased production of 17 beta estradiol	-	≈	-

Ab, Antibodies: ASRM, American Society for Reproductive Medicine; HPLC high performance liquid chromatography; IHC, immunohistochemistry; Q-PCR, Quantitative-Polymerase chain reaction, ↑/↓ statistically significant results: ≈, no difference.

HSD17B2 expression on human tissue

The HSD17B2 enzyme is involved in the oxidative reaction and is responsible for the metabolization of potent E2 to the less active E1. The evaluation of HSD17B2 at the protein level is scanty and based only on two studies with conflicting results (39, 42–46), showing lower expression (39) or no changes (45) in endometriotic tissues (mixed lesions). Concerning mRNA expression, HSD17B2 appeared to be reduced in all types of endometriotic lesions compared to controls (39, 44, 46). Deep endometriosis shows undetectable levels of HSD17B2 mRNA type 2 in 50% of patients (8/16), and low levels were found in the remaining patients (40). In the study of Colette et al., where the protein level did not differ between samples, lower mRNA expression in rectovaginal and ovarian lesions but not in superficial peritoneal lesions was observed (45). Surprisingly, in endometrioma tissues evaluated by Smuc et al. (42, 43), no statistically significant difference was found with respect to healthy patients.

The heterogeneity, in terms of the method used to evaluate mRNA and protein expression, of the abovementioned studies prevent a proper comparison. Moreover, from a clinical perspective, given the lack of data regarding the stage of disease (only Delvoux et al. specified the characteristics of their patients) and the correlation with the severity of symptoms, it is difficult to estimate which patient can really benefit from future targeted enzyme therapy.

HSD17B5 expression on human tissue

Worth of mention for its ability to influence multiple signaling pathways in endometriosis is 17β-hydroxysteroid dehydrogenase type 5 (17BHSD5) also known as AKR1C3. This steroidogenic enzyme can function as a PGF2α synthases, increasing the concentration of prostaglandins in peritoneal fluid (47), and catalyze the reduction of progesterone to the less active 20α-hydroxyprogesterone, leading to a defective

progesterone action and contributing to the progesterone-resistant state (48). Concerning its roles in androgen and estrogen biosynthesis it has a very high catalytic efficiency for the conversion of androstenedione to testosterone which may finally act as a substrate for aromatase, having thus an indirect role in estradiol formation (49). In term of protein levels using IHC, two studies reported, the presence of AKR1C3 in endometriomas (43) and peritoneal endometriotic lesions (50). However, when scoring of AKR1C3 staining was performed, no significant differences in endometriosis lesions (ovarian endometriomas) compared to the endometrium of control patients were revealed (47, 51). Data provided on mRNA levels in endometriotic tissue are even more discordant. In a study evaluating 24 samples of ovarian endometrioma mRNA reported a higher expression respect to controls (43), whereas in a study reporting the analysis of 31 ovarian endometrioma only a slight difference was observed (51). Furthermore no data on pain symptoms or endometriosis stage are reported, making difficult further comparison. Interesting results from a study where peritoneal endometriosis samples were classified according to menstrual cycle phase (50): increased expression of AKR1C3 was observed in women with disease stages I–II and during the proliferative phase of the menstrual cycle. However, when all type of endo are analyzed together, only minor differences of mRNA expression (44) were detected. As consequence, further evidence to confirm the clinical relevance of AKR1C3 as a target in endometriosis are then needed.

HSD17B inhibitors

Some inhibitors of HSD17B1 were developed in the past to target the biosynthesis of bioactive E2 in breast cancer (52). However, only a few compounds have been applied *in vivo* (53). Differences between enzymes in humans and other species are one of the main reasons that preclinical *in vivo* evaluation has been hindered.

A novel HSD17B1 inhibitor, FOR-6219, recently successfully completed a Phase 1a study in which the safety, tolerability, and pharmacokinetics of single and multiple ascending doses in 36 healthy postmenopausal women were investigated (NCT03709420). In Phase 1b, 36 premenopausal healthy women were investigated to expand the safety data and explore secondary outcome measures; interestingly, these women continued to experience normal ovulatory menstrual cycles (Report No.: NCT03709420. Available from: https://clinicaltrials.gov/ct2/show/NCT03709420).

Forendo Pharma is now planning a Phase 2 program including endometriosis patients in the US (available from: https://forendo.com/forendo-pharma-successfully-completes-phase-1-studies-of-for-6219-in-endometriosis-aiming-to-advance-program-into-phase-2-clinical-studies/). A steroidal inhibitor of AKR1C3, BAY1128688, was tested in a phase I clinical trial (NCT02434640) to investigate its safety, tolerability and pharmacokinetics in healthy women, and it appeared to be well tolerated up to a high dose of 60 mg twice per day. In a phase II clinical trial (NCT03373422) designed to evaluate the reduction of pain and the incidence of adverse events, it was planned to treat symptomatic women with endometriosis over a 12-week treatment period. Unfortunately, the trial was stopped in advance due to hepatotoxicity. A recent review, however, concluded that hepatotoxic effects can be compound-related, and AKR1C3 should not be precluded as a potential target (48). The development of other drugs targeting this enzyme is ongoing.

Sulfatase and sulfotransferase

Sulfatase (STS) is an enzyme involved in another critical alternative pathway that contributes to the increased bioavailability of regionally active estrogens. Hydrolysis transforms dehydroepiandrosterone sulfate (DHEA-S), estrone sulfate (E1S), the most abundant circulating estrogen metabolite, and estradiol sulfate (E2S) into their bioactive metabolites (DHEA, E1 and E2, respectively). Despite its potential pivotal roles in local estrogen formation, data on metabolic activity of this enzyme are not very conclusive. An analysis of 27 peritoneal endometriosis implants showed lower overall STS activity in ectopic endometrium than in eutopic endometrium (54). The authors attributed this to the relatively lower enzyme activity levels in endometriotic lesions from patients with minimal to mild disease, and indeed, with further analysis, they observed that STS activity in endometriosis implants correlates with the severity of this disease, and a significantly higher activity of STS was found in patients with moderate to severe disease with respect to controls, indicating that women with severe endometriosis may be particularly amenable to STS inhibitor therapy. However, Delvoux et al. did not find a difference in terms of STS enzyme activity between ectopic and eutopic endometrial tissue despite the analysis being provided in women affected by moderate to severe endometriosis from all three types of endometriosis (23).

Sulfatase expression on human tissue

To date, evidence on the expression of *STS* in endometriosis has remained relatively contradictory (Table 5). Only two studies evaluated STS at the protein level (41, 45), reporting no difference between cases and controls. STS mRNA expression was greater in superficial, ovarian and deep-infiltrating lesions (no significant differences were found between these two types of lesions) of endometriosis samples than in eutopic endometrium of subjects without endometriosis (43, 45, 56). Conflicting findings have been published by from Dassen et al., that, evaluated 14 women with rectovaginal endometriosis and did not observe any differences between STS mRNA levels of endometriosis and healthy tissues (P, 0.05) (41).

Sulfotransferase expression in human tissue

Estrone sulfotransferase (SULT1E1), in contrast to STS, antagonizes the action of STS by sulfating estrone into esterone sulfate, thus converting estrogens into less active metabolites. However, the expression of this enzyme in endometriosis lesions demonstrated a contradictory pattern. The evaluation of patients with mild and moderate endometriosis shows no significant differences between the expression levels of SULT1E1 protein in uterine and ectopic samples in comparison to the endometrium of healthy women (18). In endometrioma samples, Hevir et al. found that SULT1E1 mRNA levels were significantly decreased compared to controls (55), whereas Smuc et al. registered no significant difference in its expression (42). Colette et al. showed that in rectovaginal endometriosis, although no difference was encountered in SULT1E1 mRNA expression, there was a high ratio between STS and SULT1E1, giving rise to the view that in endometriosis lesions, the sulfatase pathway is overactive (45). Interestingly, two studies reported a higher expression of SULT1E1 in endometriotic lesions than in normal endometrium: rectovaginal endometriosis lesions (41) and superficial peritoneal lesions (56) showed higher expression of EST compared to the control and had a positive correlation with STS expression. If STS abounds over SULT1E1, the increased net production of estradiol in endometriosis is the directed consequence; in this way, sufficient sulfated estrogens can be continuously hydrolyzed (desulfated) and sulfated *in situ*, maintaining a highly local estrogenic milieu.

Sulfatase inhibitors

Based on the aforementioned data, STS can be considered an attractive molecular target with potential therapeutic value in endometriosis, and targeting this enzyme may benefit patients with resistance to other hormonal treatments. Purohit et al. tested a recent irreversible STS inhibitor, 667COUMATE (also called Irosustat), which was already assessed in postmenopausal women with metastatic breast cancer. It proved to be very effective at inhibiting STS activity in endometriotic cell lysates, reducing enzyme activity by 99% in both eutopic and ectopic endometrial tissue samples (54). Another inhibitor, estradiol-3-O-sufamate (E2MATE), also encoding PGL2001, was proven to effectively inhibit STS activity when tested *in vitro* on endometrial fragments of ten patients affected by benign pathologic conditions other than endometriosis (STS activity inhibition after 24 h of culture: 66.5 + 10.3%, P, 0.001). Endometriosis was then induced in mice to evaluate the inhibition *in vivo* of this enzyme. After twenty-one days of therapy, lesion sizes were found to be significantly decreased (control mice: 44.5 ±30.2 mm2; 1 mg/kg-treated mice: 26.3±20.1 mm2; 0.5 mg/kg treated mice: 22.8±15.3 mm2) (57). As an additional benefit, progesterone (PR) expression in endometriotic lesions was found to be increased, and the absence of an effect on circulating estradiol levels opens up new perspectives in endometriosis treatment. E2MATE was then evaluated in a phase I double-blind study (58).Given that the majority of estrogens are produced in the ovaries, the authors focused their evaluation on a combination STS-I plus progestin in order to reduce both the local and ovarian estrogen production. Twenty-four healthy volunteer women were randomized to E2MATE (4 mg/week), NETA or the combination E2MATE

TABLE 5 STS and SULT1E1 mRNA, protein expression in endometriotic tissue.

	Patient with endometriosis	Patient characteristics	Method	STS expression (compared to healthy endometrial tissue)		SULT1E1 expression (compared to healthy endometrial tissue)	
				mRNA	Protein (Ab)	mRNA	Protein (Ab)
Hudelist et al 2007 (18)	35	Mixed lesions. Mild to moderate endometriosis	QPCR IHC	-	-	≈	≈ (NeoMarkers)
Dassen et al 2007 (41)	14	Rectovaginal endometriosis	QPCR IHC	≈	≈ (Pineda)	↑	-
Smuc et al 2007 (42)	16	Endometrioma (stage III, IV)	QPCR	↑	-	≈	-
Colette et al 2013 (45)	79	Mixed lesions	Q-PCR IHC	↑ Rectovaginal lesion	≈ (Atlas)	≈	-
Hevir et al 2013 (55)	31	Ovarian endometriomas	QPCR	-	-	↓	-
Piccinato et al 2016 (56)	62	Peritoneal Deep lesions	Q-PCR	↑	-	↑	-

Ab, Antibodies: ASRM, American Society for Reproductive Medicine; HPLC high performance liquid chromatography; IHC, immunohistochemistry; Q-PCR, Quantitative-Polymerase chain reaction; STS, steroid sulfatase, ↑/↓ statistically significant results: ≈ , no difference.

+NETA. Treatment lasted 4 weeks with a 12-week follow-up. E2MATE associated with NETA showed a synergistic effect: the mean percentage of STS inhibition in the endometrium was 91% and 96% in the PGL2001 and PGL2001 plus NETA groups, respectively, compared to 42% in the NETA group, and due to its potent irreversible binding and long half-life one month after stopping treatment, the percentage inhibition remained high at 88% and 93% in the PGL2001 and PGL2001 plus NETA groups, respectively, with no inhibition seen in the NETA group. Treatment was well tolerated, with no relevant differences between the treatment regimens in terms of adverse events, and no impact on circulating estradiol levels was registered compared to the NETA groups. E2MATE and NETA have been further studied in endometriosis patients in a phase II study (NCT01631981, available at: https://clinicaltrials.gov/ct2/show/NCT01631981), although at present, no results are publicly available. An interesting recent development is the establishment of multiple designed ligands that effectively inhibit both STS and HSD17B1 (59).Such dual inhibitors can further decrease intracellular E2 levels more efficiently than selective inhibitors of HSD17B1 and may therefore be a superior therapeutic strategy for endometriosis.

Effect of current hormonal treatments on intracrinology regulation

Current first-line hormonal treatments in endometriosis (oral contraceptives and progestins) were originally developed using the normal endometrium as the main experimental tissue to investigate their reproductive effects and were adopted only afterwards for the treatment of endometriosis-associated pain. There are several patients for whom current first-line hormonal treatments for endometriosis do not provide enough or a sustained solution to pain. A recent review demonstrated that the median proportion of women with no decrease in pain was 11% to 19%; when the therapy ended, 5% to 59% had persistent pain; and in the follow-up, 17% to 34% felt recurrence of pain symptoms (60). The recent evidence that women, despite the hypogonatrophic effect obtained from combined oral contraceptives (COCs), have increased hormone levels in endometriosis implants compared to controls (61) highlights the crucial role of intracrinology as a mechanism of endometriosis development and drug resistance. There are only a few available research studies that have investigated the influence of the commonly used therapy against the enzymes involved in the intracrinology of the endometriosis. Few are based on progestins, and no research studies have investigated the effects of COC. Most studies, in addition, have been conducted *in vitro* using immortalized cells, limiting a realistic interpretation of the results. Dienogest (DNG), a synthetic progestin largely employed for therapy, has received the greatest attention. DNG has been shown to repress aromatase expression in human immortalized endometrial epithelial cells and primary cultured endometriotic stromal cells (SCs) (62, 63). Moreover, DNG has been shown to inhibit HSD17B1 expression and enzymes in cultured ovarian endometrioma cells, whereas no effect has been demonstrated on HSD17B2, HSD17B7, HSD17B12, steroid sulfatase (STS), and estrogen sulfotransferase (EST) activity (64). There are few details with respect to GnRH agonist and antagonist effects on endometrial intracrinology. While GnRH agonists have been shown to decrease serum E2 levels by approximately 97%, intracrinological changes in endometriosis lesions are not known. Even if GnRH agonists are responsible for decreasing tissue inflammation and angiogenesis and increasing apoptosis in endometriosis (65), 14% (0-20%) of patients did not show improvement of symptoms, and nearly one-third of patients who received GnRH analog treatment postsurgery experienced pain symptoms when medical treatment ended (60). Interestingly, one-year therapy with a GnRH analog decreases the adrenal DHEA-S combination by only 16%, leaving open the possibility of its metabolism in peripheral tissue and eventually inducing resistance to treatments (66). GnRH agonists have been shown to reduce aromatase cytochrome P450 expression in at least eutopic endometrium from patients with endometriosis (67) and hinder E1 sulfatase expression in endometrioma (68). Recently, GnRH antagonists have been augmented in the armamentarium of gynecologists to cure endometriosis and resolve the side effects of GnRH agonists based on the "estrogen threshold hypothesis," where estrogen may be regulated to a level that is enough to reduce pain without causing clinical hypoestrogenic effects. Elagolix did not fully repress ovulation at doses of 150 and 200 mg/day, 56% of women had proliferative endometrium after 6 months of therapy at a dose of 150 mg, and 61% had normal dormant or least stimulated endometrium at a dose of 200 mg. Even if the majority of patients were satisfied with this therapy, as many as 40% of patients indicated unsatisfactory improvement of pain symptoms (69). On the basis of the complexity of the intracrinology of endometriosis and the fact that GnRH antagonists have no direct effect on the endometrium, we can then hypothesize that the intracrine features of endometriosis may represent a mechanism creating an incomplete response to symptoms of pain in 30-35% of patients

Discussion

The study of intracrinology in endometriosis highlights important limitations in the current knowledge of the disease, from its developmental and initial phases to the macroscopic appearance of advanced deep nodules. It is clear that the current macroscopic classification is insufficient to properly characterize heterogeneous lesions. A closer look at endocrine aspects of lesions may shed new light on disease features, enabling precision medicine in endometriosis care. However, current

methodological limitations (i.e., number of patients enrolled in the studies) or appropriateness of investigations (i.e., contamination of endometrioma cysts with ovarian cortex) has limited the implementation of endocrine phenotyping in daily practice. Furthermore, the accurate evaluation of the expression of these enzymes is a complex task; for instance, concerning aromatase protein expression, some authors argued that what was believed to be aromatase protein in a previous study was mainly endogenous biotic labeling or iron deposits (20). Even if various enzymatic pathways are aberrantly regulated in endometriosis, the recognition of which enzymatic pathways are more critical for promoting the hyperestrogenic environment is less clear. This is important for the development of new drugs aimed at decreasing local estrogenic activity with a minimal effect on gonadal activity.

Strengths and limitations

The strength of this review lies in the evaluation of intracrinology of endometriosis from a clinical prospective. The main limitation was that heterogeneity of the studies included and a lack a particular model to investigate the role of local modulation of these enzymatic pathways.

Conclusion and new perspectives

Unbalanced intracrinology is a critical feature of endometriosis implants and a complex mechanism that supports local hyperestrogenism partially independent from gonadal function. This has far-reaching implications in clinical practice, since all available therapies induce a reduction in gonadal activity as main mechanism of action. Recently, the development of harmonization initiatives, such as EPHect, Endometriosis Phenome and Biobanking Harmonisation Project, has represented a new systematic approach to stratify predefined outcomes in endometriosis research with family history, symptoms, clinical examination, dynamic imaging/pain reporting, surgical staging, and systemic or tissue biomarkers. From a clinical perspective, current knowledge of intracrinology in endometriosis in the actual classification of the disease has identified that endometriosis lesions on the ovary are likely the most endocrine active and responsive to steroids. As a result, they are characterized by a higher incidence of recurrence following surgical excision. In addition, enzymatic pathways expressed in endometriosis are likely consequences of epigenetic changes and inflammation signals. Again, a closer look at intracrinology could facilitate lesion phenotyping and estimate the aggressiveness of the disease. However, current methodological limitations and heterogeneity in the evaluation of mRNA and protein expression make it hard to draw definitive conclusions. In certain cases, contradictory results can be explained by the close proximity of healthy tissue to the endometriotic lesion, influencing the results obtained by the whole tissue specimens and highlighting the need for a careful histopathological characterization of the specimens studied (laser capture microdissection may therefore be envisaged to fully isolate endometriotic glands). Moreover, a large number of studies miss correlations with the severity of symptoms, stage and localization of disease, making it difficult to estimate which patient can benefit the best from future targeted enzyme therapy. Although AIs are not realistically useful in clinical practice, intracrinology offers interesting new drug targets that can incorporate many of the above ambitious features. Some molecules are already in the pipeline of the pharma industry in the next 10 years. In conclusion, intracrinology of endometriosis is relevant in clinical practice as a major main endometriosis developmental feature, a basis for phonotypical characterization, a potential mechanism of drug resistance and a source of new therapeutic targets.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

AM and NP undertook the searches, data extraction and drafted the manuscript. NP, AM, PG, PD, and AR participated in data analysis and interpretation, preparation of. the manuscript and critically revising the paper. NP and AR conceived the idea of the manuscript. All authors approved the final version of the manuscript.

Funding

Open access funding was provided by the University of Geneva.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Zhang Y, Ma NY. Environmental risk factors for endometriosis: An umbrella review of a meta-analysis of 354 observational studies with over 5 million populations. *Front Med (Lausanne).* (2021) 8:680833. doi: 10.3389/fmed.2021.680833

2. Zondervan KT, Becker CM, Koga K, Missmer SA, Taylor RN, Vigano P. Endometriosis. *Nat Rev Dis Primers.* (2018) 4(1):9. doi: 10.1038/s41572-018-0008-5

3. Vercellini P, Vigano P, Somigliana E, Fedele L. Endometriosis: pathogenesis and treatment. *Nat Rev Endocrinol* (2014) 10(5):261–75. doi: 10.1038/nrendo.2013.255

4. Chantalat E, Valera MC, Vaysse C, Noirrit E, Rusidze M, Weyl A, et al. Estrogen receptors and endometriosis. *Int J Mol Sci* (2020) 21(8):2815. doi: 10.3390/ijms21082815

5. Bulun SE. Endometriosis. *N Engl J Med* (2009) 360(3):268–79. doi: 10.1056/NEJMra0804690

6. Pluchino N, Mamillapalli R, Wenger JM, Ramyead L, Drakopoulos P, Tille JC, et al. Estrogen receptor-α immunoreactivity predicts symptom severity and pain recurrence in deep endometriosis. *Fertil Steril.* (2020) 113(6):1224–31. doi: 10.1016/j.fertnstert.2020.01.036

7. Colgrave EM, Bittinger S, Healey M, Dior UP, Rogers PAW, Keast JR, et al. Superficial peritoneal endometriotic lesions are histologically diverse and rarely demonstrate menstrual cycle synchronicity with matched eutopic endometrium. *Hum Reprod* (2020) 3512:2701–14. doi: 10.1093/humrep/deaa249

8. Labrie F. Intracrinology. *Mol Cell Endocrinol* (1991) 78(3):C113–8. doi: 10.1016/0303-7207(91)90116-A

9. Pluchino N, Drakopoulos P, Bianchi-Demicheli F, Wenger JM, Petignat P, Genazzani AR. Neurobiology of DHEA and effects on sexuality, mood and cognition. *J Steroid Biochem Mol Biol* (2015) 145:273–80. doi: 10.1016/j.jsbmb.2014.04.012

10. Labrie F. Intracrinology and menopause: the science describing the cell-specific intracellular formation of estrogens and androgens from DHEA and their strictly local action and inactivation in peripheral tissues. *Menopause (New York NY)* (2019) 26(2):220–24. doi: 10.1097/GME.0000000000001177

11. L Gibson DA, Simitsidellis I, Collins F, Saunders PTK. Endometrial intracrinology: Oestrogens, androgens and endometrial disorders. *Int J Mol Sci* (2018) 19:3276. doi: 10.3390/ijms19103276

12. Cornel KMC, MY B, Kruitwagen RPFM, Romano A. Local estrogen metabolism (intracrinology) in endometrial cancer: A systematic review. *Mol Cell Endocrinol* (2019) 489:45–65. doi: 10.1016/j.mce.2018.10.004

13. Konings G, Brentjens L, Delvoux B, Linnanen T, Cornel K, Koskimies P, et al. Intracrine regulation of estrogen and other sex steroid levels in endometrium and non-gynecological tissues; pathology, physiology, and drug discovery. *Front Pharmacol* (2018) 9:940. doi: 10.3389/fphar.2018.00940

14. Noble LS, Takayama K, Zeitoun KM, Putman JM, Johns DA, Hinshelwood MM, et al. Bulun, prostaglandin E2 stimulates aromatase expression in endometriosis-derived stromal cells. *J Clin Endocrinol Metab* (1997) 822:600–6. doi: 10.1210/jcem.82.2.3783

15. Noble LS, Takayama K, Zeitoun KM, Putman JM, Johns DA, Hinshel- wood MM, et al. Prostaglandin E2 stimulates aromatase expression in endometriosis-derived stromal cells. *J Clin Endocrinol Metab* (1997) 82:600–6. doi: 10.1210/jc.82.2.600

16. Attar E, Bulun SE. Aromatase and other steroidogenic genes in endometriosis: translational aspects. *Hum Reprod* (2006) 12:49–56. doi: 10.1093/humupd/dmi034

17. Buccitelli C, Selbach M. mRNAs, proteins and the emerging principles of gene expression control. *Nat Rev Genet* (2020) 21:630–44. doi: 10.1038/s41576-020-0258-4

18. Hudelist G, Czerwenka K, Keckstein J, Haas C, Fink-Retter A, Gschwantler-Kaulich D, et al. Expression of aromatase and estrogen sulfotransferase in eutopic and ectopic endometrium: evidence for unbalanced estradiol production in endometriosis. *Reprod Sci* (2007) 14(8):798–805. doi: 10.1177/1933719107309120

19. Acién P, Velasco I, Gutiérrez M, Martínez-Beltrán M. Aromatase expression in endometriotic tissues and its relationship to clinical and analytical findings. *Fertil Steril* (2007) 88(1):32–8. doi: 10.1016/j.fertnstert.2006.11.188

20. Colette S, Lousse JC, Defrère S, Curaba M, Heilier JF, Van Langendonckt A, et al. Absence of aromatase protein and mRNA expression in endometriosis. *Hum Reprod* (2009) 24(9):2133–41. doi: 10.1093/humrep/dep199

21. Maia HJr, Haddad C, Casoy J. Correlation between aromatase expression in the eutopic endometrium of symptomatic patients and the presence of endometriosis. *Int J Womens Health* (2012) 4:61–5. doi: 10.2147/IJWH.S29154

22. Koninckx PR, Ussia A, Adamyan L, Gomel V, Martin DC. Peritoneal fluid progesterone and progesterone resistance in superficial endometriosis lesions. *Hum Reprod* (2022) 37(2):203–11. doi: 10.1093/humrep/deab258

23. Delvoux B, Groothuis P, D'Hooghe T, Kyama C, Dunselman G, Romano A. Increased production of 17beta-estradiol in endometriosis lesions is the result of impaired metabolism. *J Clin Endocrinol Metab* (2009) 94(3):876–83. doi: 10.1210/jc.2008-2218

24. Takayama K, Zeitoun K, Gunby RT, Sasano H, Carr BR, Bulun SE. Treatment of severe postmenopausal endometriosis with an aromatase inhibitor. *Fertil Steril.* (1998) 69(4):709–13. doi: 10.1016/S0015-0282(98)00022-3

25. Pavone ME, Bulun SE. Aromatase inhibitors for the treat- ment of endometriosis [Review]. *Fertil Steril.* (2012) 98(6):1370–9. doi: 10.1016/j.fertnstert.2012.08.053

26. Soysal S, Soysal ME, Ozer S, Gul N. Gezgin T: The effects of post-surgicaladministration of goserelin plus anastrozole compared to goserelin alone in patients with severe endometriosis: a prospective randomized trial. *Hum Reprod* (2004) 19:160–7. doi: 10.1093/humrep/deh035

27. Ferrero S, Camerini G, Seracchioli R, Ragni N, Venturini PL, Remorgida V. Letrozole combined with norethisterone acetate compared with norethisterone acetate alone in the treatment of pain symptoms caused by endometriosis. *Hum Reprod* (2009) 2412:3033–41. doi: 10.1093/humrep/dep302

28. Roghaei MA, Tehrany HG, Taherian A, Koleini N. Effects of letrozole compared with danazol on patients with confirmed endometriosis: A randomized clinical trial. *IJFS* (2010) 4:67–72. Available at: https://www.sid.ir/en/journal/viewpaper.aspx?id=176450

29. Alborzi S, Hamedi B, Omidvar A, Dehbashi S, Alborzi S, Alborzi M. A comparison of the effect of short-term aromatase inhibitor (letrozole) and GnRH agonist (triptorelin) versus case control on pregnancy rate and symptom and sign recurrence after laparoscopic treatment of endometriosis. *Arch Gynecol Obstet* (2011) 284(1):105–10. doi: 10.1007/s00404-010-1599-6

30. Ferrero S, Venturini PL, Gillott DJ, Remorgida V. Letrozole and norethisterone acetate versus letrozole and triptorelin in the treatment of endometriosis related pain symptoms: a randomized controlled trial. *Reprod Biol Endocrinol* (2011) 9:88. doi: 10.1186/1477-7827-9-88

31. Ferrero S, Leone Roberti Maggiore U, Scala C, Di Luca M, PL V, Remorgida V. Changes in the size of rectovaginal endometriotic nodules infiltrating the rectum during hormonal therapies. *Arch Gynecol Obstet.* (2013) 287(3):447–53. doi: 10.1007/s00404-012-2581-2

32. Ferrero S, Remorgida V, Venturini PL, Leone Roberti Maggiore U, et al. Norethisterone acetate versus norethisterone acetatecombined with letrozole for the treatment of ovarian endometriotic cysts: a patient preference study. *Eur JObstet Gynecol Reprod Biol* (2014) 174:117–22. doi: 10.1016/j.ejogrb.2013.11.030

33. Dunselman GA, Vermeulen N, Becker C, Calhaz-Jorge C, D'Hooghe T, De Bie B, et al. European Society of human reproduction and embryology. ESHRE guideline: management of women with endometriosis. *Hum Reprod* (2014) 29 (3):400–12. doi: 10.1093/humrep/det457

34. Hussain A, Ahsan F. The vagina as a route for systemic drug delivery. *J Control Release* (2005) 103(2):301–13. doi: 10.1016/j.jconrel.2004.11.034

35. Schultze-Mosgau MH, Waellnitz K, Nave R, Klein S, Kraetzschmar J, Rautenberg T, et al. Pharmacokinetics, pharmacodynamics, safety and tolerability of an intravaginal ring releasing anastrozole and levonorgestrel in healthy premenopausal women: a phase 1 randomized controlled trial. *Hum Reprod* (2016) 318:1713–22. doi: 10.1093/humrep/dew145

36. Nave R, Mellinger U, Klein S, Höchel J, Schmitz H., et al. Absence of drug-drug-interaction of anastrozole on levonorgestrel delivered simultaneously by an intravaginal ring: results of a phase 2 trial. *J Clin Pharmacol* (2019) 59(7):1022–8. doi: 10.1002/jcph.1396

37. Hefler LA, Grimm C, van Trotsenburg M, Nagele F. Role of the vaginally administered aromatase inhibitor anastrozole in women with rectovaginal endometriosis: a pilot study. *Fertil Steril.* (2005) 84(4):1033–6. doi: 10.1016/j.fertnstert.2005.04.059

38. Pluchino N, Poppi G, Yart L, Marci R, Wenger JM, Tille JC, et al. Effect of local aromatase inhibition in endometriosis using a new chick embryo chorioallantoic membrane model. *J Cell Mol Med* (2019) 23(8):5808–12. doi: 10.1111/jcmm.14372

39. Zeitoun K, Takayama K, Sasano H, Suzuki T, Moghrabi N, Andersson S. Deficient 17-hydroxysteroid dehydrogenase type 2 expression in endometriosis: failure to metabolize 17-estradiol. *J Clin Endocrinol Metab* (1998) 83:4474–80. doi: 10.1210/jc.83.12.4474

40. Matsuzaki S, Canis M, Pouly JL, Dechelotte PJ, Mage G. Analysis of aromatase and 17-hydroxysteroid dehydroganse type 2 messenger ribonucleicacid

expression in deep endometriosis and eutopic endometrium using lasercapture microdissection. *Fertil Steril* (2006) 85:308–13. doi: 10.1016/j.fertnstert.2005.08.017

41. Dassen H, Pundayeera C, Kamps R, Delvoux B, Van Langendonckt A, Donnez J, et al. Estrogen metabolizingenzymes in endometrium and endometriosis. *Hum Reprod* (2007) 22:3148–58. doi: 10.1093/humrep/dem310

42. Smuc T, Pucelj MR, Sinkovec J, Husen B, Thole H, Lanisnik Rizner T. Expression analysis of the genes involved in estradiol and progesterone action in human ovarian endometriosis. *Gynecol Endocrinol* (2007) 23(2):105–11. doi: 10.1080/09513590601152219

43. Smuc T, Hevir N, Ribic-Pucelj M, Husen B, Thole H, Rizner TL. Disturbed estrogen and progesterone action in ovarian endometriosis. *Mol Cell Endocrinol* (2009) 301(1-2):59–64. doi: 10.1016/j.mce.2008.07.020

44. Huhtinen K, Desai R, Ståhle M, Salminen A, Handelsman DJ, Perheentupa A, et al. Endometrial and endometriotic concentrations of estrone and estradiol are determined by local metabolism rather than circulating levels. *J Clin Endocrinol Metab* (2012) 97(11):4228–35. doi: 10.1210/jc.2012-1154

45. Colette S, Defrère S, Van Kerk O, Van Langendonckt A, Dolmans MM, Donnez J. Differential expression of steroidogenic enzymes according to endometriosis type. *Fertil Steril.* (2013) 100(6):1642–9. doi: 10.1016/j.fertnstert.2013.08.003

46. Delvoux B, D'Hooghe T, Kyama C, Koskimies P, RJ H, GA D, et al. Inhibition of type 1 17beta-hydroxysteroid dehydrogenase impairs the synthesis of 17beta-estradiol in endometriosis lesions. *J Clin Endocrinol Metab* (2014) 99 (1):276–84. doi: 10.1210/jc.2013-2851

47. Sinreih M, Anko M, Kene NH, Kocbek V, Rižner TL. Expression of AKR1B1, AKR1C3 and other genes of prostaglandin F2α biosynthesis and action in ovarian endometriosis tissue and in model cell lines, chem. *Biol Interact* (2015) 234:320–31. doi: 10.1016/j.cbi.2014.11.009

48. Rižner TL, Penning TM. Aldo-keto reductase 1C3-assessment as a new target for the treatment of endometriosis. *Pharmacol Res* (2020) 152:104446. doi: 10.1016/j.phrs.2019.104446

49. Sharma KK, Lindqvist A, Zhou XJ, Auchus RJ, Penning TM, Andersson S. Deoxycorticosterone inactivation by AKR1C3 in human mineralocorticoid target tissues, mol. *Cell Endocrinol* (2006) 248(1-2):79–86. doi: 10.1016/j.mce.2005.10.024

50. Rakhila H, Carli C, Daris M, Lemyre M, Leboeuf M, Akoum A. Identification of multiple and distinct defects in prostaglandin biosynthetic pathways in eutopic and ectopic endometrium of women with endometriosis, fertil. *Steril* (2013) 100(6):1650–9. doi: 10.1016/j.fertnstert.2013.08.016

51. Hevir N, Vouk K, Sinkovec J, Ribič-Pucelj M, Rižner TL. Aldo-keto reductases AKR1C1, AKR1C2 and AKR1C3 may enhance progesterone metabolism in ovarian endometriosis, chem. *Biol Interact* (2011) 191(1-3):217–26. doi: 10.1016/j.cbi.2011.01.003

52. Poirier D. Contribution to the development of inhibitors of 17β-hydroxysteroid dehydrogenase types 1 and 7: key tools for studying and treating estrogen-dependent diseases. *J Steroid Biochem Mol Biol* (2011) 125(1-2):83–94. doi: 10.1016/j.jsbmb.2010.12.007

53. Day JM, Tutill HJ, Purohit A, Reed MJ. Design and validation of specific inhibitors of 17-hydroxysteroid dehydrogenases for therapeutic application in breast and prostate cancer, and in endometriosis. *Endocr Relat Cancer.* (2008) 15:665–92. doi: 10.1677/ERC-08-0042

54. Purohit A, Fusi L, Brosens J, Woo LWL, Potter BVL, MJ R. Inhibition of steroid sulphatase activity in endometriotic implants by 667 COUMATE: a potential new therapy. *Hum Reprod* (2008) 232:290–7. doi: 10.1093/humrep/dem308

55. Hevir N, Ribič-Pucelj M, Lanišnik Rižner T. Disturbed balance between phase I and II metabolizing enzymes in ovarian endometriosis: a source of excessive hydroxy-estrogens and ROS? *Mol Cell Endocrinol* (2013) 367(1-2):74–84. doi: 10.1016/j.mce.2012.12.019

56. Piccinato CA, Neme RM, Torres N, Sanches LR, Derogis P, Brudniewski HF, et al. Effects of steroid hormone on estrogen sulfotransferase and on steroid sulfatase expression in endometriosis tissue and stromal cells. *J Steroid Biochem Mol Biol* (2016) 158:117–26. doi: 10.1016/j.jsbmb.2015.12.025

57. Colette S, Defrere S, Lousse JC, Van Langendonckt A, Gotteland JP, Loumaye E, et al. Inhibition of steroid sulfatase decreases endometriosis in an *in vivo* murine model. *Hum Reprod* (2011) 26:1362–70. doi: 10.1093/humrep/der079

58. Pohl O, Bestel E, Gotteland JP. Synergistic effects of E2MATE and norethindrone acetate on steroid sulfatase inhibition: a randomized phase I proof-of-principle clinical study in women of reproductive age. *Reprod Sci* (2014) 21(10):1256–65. doi: 10.1177/1933719114522526

59. Salah M, Abdelsamie AS, Frotscher M. First dual inhibitors of steroid sulfatase (STS) and 17β-hydroxysteroid dehydrogenase type 1 (17β-HSD1): Designed multiple ligands as novel potential therapeutics for estrogen-dependent diseases. *J Med Chem* (2017) 60(9):4086–92. doi: 10.1021/acs.jmedchem.7b00062

60. Becker CM, Gattrell WT, Gude K, Singh SS. Reevaluating response and failure of medical treatment of endometriosis: a systematic review. *Fertility Sterility* (2017) 108(1):125–36. doi: 10.1016/j.fertnstert.2017.05.004

61. Attar E, Bulun SE. Aromatase and other steroidogenic genes in endometriosis: translational aspects. *Hum Reprod* (2006) 12:49–56. doi: 10.1093/humupd/dmi034

62. Yamanaka K, Xu B, Suganuma I, Kusuki I, Mita S, Shimizu Y, et al. Dienogest inhibits aromatase and cyclooxygenase-2 expression and prostaglandin E2 productionin human endometriotic stromal cells in spheroid culture. *Fertility Sterility* (2012) 97:477–82. doi: 10.1016/j.fertnstert.2011.11.005

63. Shimizu Y, Mita S, Takeuchi T, Notsu T, Mizuguchi K, Kyo S. Dienogest, a synthetic progestin, inhibits prostaglandin E2 production and aromatase expression by human endometrial epithelial cells in a spheroid culture system. *Steroids* (2011) 76(1-2):60–7. doi: 10.1016/j.steroids.2010.08.010

64. Mori T, Ito F, Matsushima H, Takaoka O, Koshiba A, Tanaka Y, et al. Dienogest reduces HSD17beta1 expression and activity in endometriosis. *J Endocrinol* (2015) 225(2):69–76. doi: 10.1530/JOE-15-0052

65. Khan KN, Kitajima M, Hiraki K, Fujishita A, Sekine I, Ishimaru T, et al. Changes in tissue inflammation, angiogenesis and apoptosis in endometriosis, adenomyosis and uterine myoma after GnRH agonist therapy. *Hum Reprod* (2010) 25(3):642–53. doi: 10.1093/humrep/dep437

66. Miyazawa Y, Sekine Y, Syuto T, Nomura M, Koike H, Matsui H, et al. A gonadotropin-releasing hormone antagonist reduces serum adrenal androgen levels in prostate cancer patients. *BMC Urol* (2017) 17(1):70. doi: 10.1186/s12894-017-0261-z

67. Ishihara H, Kitawaki J, Kado N, Koshiba H, Fushiki S, Honjo H. Gonadotropin-releasing hormone agonist and danazol normalize aromatase cytochrome P450 expression in eutopic endometrium from women with endometriosis, adenomyosis, or leiomyomas. *Fertil Steril* (2003) 79 Suppl 1:735–42. doi: 10.1016/S0015-0282(02)04813-6

68. Maitoko K, Sasaki H. Gonadotropin-releasing hormone agonist inhibits estrone sulfatase expression of cystic endometriosis in the ovary. *Fertil Steril.* (2004) 82(2):322–6. doi: 10.1016/j.fertnstert.2003.12.044

69. Taylor HS, LC G, BA L, MS A, Kotarski J, DF A, et al. Treatment of endometriosis-associated pain with elagolix, an oral GnRH antagonist. *New Engl J Med* (2017) 377(1):28–40. doi: 10.1056/NEJMoa1700089

frontiers | Frontiers in Endocrinology

TYPE Review
PUBLISHED 20 October 2022
DOI 10.3389/fendo.2022.945578

OPEN ACCESS

EDITED BY
Tiziano Baroni,
University of Perugia, Italy

REVIEWED BY
Sahar Houshdaran,
University of California, San Francisco, United States
Chi Chiu Wang,
The Chinese University of Hong Kong, China

*CORRESPONDENCE
Ruijin Wu
wurj@zju.edu.cn

†These authors have contributed equally to this work and share first authorship

SPECIALTY SECTION
This article was submitted to Reproduction, a section of the journal Frontiers in Endocrinology

RECEIVED 16 May 2022
ACCEPTED 05 October 2022
PUBLISHED 20 October 2022

CITATION
Zhou Y, Jin Y, Wang Y and Wu R (2022) Hypoxia activates the unfolded protein response signaling network: An adaptive mechanism for endometriosis.
Front. Endocrinol. 13:945578.
doi: 10.3389/fendo.2022.945578

Hypoxia activates the unfolded protein response signaling network: An adaptive mechanism for endometriosis

Yong Zhou†, Yang Jin†, Yuan Wang and Ruijin Wu*

Women's Hospital, Zhejiang University School of Medicine, Hangzhou, China

Endometriosis (EMS) is a chronic gynecological disease that affects women of childbearing age. However, the exact cause remains unclear. The uterus is a highly vascularized organ that continuously exposes endometrial cells to high oxygen concentrations. According to the "planting theory" of EMS pathogenesis, when endometrial cells fall from the uterine cavity and retrograde to the peritoneal cavity, they will face severe hypoxic stress. Hypoxic stress remains a key issue even if successfully implanted into the ovaries or peritoneum. In recent years, increasing evidence has confirmed that hypoxia is closely related to the occurrence and development of EMS. Hypoxia-inducible factor-1α (HIF-1α) can play an essential role in the pathological process of EMS by regulating carbohydrate metabolism, angiogenesis, and energy conversion of ectopic endometrial cells. However, HIF-1α alone is insufficient to achieve the complete program of adaptive changes required for cell survival under hypoxic stress, while the unfolded protein response (UPR) responding to endoplasmic reticulum stress plays an essential supplementary role in promoting cell survival. The formation of a complex signal regulation network by hypoxia-driven UPR may be the cytoprotective adaptation mechanism of ectopic endometrial cells in unfavorable microenvironments.

KEYWORDS

hypoxia, unfolded protein response, endometriosis, endoplasmic reticulum stress, hypoxia-inducible factor

Introduction

Endometriosis is a common benign gynecological disorder characterized by the presence and growth of functional endometrial glands and stroma outside the uterine cavity, usually accompanied by reactive fibrosis and muscular metaplasia of affected organs (1, 2). Unfortunately, only a few studies estimated the prevalence and incidence of endometriosis in the general population, reporting that the prevalence of symptomatic

endometriosis is about 10%, and approximately 2-7/1000 women are diagnosed with endometriosis every year. However, 11% of cases remain undiagnosed (3, 4).

EMS is closely related to dysmenorrhea, painful intercourse, painful defecation, and infertility in women of reproductive age and may seriously affect their quality of life (5, 6). In addition, patients and physicians face the great challenge of high recurrence rates after conservative EMS surgery (7, 8). Lesions are most commonly found in the pelvic cavity, including the ovaries, uterus, sacral ligaments, vaginal septum, bladder, rectum, and ureters. EMS may also invade organs other than the pelvic cavity, such as abdominal incision (9), diaphragm, and even lungs (10).

Theoretical basis of hypoxia in EMS

The exact pathogenesis of EMS is still unclear. However, the implant theory is still widely accepted as the mainstream theory (11). The hypothesis is based on the concept that EMS lesions' matrix and glands are derived from ectopic endometrium. Thus, EMS is considered a benign metastasis of ectopic endometrium, which is transferred from the uterine cavity to another position in the body through different pathways. The theory of menstrual blood reflux, based on clinical and anatomical observations, was first proposed by Sampson in 1927 (12). It is believed that most EMS were induced by endometrial fragments entering the pelvic cavity through fallopian tube reflux during menstruation, the transition from the endometrium to ectopic endometrium, and growth on peritoneum as well as ovary.

Although menstrual reflux theory cannot explain all forms of EMS, it remains the most accepted hypothesis of EMS. Some scientists found that 76~90% of women have the phenomenon of menstrual blood regurgitation of fallopian tubes (13), and laparoscopy has revealed that endometrial epithelial cells can be isolated from abdominal fluid at the early stage of endometrial hyperplasia (14). In addition, some researchers have identified viable single endometrial cells and glandular structures from the shedding of menstrual blood (15). The "eutopic endometrium determinism", proposed by Lang (16), believes that the onset of EMS depends on the characteristics of eutopic endometrial cells in the uterine, so eutopic endometrial cells of patients with EMS may have stronger survival and proliferation ability after falling off. Thus, they are more likely to become ectopic lesions. Among this process, retrograde menstruation is the only bridge from the potential pathogenicity of endometrial cells to the onset of EMS. Recently, based on the physiological phenomenon of periodic stripping and regeneration of endometrium, it is speculated that endometrial stem cells with differentiation potential may exist in menstrual blood. Therefore, some scientists proposed the "stem cell theory" of EMS pathogenesis (17). Subsequently, relevant studies also confirmed that after menstrual blood enters the pelvic cavity from the fallopian tube, endometrial cell fragments with stem cell characteristics can adhere to mesothelial cells and promote the growth of EMS lesions, leading to the occurrence and development of EMS (18, 19). These theories enriched the theoretical basis of planting theory.

The anatomical characteristics of the uterine vessels are that the body branches of the uterine arteries vertically discharge the arch artery along with the muscle layer of the lateral wall of the uterus. The uterine spiral artery then extends vertically into the endometrium, finally forming a blood-rich capillary network in the endometrium (as shown in Figure 1). During menstruation, endometrial vasospasm causes acute hypoxia of the endometrium, leading to necrosis and endometrial dissection. Finally, blood vessels rupture and a mixture of blood and exfoliated endometrium fragments are formed. The main pathogenesis of EMS, including implant theory, menstrual blood reflux theory, endometrial determinism, and stem cell theory, all began with the shedding of endometrial fragments into the pelvic cavity. Under normal circumstances, the shedding endometrial cells immediately convert into a state of severe hypoxia when the blood supply of the endometrial capillaries is lost. Then relevant apoptotic signals are activated to induce apoptosis and endometrial cells are finally cleared by the immune cells in the pelvic. At the same time, this transient physiological hypoxia could promote the timely repair of the exfoliated endometrial surface and prevent excessive menstrual bleeding, which is mostly meditated by hypoxia-induced high expression of vascular endothelial growth factor (VEGF) (20). However, in patients with EMS, various changes of cell biological functions (21) may occur in the shedding endometrial cells under hypoxic stress, such as increased adhesive and invasive capacity, enhanced angiogenesis, and dysregulated immuno-clearance system, thus improving their ability to resist hypoxia or activating certain functions to resist apoptosis, adapt to the hypoxic environment, and survive. Eventually, they will develop into EMS lesions. Therefore, hypoxia can also be the driving factor for EMS, but the relevant pathological mechanism remains unclear.

The role of hypoxia-inducible factors in endometriosis

Hypoxia (22) generally refers to the pathological process in which oxygen is not adequately supplied or consumed excessively, resulting in insufficient oxygen concentration, reduced availability, and the inability to maintain normal cellular functions. If hypoxia persists, it will lead to a metabolic crisis and ultimately threaten the cell's survival. The concentration of oxygen in the atmosphere is approximately 20%, but the content of oxygen in human tissues is much lower. Generally, the normal oxygen content of human tissues is approximately 3%~5% (as shown in Figure 2) (23), which is essential for the maintenance of cells' normal life. When the intracellular oxygen content ranges in 1%~3%, it is called mild

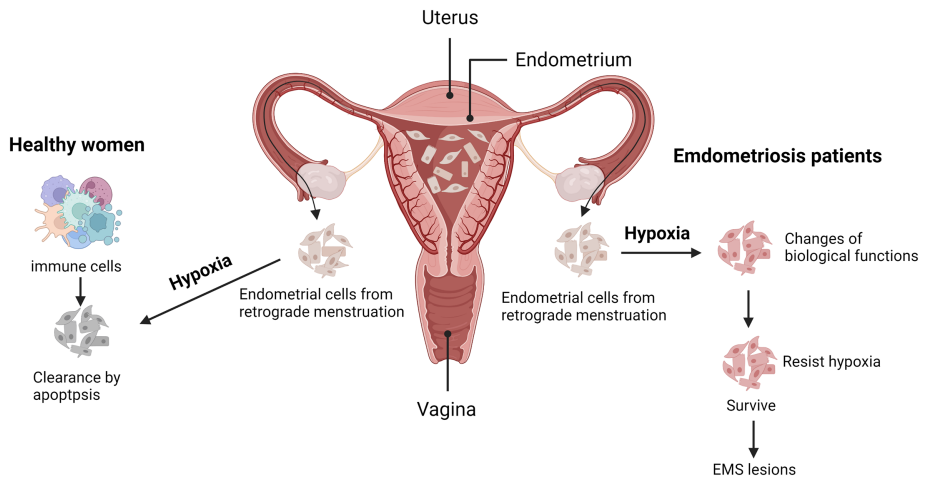


FIGURE 1
Possible adaptive mechanism for endometriosis under hypoxia. During menstruation, the exfoliated endometrium fragments flow into the pelvic cavity with retrograde menstruation. Under normal circumstances (as shown in the left part), the shedding endometrial cells will face acute hypoxic stress, activating apoptotic signals and being cleared by the immune cells. Conversely, those endometrial cells of endometriosis (EMS) patients (as shown in the right part), possess with altered biological functions, may resist hypoxia or apoptosis and finally develop into EMS lesions. Created with BioRender.com.

hypoxia. In this condition, molecular O2 will play a role as a critical signal to regulate cell fate, activating various adaptive mechanisms to promote cell survival and proliferation.

However, in a state of moderate hypoxia, which means that the oxygen content is less than 1%, many cells can still survive. To survive in a moderate hypoxic environment, the expression of various genes is needed to allow cells to adapt to the stress response induced by hypoxia. This process is heavily controlled by the hypoxia-inducible factors (HIFs) family. HIFs, first discovered by Semenza et al. (24) in 1992, are activated through a transcription mechanism under hypoxic conditions and then promote the expression of many hypoxia-regulated gene products to form an adaptive mechanism for cell protection. Acting as the master switch in the body in response to hypoxia, the downstream mechanisms of HIFs are multifaceted (25). For example, HIFs promotes increased secretion of erythropoietin to accelerate red blood cell production, enhancing tissue's ability to transport oxygen, ensuring that cells have an adequate supply of oxygen (26). In addition, switching of metabolic patterns during hypoxia is essential to reduce oxygen consumption. HIFs can activate glycolytic genes and inhibit tricarboxylic acid cycle metabolism, thereby reducing oxygen consumption and maintaining cell survival in hypoxia condition (27). HIFs can also induce increased secretion of vascular endothelial growth factor (VEGF) in cells and increase the blood supply of tissues by promoting angiogenesis, thus protecting cells from ischemic damage (28). Hypoxia can also promote the synthesis of some glucose transporters and maintain the production of high-energy molecular ATP in cells (29). Overall, HIFs are the most sensitive and important nuclear transcription regulators response to hypoxia. They are prevalent in various mammalian cells even in the simplest animal (30). HIFs regulate the expression of various genes, and they are widely involved in regulating oxygen homeostasis in response to hypoxia and other changes in the cell's internal environment of the cell. Thus, HIF-1 is a key oxygen sensor and a hypoxic adaptive response regulator.

Regarding EMS, when endometrial cells enter the pelvic cavity with retrograde menstrual blood and have not established effective blood circulation, continuous hypoxia can induce the overexpression of HIF-1 in endometrial cells, thus inducing the secretion of various factors within the endometrial environment and prompting the development of endometriosis through multiple mechanisms (31). Angiogenesis is the primary challenge for the survival of endometrial cells flowed into the pelvis, and it is also a major prerequisite for the initiation and progression of endometriosis (32). In this process, HIFs can induce the expression of numerous downstream factors to promote the angiogenesis of endometrial cells. Among them, VEGF family are the most classical factors which were found up-regulated both *in vivo* and *in vitro* (33, 34). Other cytokines and chemokines, such as IL-8, leptin, CYR61 and osteopontin also participant in the HIF-induced angiogenesis process (35–37). In addition, HIFs could also enhance cell adhesive ability through the regulation of Transforming growth factor β1 (TGF-β1), Enhancer of zeste homolog 2 (EZH2), and anthrax toxin receptor 2 (ANTXR2) (34, 38). Hypoxia also participants in the regulation of inflammation and immune system *via* mediating the expression of IL-6, DUPS2, and COX-2 in

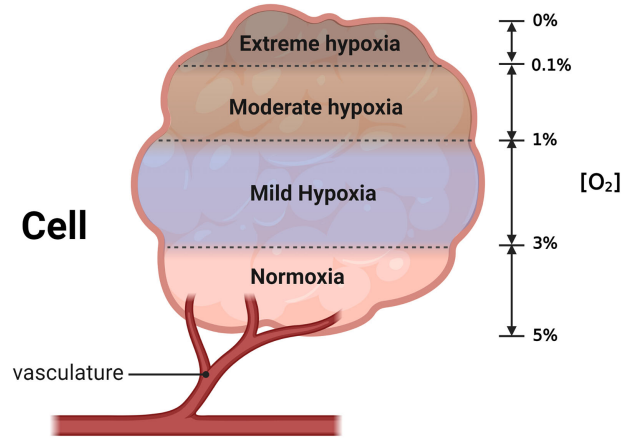


FIGURE 2
Grading of hypoxia. The normal oxygen content in human cell is 3%~5%; mild hypoxia refers to the oxygen concentration raging in 1%-3%; Oxygen content less than 1% is defined as severe hypoxia. Created with BioRender.com.

endometrial cells (39). Furthermore, although EMS is a benign disorder, it displays some pathogenic characteristics of malignant diseases (40), such as the tendency to invade and relapse, and adaptation to hypoxia (41). A latest research showed that the expression of early growth response 1 (EGR1) and its downstream target carbonic anhydrase 9 (CA9), which were up-regulated in hypoxic cancers, are significantly increased in ectopic lesions (42). In addition, malignant cells possess a unique energy metabolism feature which is called the "Warburg effect" (43), also known as aerobic glycolysis. Specifically, even in anoxic conditions, malignant tumor cells could remain powered by glycolysis, increasing glucose consumption and lactic acid production. This progress also provides abundant energy for the growth of malignant cells, ensuring their ability to proliferate rapidly, and HIF-1α plays a vital role in this energy metabolic pathway and may protect cells from hypoxia (44). Interestingly, Young et al. (45) found that the "Warburg effect" also existed in the EMS lesion, and HIF-1α also showed same effect on the aerobic glycolysis of EMS.

Hypoxia activates the unfolded protein response

Recent evidence suggests that HIFs alone cannot achieve the whole program of adaptive changes required for cell survival under hypoxic stress and the unfolded protein response (UPR) under endoplasmic reticulum stress (ERS) plays an essential complementary role in this process (46, 47). UPR and HIF pathway interacts with HIF-independent pathways, forming a highly correlated regulatory network under hypoxia stress.

The endoplasmic reticulum (ER) is an extensive intracellular membranous network extending to the entire cytoplasm. The key site for lipid and glucose metabolism, calcium homeostasis, detoxifying drugs, and metabolisms is the central processing unit responsible for protein translation, folding, and modification (48). In protein synthesis, folding the protein spatial structure depends on the oxygen content, which is also called oxidizing protein folding (49). When intracellular oxygen availability is reduced (hypoxia), protein folding will be disturbed, leading to accumulation of misfolded or unfolded proteins. These changes break the protein dynamics in the endoplasmic reticulum and activate the UPR signaling network, thus inducing the production of self-protection mechanisms in cells. This process is called endoplasmic reticulum stress (ERS), which aims to restore homeostasis and function of the intracellular environment (50–52).

UPR is mediated by a partner molecule specific for the endoplasmic reticulum, namely glucose-regulated protein78 (GRP78), and three transmembrane protein stress sensors (53), namely protein kinase RNA-like endoplasmic reticulum kinase (PERK), activating transcription factor 6 (ATF6) and inositol-requiring protein 1α (IRE1α)(as shown in Figure 3). UPR can avoid the ERS-caused damage by reducing the accumulation of unfolded proteins and improving the correct folding of proteins, and restoring normal physiological functions of cells. However, if ERS becomes persistent or damage is too severe, the signal will change from pro-survival to pro-apoptosis

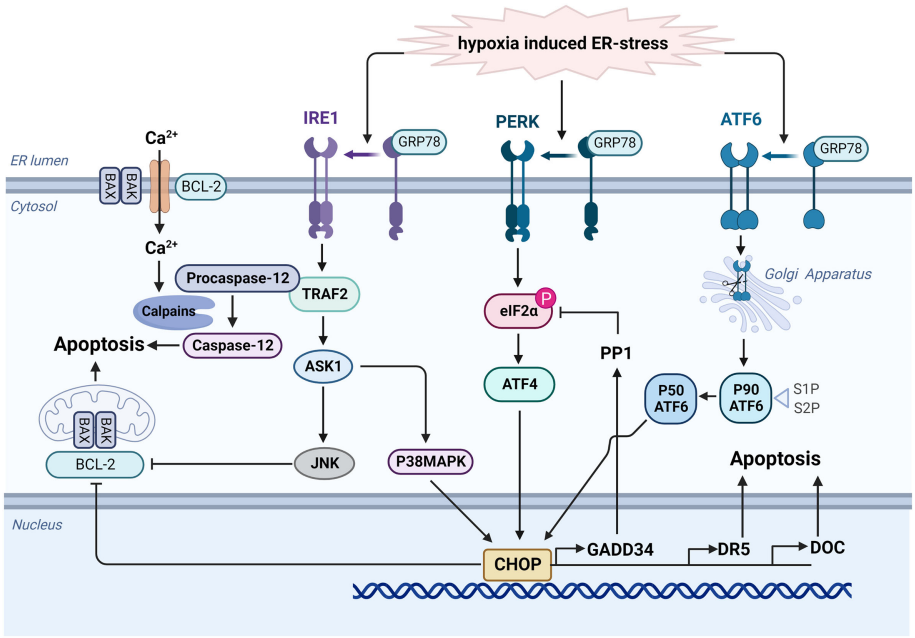


FIGURE 3
Hypoxia activates the UPR signaling network to mediate apoptosis. When ERS damage occurs, GRP78 dissociates from PERK, ATF6 and IRE1α. Activated ATF6 can be transported to Golgi apparatus. After modification, it could induce CHOP expression and lead to UPR-related apoptosis *via* the inhibition of BCL-2 protein family and the activation of multiple genes including GADD34, DR5, and DOC. Similarly, activated PERK phosphorylates the α subunit of eIF2 and initiates translation of ATF4, then promoting the transcription of CHOP. Additionally, activated IRE1α stimulates the expression of TRAF2, then activate ASK1 and JNK to promote apoptosis. TRAF2 also promotes clustering of procaspase-12, and it will be activated into caspase 12 by calpains under ER stress. Created with BioRender.com.

(54). Under normal physiological conditions, these three UPR-related transmembrane proteins all bind to GRP78 and remain in an inactive state. When ERS damage occurs, GRP78 will dissociate from these transmembrane proteins (55). Activated PERK phosphorylates the α subunit of eukaryotic initiation factor 2 (eIF2), initiating selective translation of activating transcription factor 4 (ATF4) under stress conditions. ATF4 is mainly involved in regulating peptide chain biosynthesis, antioxidant action, protein folding, and gene expression related to the maintenance of redox homeostasis (56). If the stress persists, ATF4 can also promote the transcription of C/EBP homologous protein (CHOP), a pro-apoptotic protein, to induce cell apoptosis (57). Activated ATF6 can also initiate related transcriptional procedures to restore endoplasmic reticulum homeostasis, including inducing GRP78 expression, promoting protein chaperone and lipid synthesis, stimulating endoplasmic reticulum degradation, and improving N-glycosylation (58, 59), and ATF6 also induces CHOP expression, leading to UPR-related apoptosis (60, 61).

Activated IRE1α regulates gene expression by increasing ER protein folding capacity *via* TRAF2. It also promotes the expression of proteins related to disulfide bond formation and molecular chaperones and proteins involved in ER degradation and vesicle transport (62). TRAF2 also promotes clustering of procaspase-12. Under ER stress, sustained Ca2+ release from the ER and activate calpains, which may induce the activation of caspase 12 to mediate apoptosis (63). In addition, IRE1α kinase can up-regulated the expression of apoptosis signal regulated kinase 1(ASK1) and then activate c-Jun N-terminal kinase (JNK) to promote apoptosis through the inhibition of BCL-2 protein family (64, 65). CHOP, a member of C/EBP transcription factor family, is an ERS-specific effector molecule representing an important cell transition signal to apoptosis (66). Under normal physiological conditions, CHOP expression remains at a very low level, while in the ERS state, activation of PERK, ATF6, and IRE1α can induce CHOP transcription, significantly increasing its expression and migration into the nucleus, promoting cell apoptosis *via* the inhibition of BCL-2 protein family (67, 68). CHOP also prompt the expression of GADD34 (69), which complexes with protein phosphatase 1 (PP1) to induce the dephosphorylation of eIF2α, thus forming a negative feedback loop. Other downstream target genes, including death receptor 5 (DR5) and downstream of CHOP (DOC) have also been proposed to lead to apoptosis (70).

Since protein synthesis and oxygen-dependent protein folding are energy-intensive processes, and hypoxia significantly reduces the level of intracellular ATP, regulating mRNA translation is an important cellular response to hypoxia

(27). Hypoxia activates PERK, leading to the inhibition of eIF2α phosphorylation and overall translation, while ATF4 translation increases after PERK/eIF2α is activated (71, 72). This is a rapid response independent of HIF-1α and usually occurs within minutes of exposure to hypoxic conditions in cells. The phosphorylation of eIF2α is transient, attributed to the negative feedback caused by GADD34 dependent on ATF4 upregulation. Dephosphorylation of eIF2α will increase the production of various intracellular reactive oxygen species (ROS), thus stimulating various biological reactions, while mitochondria are the main source of oxygen-deficient ROS (73). Mitochondrial hypoxic ROS activates and integrates the stress response to maintain energy and REDOX homeostasis and then constitutes an early adaptive response to hypoxia. Some enzyme antioxidants, such as catalase and glutathione peroxidase, can reduce eIF2α phosphorylation due to hypoxia. In contrast, ATF4 can promote cell survival by enhancing the upregulation of HIF-1α-mediated downstream targets (74). Brief exposure to ERS can modulate cells and enable them to survive in more severe stress. Survival-promoting genes may induce this pre-adaptation, and integration of stress response is also a sufficient survival-promoting mechanism under hypoxia. Cells with impaired PERK-eIF2a-ATF4 signal transduction are more sensitive to hypoxic stress *in vitro*, indicating that the PERK-eIF2a-ATF4 pathway provides a survival advantage for cells under hypoxic conditions (75, 76), which is crucial for resistance to intracellular hypoxia, metabolic stress, and starvation.

Scientists have analyzed variations of gene expression under hypoxia conditions, but UPR-related genes can be excessively induced in the state of "extreme" hypoxia or "moderate" hypoxia. For example, X-box binding protein 1 (XBP1), a transcription factor containing zinc finger structure, is critical for UPR signaling network and acts on the folding of multiple proteins regulated by downstream target molecules. Under normal conditions, XBP1 exists in XBP1 unspliced (XBP1u). In the hypoxia state, XBP1u is activated into XBP1 spliced (XBP1s) by IRE1, enabling effective transcription of multiple target genes in the nucleus. Meanwhile, hypoxia can also induce XBP1 expression and activate its mRNA splicing in a HIF-1α dependent pathway, leading to increased XBP1s (77). When XBP1s were co-localized in tumors with hypoxia markers, the loss of XBP1 increased the sensitivity of transformed cells to hypoxia-induced apoptosis and inhibited tumor growth (78). Studies have demonstrated that XBP1 is critical for carcinogenicity and progression of triple-negative breast cancer (TNBC). TNBC is a type of breast cancer that lacks estrogen receptors, progesterone, and HER2, in which HIF-1α is overactivated. However, XBP1 splicing is not directly regulated by HIF-1α. XBP1 mainly enhances the transcriptional activity of HIF-1α and regulates its transcriptional program by binding to HIF-1α and forming the transcription complex to drive the carcinogenicity of TNBC. In contrast, XBP1 knockout can reduce the formation of breast cancer lesions under hypoxia conditions. The characteristics of XBP1 gene expression in TNBC patients are closely related to those in the state of hypoxia, indicating a poor prognosis.

The role of UPR in EMS

UPR is a protection mechanism to maintain the stability of the intracellular environment under hypoxia circumstances, and its activation will promote cell survival. To determine the role of UPR in the pathogenesis of EMS, Guzel (79) et al. detected GRP78 expression in normal and ectopic endometrial cells, finding that the level of GRP78 in ectopic endometrial cells is significantly higher than that of normal endometrial cells. This indicated that the UPR cascade reaction was activated in EMS, and the upregulated expression of GRP78 significantly reduced the sensitivity of ectopic endometrial cells to apoptosis and increased their anti-apoptosis ability, which was beneficial for the survival of ectopic endometrial cells. Other UPR proteins, p-IRE1 and p-PERK, were also found increased in endometrial cells when they were treated with peritoneal fluid obtained from women with endometriosis (80). Taylor (81) et al. also reported that UPR induced the increased expression of IL-8, indicating that UPR may be involved in the pathogenesis of EMS by promoting neovascularization and cell survival *via* IL-8 related pathways. The protein kinase B (AKT)/mammalian target of rapamycin (mTOR) signaling pathway plays an essential role in enhancing the invasiveness of cells, and inhibition of this pathway can effectively reduce the invasiveness of various cancer cells (82). Several studies (83, 84) have displayed that UPR can inhibit AKT/mTOR pathway through CCAAT/CHOP/(Tribbles Homolog 3, TRIB3) signal transduction and regulate the invasiveness of ectopic endometrial cells. EMS is an estrogen-dependent disease and is related to progesterone resistance (85). UPR-induced apoptosis can be inhibited by estrogen, thus promoting endometrial cell survival (86). In contrast, in the secretory phase, due to the antagonistic effect of progesterone, the inhibition effect of estrogen on the UPR-mediated apoptotic pathway can be reduced. Then, UPR upregulation may help reduce the invasiveness of endometrial cells (87). However, progesterone resistance exists in ectopic and endometrial stromal cells of women with EMS, and in the secretory phase, progesterone resistance in ectopic endometrium stromal cells may alter the effect induced by UPR as described above (88, 89). *In vitro* studies on UPR regulating endometrial cell invasiveness also suggest that (90), in endometrial cells with estrogen added alone in the proliferative phase, the expressions of GRP78, CHOP, and TRIB3 increased significantly with increased progesterone during the secretory phase. In contrast, endometrial cell invasiveness was significantly suppressed when AKT and mTOR activity was inhibited. Thus, progesterone can upregulate the expression of UPR-related CHOP and TRIB3 by

antagonizing estrogen and inhibiting the AKT/mTOR pathway, reducing the invasiveness of endometrial cells. These progesterone-induced signal regulations can occur in normal endometrial cells. In ectopic cells, progesterone has no significant effect on CHOP/TRIB3 or AKT/mTOR signaling, so it does not play a role in the invasiveness of endometrial cells.

Discussion

EMS severely affects the physical and mental health and quality of life of women of reproductive age. The implantation theory on the pathogenesis of EMS indicates the key role of hypoxia in the occurrence and development of EMS, which involves cell biological processes such as apoptosis, adhesion, proliferation, invasion, and metastasis of ectopic endometrial cells. The mechanism of EMS is complex, and its etiology and pathology cannot be clearly explained with a onefold theory. Hypoxia-induced UPR may be one of the potential mechanisms by which ectopic endometrium cells could resist apoptosis and develop into endometriotic lesions, and some drugs (91, 92) targeting this mechanism may become potential effective therapeutic agents for the treatment of the disease. But the exact mechanism of hypoxia-stimulated UPR in EMS remains to be explored, and we look forward to new break-throughs in this field.

Author contributions

YZ and YJ contributed equally to the manuscript. YZ contributed to the conception and design of the review. YZ and YJ drafted the manuscript. YJ performed the descriptive figures. YW and RW revised the manuscript and provided critical advice on the content of the manuscript. All authors read and approved the final manuscript.

Funding

This work was supported by National Natural Science foundation of China (82101723), Department of Education of Zhejiang Province (Y202045691), and Zhejiang Province Key R&D Program (2021C03095).

Acknowledgments

We gratefully acknowledge the assistance of Home for Researchers for language modification.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Giudice LC. Clinical practice. endometriosis. *N Engl J Med* (2010) 362 (25):2389–98. doi: 10.1056/NEJMcp1000274

2. Vercellini P, Viganò P, Somigliana E, Fedele L. Endometriosis: pathogenesis and treatment. *Nat Rev Endocrinol* (2014) 10(5):261–75. doi: 10.1038/nrendo.2013.255

3. Viganò P, Parazzini F, Somigliana E, Vercellini P. Endometriosis: epidemiology and aetiological factors. *Best Pract Res Clin Obstet Gynaecol* (2004) 18(2):177–200. doi: 10.1016/j.bpobgyn.2004.01.007

4. Buck Louis GM, Hediger ML, Peterson CM, Croughan M, Sundaram R, Stanford J, et al. Incidence of endometriosis by study population and diagnostic method: the ENDO study. *Fertil Steril* (2011) 96(2):360–5. doi: 10.1016/j.fertnstert.2011.05.087

5. Laganà AS, La Rosa V, Petrosino B, Vitale SG. Comment on "Risk of developing major depression and anxiety disorders among women with endometriosis: A longitudinal follow-up study". *J Affect Disord* (2017) 208:672–3. doi: 10.1016/j.jad.2016.07.016

6. Matalliotakis M, Zervou MI, Matalliotaki C, Rahmioglu N, Koumantakis G, Kalogiannidis I, et al. The role of gene polymorphisms in endometriosis. *Mol Med Rep* (2017) 16(5):5881–6. doi: 10.3892/mmr.2017.7398

7. Raffaelli R, Garzon S, Baggio S, Genna M, Pomini P, Laganà AS, et al. Mesenteric vascular and nerve sparing surgery in laparoscopic segmental intestinal resection for deep infiltrating endometriosis. *Eur J Obstet Gynecol Reprod Biol* (2018) 231:214–9. doi: 10.1016/j.ejogrb.2018.10.057

8. Thomin A, Belghiti J, David C, Marty O, Bornes M, Ballester M, et al. Maternal and neonatal outcomes in women with colorectal endometriosis. *Bjog* (2018) 125(6):711–8. doi: 10.1111/1471-0528.14221

9. Khan Z, Zanfagnin V, El-Nashar SA, Famuyide AO, Daftary GS, Hopkins MR. Risk factors, clinical presentation, and outcomes for abdominal wall endometriosis. *J Minim Invasive Gynecol* (2017) 24(3):478–84. doi: 10.1016/j.jmig.2017.01.005

10. Chamié LP, Ribeiro D, Tiferes DA, Macedo Neto AC, Serafini PC. Atypical sites of deeply infiltrative endometriosis: Clinical characteristics and imaging findings. *Radiographics* (2018) 38(1):309–28. doi: 10.1148/rg.2018170093

11. Sampson JA. The development of the implantation theory for the origin of peritoneal endometriosis. *Am J Obstet Gynecol* (1940) 40:549–57. doi: 10.1016/s0002-9378(40)91238-8

12. Sampson JA. Peritoneal endometriosis due to the menstrual dissemination of endometrial tissue into the peritoneal cavity. *Am J Obstet Gynecol* (1927) 14:422–69. doi: 10.1016/s0002-9378(15)30003-x

13. Halme J, Hammond MG, Hulka JF, Raj SG, Talbert LM. Retrograde menstruation in healthy women and in patients with endometriosis. *Obstet Gynecol* (1984) 64(2):151–4. Available at: https://pubmed.ncbi.nlm.nih.gov/6234483/.

14. Kruitwagen RF, Poels LG, Willemsen WN, Jap PH, Thomas CM, Rolland R. Retrograde seeding of endometrial epithelial cells by uterine-tubal flushing. *Fertil Steril* (1991) 56(3):414–20. doi: 10.1016/s0015-0282(16)54533-6

15. Koks CA, Dunselman GA, De Goeij AF, Arends JW, Evers JL. Evaluation of a menstrual cup to collect shed endometrium for *in vitro* studies. *Fertil Steril* (1997) 68(3):560–4. doi: 10.1016/s0015-0282(97)00250-1

16. Lang J. A new milestone in endometriosis research. *Chin J Obstet Gynecol* (2005) 40:3–4. doi: 10.3760/j.issn:0529-567X.2005.01.002

17. Figueira PG, Abrão MS, Krikun G, Taylor HS. Stem cells in endometrium and their role in the pathogenesis of endometriosis. *Ann N Y Acad Sci* (2011) 1221 (1):10–7. doi: 10.1111/j.1749-6632.2011.05969.x

18. Hapangama DK, Kamal A, Saretzki G. Implications of telomeres and telomerase in endometrial pathology. *Hum Reprod Update* (2017) 23(2):166–87. doi: 10.1093/humupd/dmw044

19. Liu Y, Zhang Z, Yang F, Wang H, Liang S, Wang H, et al. The role of endometrial stem cells in the pathogenesis of endometriosis and their application to its early diagnosis†. *Biol Reprod* (2020) 102(6):1153–9. doi: 10.1093/biolre/ioaa011

20. Maybin JA, Murray AA, Saunders PTK, Hirani N, Carmeliet P, Critchley HOD. Hypoxia and hypoxia inducible factor-1α are required for normal endometrial repair during menstruation. *Nat Commun* (2018) 9(1):295. doi: 10.1038/s41467-017-02375-6

21. Li WN, Wu MH, Tsai SJ. HYPOXIA AND REPRODUCTIVE HEALTH: The role of hypoxia in the development and progression of endometriosis. *Reproduction* (2021) 161(1):F19–f31. doi: 10.1530/rep-20-0267

22. Simon MC, Keith B. The role of oxygen availability in embryonic development and stem cell function. *Nat Rev Mol Cell Biol* (2008) 9(4):285–96. doi: 10.1038/nrm2354

23. Koumenis C, Wouters BG. "Translating" tumor hypoxia: unfolded protein response (UPR)-dependent and UPR-independent pathways. *Mol Cancer Res* (2006) 4(7):423–36. doi: 10.1158/1541-7786.Mcr-06-0150

24. Semenza GL, Wang GL. A nuclear factor induced by hypoxia via de novo protein synthesis binds to the human erythropoietin gene enhancer at a site required for transcriptional activation. *Mol Cell Biol* (1992) 12(12):5447–54. doi: 10.1128/mcb.12.12.5447-5454.1992

25. West JB. Physiological effects of chronic hypoxia. *N Engl J Med* (2017) 376 (20):1965–71. doi: 10.1056/NEJMra1612008

26. Kourembanas S, Marsden PA, Mcquillan LP, Faller DV. Hypoxia induces endothelin gene expression and secretion in cultured human endothelium. *J Clin Invest* (1991) 88(3):1054–7. doi: 10.1172/jci115367

27. Kim JW, Tchernyshyov I, Semenza GL, Dang CV. HIF-1-mediated expression of pyruvate dehydrogenase kinase: A metabolic switch required for cellular adaptation to hypoxia. *Cell Metab* (2006) 3(3):177–85. doi: 10.1016/j.cmet.2006.02.002

28. Shweiki D, Itin A, Soffer D, Keshet E. Vascular endothelial growth factor induced by hypoxia may mediate hypoxia-initiated angiogenesis. *Nature* (1992) 359(6398):843–5. doi: 10.1038/359843a0

29. Semenza GL, Roth PH, Fang HM, Wang GL. Transcriptional regulation of genes encoding glycolytic enzymes by hypoxia-inducible factor 1. *J Biol Chem* (1994) 269(38):23757–63. doi: 10.1016/S0021-9258(17)31580-6

30. Loenarz C, Coleman ML, Boleininger A, Schierwater B, Holland PW, Ratcliffe PJ, et al. The hypoxia-inducible transcription factor pathway regulates oxygen sensing in the simplest animal, trichoplax adhaerens. *EMBO Rep* (2011) 12 (1):63–70. doi: 10.1038/embor.2010.170

31. Wu MH, Hsiao KY, Tsai SJ. Hypoxia: The force of endometriosis. *J Obstet Gynaecol Res* (2019) 45(3):532–41. doi: 10.1111/jog.13900

32. Laschke MW, Menger MD. *In vitro* and *in vivo* approaches to study angiogenesis in the pathophysiology and therapy of endometriosis. *Hum Reprod Update* (2007) 13(4):331–42. doi: 10.1093/humupd/dmm006

33. Lu Z, Zhang W, Jiang S, Zou J, Li Y. Effect of oxygen tensions on the proliferation and angiogenesis of endometriosis heterograft in severe combined immunodeficiency mice. *Fertil Steril* (2014) 101(2):568–76. doi: 10.1016/j.fertnstert.2013.10.039

34. Yu YX, Xiu YL, Chen X, Li YL. Transforming growth factor-beta 1 involved in the pathogenesis of endometriosis through regulating expression of vascular endothelial growth factor under hypoxia. *Chin Med J (Engl)* (2017) 130(8):950–6. doi: 10.4103/0366-6999.204112

35. Wu MH, Chen KF, Lin SC, Lgu CW, Tsai SJ. Aberrant expression of leptin in human endometriotic stromal cells is induced by elevated levels of hypoxia inducible factor-1alpha. *Am J Pathol* (2007) 170(2):590–8. doi: 10.2353/ajpath.2007.060477

36. Lin SC, Wang CC, Wu MH, Yang SH, Li YH, Tsai SJ. Hypoxia-induced microRNA-20a expression increases ERK phosphorylation and angiogenic gene expression in endometriotic stromal cells. *J Clin Endocrinol Metab* (2012) 97(8): E1515–1523. doi: 10.1210/jc.2012-1450

37. Hsiao KY, Chang N, Lin SC, Li YH, Wu MH. Inhibition of dual specificity phosphatase-2 by hypoxia promotes interleukin-8-mediated angiogenesis in endometriosis. *Hum Reprod* (2014) 29(12):2747–55. doi: 10.1093/humrep/deu255

38. Lin SC, Lee HC, Hsu CT, Huang YH, Li WN, Hsu PL, et al. Targeting anthrax toxin receptor 2 ameliorates endometriosis progression. *Theranostics* (2019) 9(3):620–32. doi: 10.7150/thno.30655

39. Hsiao KY, Chang N, Tsai JL, Lin SC, Tsai SJ, Wu MH. Hypoxia-inhibited DUSP2 expression promotes IL-6/STAT3 signaling in endometriosis. *Am J Reprod Immunol* (2017) 78(4):e12690. doi: 10.1111/aji.12690

40. Terzic M, Aimagambetova G, Kunz J, Bapayeva G, Aitbayeva B, Terzic S, et al. Molecular basis of endometriosis and endometrial cancer: Current knowledge and future perspectives. *Int J Mol Sci* (2021) 22(17):9274. doi: 10.3390/ijms22179274

41. Liang Y, Wu J, Wang W, Xie H, Yao S. Pro-endometriotic niche in endometriosis. *Reprod BioMed Online* (2019) 38(4):549–59. doi: 10.1016/j.rbmo.2018.12.025

42. Maurya VK, Szwarc MM, Fernandez-Valdivia R, Lonard DM, Yong S, Joshi N, et al. Early growth response 1 transcription factor is essential for the pathogenic properties of human endometriotic epithelial cells. *Reproduction* (2022) 164(2):41–54. doi: 10.1530/rep-22-0123

43. Vander Heiden MG, Cantley LC, Thompson CB. Understanding the warburg effect: the metabolic requirements of cell proliferation. *Science* (2009) 324(5930):1029–33. doi: 10.1126/science.1160809

44. Li R, Li H, Zhu L, Zhang X, Liu D, Li Q, et al. Reciprocal regulation of LOXL2 and HIF1α drives the warburg effect to support pancreatic cancer aggressiveness. *Cell Death Dis* (2021) 12(12):1106. doi: 10.1038/s41419-021-04391-3

45. Young VJ, Brown JK, Maybin J, Saunders PT, Duncan WC, Horne AW. Transforming growth factor-β induced warburg-like metabolic reprogramming may underpin the development of peritoneal endometriosis. *J Clin Endocrinol Metab* (2014) 99(9):3450–9. doi: 10.1210/jc.2014-1026

46. Xu C, Bailly-Maitre B, Reed JC. Endoplasmic reticulum stress: cell life and death decisions. *J Clin Invest* (2005) 115(10):2656–64. doi: 10.1172/jci26373

47. Zhao L, Ackerman SL. Endoplasmic reticulum stress in health and disease. *Curr Opin Cell Biol* (2006) 18(4):444–52. doi: 10.1016/j.ceb.2006.06.005

48. Gidalevitz T, Stevens F, Argon Y. Orchestration of secretory protein folding by ER chaperones. *Biochim Biophys Acta* (2013) 1833(11):2410–24. doi: 10.1016/j.bbamcr.2013.03.007

49. Eletto D, Chevet E, Argon Y, Appenzeller-Herzog C. Redox controls UPR to control redox. *J Cell Sci* (2014) 127(Pt 17):3649–58. doi: 10.1242/jcs.153643

50. Walter P, Ron D. The unfolded protein response: from stress pathway to homeostatic regulation. *Science* (2011) 334(6059):1081–6. doi: 10.1126/science.1209038

51. Cubillos-Ruiz JR, Bettigole SE, Glimcher LH. Tumorigenic and immunosuppressive effects of endoplasmic reticulum stress in cancer. *Cell* (2017) 168(4):692–706. doi: 10.1016/j.cell.2016.12.004

52. Dufey E, Bravo-San Pedro JM, Eggers C, González-Quiroz M, Urra H, Sagredo AI, et al. Genotoxic stress triggers the activation of IRE1α-dependent RNA decay to modulate the DNA damage response. *Nat Commun* (2020) 11(1):2401. doi: 10.1038/s41467-020-15694-y

53. Marciniak SJ. Endoplasmic reticulum stress: A key player in human disease. *FEBS J* (2019) 286(2):228–31. doi: 10.1111/febs.14740

54. Lebeaupin C, Vallée D, Hazari Y, Hetz C, Chevet E, Bailly-Maitre B. Endoplasmic reticulum stress signalling and the pathogenesis of non-alcoholic fatty liver disease. *J Hepatol* (2018) 69(4):927–47. doi: 10.1016/j.jhep.2018.06.008

55. Pan WW, Myers MGJr. Leptin and the maintenance of elevated body weight. *Nat Rev Neurosci* (2018) 19(2):95–105. doi: 10.1038/nrn.2017.168

56. Han J, Back SH, Hur J, Lin YH, Gildersleeve R, Shan J, et al. ER-stress-induced transcriptional regulation increases protein synthesis leading to cell death. *Nat Cell Biol* (2013) 15(5):481–90. doi: 10.1038/ncb2738

57. Yang Z, Tsuchiya H, Zhang Y, Lee S, Liu C, Huang Y, et al. REV-ERBα activates C/EBP homologous protein to control small heterodimer partner-mediated oscillation of alcoholic fatty liver. *Am J Pathol* (2016) 186(11):2909–20. doi: 10.1016/j.ajpath.2016.07.014

58. Li M, Baumeister P, Roy B, Phan T, Foti D, Luo S, et al. ATF6 as a transcription activator of the endoplasmic reticulum stress element: thapsigargin stress-induced changes and synergistic interactions with NF-y and YY1. *Mol Cell Biol* (2000) 20(14):5096–106. doi: 10.1128/mcb.20.14.5096-5106.2000

59. Zhang K, Kaufman RJ. Signaling the unfolded protein response from the endoplasmic reticulum. *J Biol Chem* (2004) 279(25):25935–8. doi: 10.1074/jbc.R400008200

60. Iurlaro R, Muñoz-Pinedo C. Cell death induced by endoplasmic reticulum stress. *FEBS J* (2016) 283(14):2640–52. doi: 10.1111/febs.13598

61. Bartoszewski R, Gebert M, Janaszak-Jasiecka A, Cabaj A, Króliczewski J, Bartoszewska S, et al. Genome-wide mRNA profiling identifies RCAN1 and GADD45A as regulators of the transitional switch from survival to apoptosis during ER stress. *FEBS J* (2020) 287(14):2923–47. doi: 10.1111/febs.15195

62. Yoshida H, Matsui T, Yamamoto A, Okada T, Mori K. XBP1 mRNA is induced by ATF6 and spliced by IRE1 in response to ER stress to produce a highly active transcription factor. *Cell* (2001) 107(7):881–91. doi: 10.1016/s0092-8674(01)00611-0

63. Yoneda T, Imaizumi K, Oono K, Yui D, Gomi F, Katayama T, et al. Activation of caspase-12, an endoplastic reticulum (ER) resident caspase, through tumor necrosis factor receptor-associated factor 2-dependent mechanism in response to the ER stress. *J Biol Chem* (2001) 276(17):13935–40. doi: 10.1074/jbc.M010677200

64. Han D, Lerner AG, Vande Walle L, Upton JP, Xu W, Hagen A, et al. IRE1alpha kinase activation modes control alternate endoribonuclease outputs to determine divergent cell fates. *Cell* (2009) 138(3):562–75. doi: 10.1016/j.cell.2009.07.017

65. Wu Q, Wu W, Jacevic V, Franca TCC, Wang X, Kuca K. Selective inhibitors for JNK signalling: A potential targeted therapy in cancer. *J Enzyme Inhib Med Chem* (2020) 35(1):574–83. doi: 10.1080/14756366.2020.1720013

66. Liu R, Li X, Huang Z, Zhao D, Ganesh BS, Lai G, et al. C/EBP homologous protein-induced loss of intestinal epithelial stemness contributes to bile duct ligation-induced cholestatic liver injury in mice. *Hepatology* (2018) 67(4):1441–57. doi: 10.1002/hep.29540

67. Rutkowski DT, Kaufman RJ. All roads lead to ATF4. *Dev Cell* (2003) 4(4):442–4. doi: 10.1016/s1534-5807(03)00100-x

68. Scull CM, Tabas I. Mechanisms of ER stress-induced apoptosis in atherosclerosis. *Arterioscler Thromb Vasc Biol* (2011) 31(12):2792–7. doi: 10.1161/atvbaha.111.224881

69. Novoa I, Zeng H, Harding HP, Ron D. Feedback inhibition of the unfolded protein response by GADD34-mediated dephosphorylation of eIF2alpha. *J Cell Biol* (2001) 153(5):1011–22. doi: 10.1083/jcb.153.5.1011

70. Malhi H, Kaufman RJ. Endoplasmic reticulum stress in liver disease. *J Hepatol* (2011) 54(4):795–809. doi: 10.1016/j.jhep.2010.11.005

71. Bi M, Naczki C, Koritzinsky M, Fels D, Blais J, Hu N, et al. ER stress-regulated translation increases tolerance to extreme hypoxia and promotes tumor growth. *EMBO J* (2005) 24(19):3470–81. doi: 10.1038/sj.emboj.7600777

72. Wouters BG, Koritzinsky M. Hypoxia signalling through mTOR and the unfolded protein response in cancer. *Nat Rev Cancer* (2008) 8(11):851–64. doi: 10.1038/nrc2501

73. Liu L, Wise DR, Diehl JA, Simon MC. Hypoxic reactive oxygen species regulate the integrated stress response and cell survival. *J Biol Chem* (2008) 283(45):31153–62. doi: 10.1074/jbc.M805056200

74. Pereira ER, Frudd K, Awad W, Hendershot LM. Endoplasmic reticulum (ER) stress and hypoxia response pathways interact to potentiate hypoxia-inducible factor 1 (HIF-1) transcriptional activity on targets like vascular endothelial growth factor (VEGF). *J Biol Chem* (2014) 289(6):3352–64. doi: 10.1074/jbc.M113.507194

75. Fels DR, Koumenis C. The PERK/eIF2alpha/ATF4 module of the UPR in hypoxia resistance and tumor growth. *Cancer Biol Ther* (2006) 5(7):723–8. doi: 10.4161/cbt.5.7.2967

76. Dekervel J, Bulle A, Windmolders P, Lambrechts D, Van Cutsem E, Verslype C, et al. Acriflavine inhibits acquired drug resistance by blocking the epithelial-to-Mesenchymal transition and the unfolded protein response. *Transl Oncol* (2017) 10(1):59–69. doi: 10.1016/j.tranon.2016.11.008

77. Spiotto MT, Banh A, Papandreou I, Cao H, Galvez MG, Gurtner GC, et al. Imaging the unfolded protein response in primary tumors reveals microenvironments with metabolic variations that predict tumor growth. *Cancer Res* (2010) 70(1):78–88. doi: 10.1158/0008-5472.Can-09-2747

78. Chen X, Iliopoulos D, Zhang Q, Tang Q, Greenblatt MB, Hatziapostolou M, et al. XBP1 promotes triple-negative breast cancer by controlling the HIF1α pathway. *Nature* (2014) 508(7494):103–7. doi: 10.1038/nature13119

79. Guzel E, Arlier S, Guzeloglu-Kayisli O, Tabak MS, Ekiz T, Semerci N, et al. Endoplasmic reticulum stress and homeostasis in reproductive physiology and pathology. *Int J Mol Sci* (2017) 18(4):792. doi: 10.3390/ijms18040792

80. Ekiz-Yilmaz T, Isildar B, Gezer A, Kankaya D, Cansiz-Ersoz C, Kayisli UA, et al. The role of unfolded protein response in the pathogenesis of endometriosis: contribution of peritoneal fluid. *Reprod BioMed Online* (2021) 42(1):1–15. doi: 10.1016/j.rbmo.2020.09.012

81. Taylor RN, Yu J, Torres PB, Schickedanz AC, Park JK, Mueller MD, et al. Mechanistic and therapeutic implications of angiogenesis in endometriosis. *Reprod Sci* (2009) 16(2):140–6. doi: 10.1177/1933719108324893

82. Alzahrani AS. PI3K/Akt/mTOR inhibitors in cancer: At the bench and bedside. *Semin Cancer Biol* (2019) 59:125–32. doi: 10.1016/j.semcancer.2019.07.009

83. Lin S, Yang L, Shi H, Du W, Qi Y, Qiu C, et al. Endoplasmic reticulum-targeting photosensitizer hypericin confers chemo-sensitization towards oxaliplatin through inducing pro-death autophagy. *Int J Biochem Cell Biol* (2017) 87:54–68. doi: 10.1016/j.biocel.2017.04.001

84. Xu X, Huang E, Tai Y, Zhao X, Chen X, Chen C, et al. Nupr1 modulates methamphetamine-induced dopaminergic neuronal apoptosis and autophagy through CHOP-Trib3-Mediated endoplasmic reticulum stress signaling pathway. *Front Mol Neurosci* (2017) 10:203. doi: 10.3389/fnmol.2017.00203

85. Kapoor R, Stratopoulou CA, Dolmans MM. Pathogenesis of endometriosis: New insights into prospective therapies. *Int J Mol Sci* (2021) 22(21):11700. doi: 10.3390/ijms222111700

86. Reis FM, Petraglia F, Taylor RN. Endometriosis: hormone regulation and clinical consequences of chemotaxis and apoptosis. *Hum Reprod Update* (2013) 19(4):406–18. doi: 10.1093/humupd/dmt010

87. Choi JY, Jo MW, Lee EY, Lee DY, Choi DS. Ovarian steroid dependence of endoplasmic reticulum stress involvement in endometrial cell apoptosis during the human endometrial cycle. *Reproduction* (2018) 155(6):493–503. doi: 10.1530/rep-17-0713

88. Attia GR, Zeitoun K, Edwards D, Johns A, Carr BR, Bulun SE. Progesterone receptor isoform a but not b is expressed in endometriosis. *J Clin Endocrinol Metab* (2000) 85(8):2897–902. doi: 10.1210/jcem.85.8.6739

89. Bulun SE, Cheng YH, Yin P, Imir G, Utsunomiya H, Attar E, et al. Progesterone resistance in endometriosis: link to failure to metabolize estradiol. *Mol Cell Endocrinol* (2006) 248(1-2):94–103. doi: 10.1016/j.mce.2005.11.041

90. Choi J, Jo M, Lee E, Lee DY, Choi D. Dienogest regulates apoptosis, proliferation, and invasiveness of endometriotic cyst stromal cells via endoplasmic reticulum stress induction. *Mol Hum Reprod* (2020) 26(1):30–9. doi: 10.1093/molehr/gaz064

91. Dioufa N, Kassi E, Papavassiliou AG, Kiaris H. Atypical induction of the unfolded protein response by mifepristone. *Endocrine* (2010) 38(2):167–73. doi: 10.1007/s12020-010-9362-0

92. Park S, Lim W, Bazer FW, Song G. Apigenin induces ROS-dependent apoptosis and ER stress in human endometriosis cells. *J Cell Physiol* (2018) 233(4):3055–65. doi: 10.1002/jcp.26054v

frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 20 October 2022
DOI 10.3389/fendo.2022.942368

OPEN ACCESS

EDITED BY
Rajakumar Anbazhagan,
Eunice Kennedy Shriver National Institute of Child Health and Human Development (NIH), United States

REVIEWED BY
Asamanja Chattoraj,
Kazi Nazrul University, India
Jenny Fung,
School of Biomedical Sciences, The University of Queensland, Australia
Ramya Billur,
University of Pennsylvania, United States

*CORRESPONDENCE
Jongkil Joo
jkjoo@pusan.ac.kr
Ki-Tae Ha
hagis@pusan.ac.kr

†These authors have contributed equally to this work

SPECIALTY SECTION
This article was submitted to Reproduction, a section of the journal Frontiers in Endocrinology

RECEIVED 12 May 2022
ACCEPTED 07 October 2022
PUBLISHED 20 October 2022

CITATION
Bae S-J, Jo Y, Cho MK, Jin J-S, Kim J-Y, Shim J, Kim YH, Park J-K, Ryu D, Lee HJ, Joo J and Ha K-T (2022) Identification and analysis of novel endometriosis biomarkers *via* integrative bioinformatics. *Front. Endocrinol.* 13:942368. doi: 10.3389/fendo.2022.942368

Identification and analysis of novel endometriosis biomarkers *via* integrative bioinformatics

Sung-Jin Bae[1†], Yunju Jo[2†], Min Kyoung Cho[3], Jung-Sook Jin[3], Jin-Young Kim[4], Jaewon Shim[5], Yun Hak Kim[6,7], Jang-Kyung Park[8], Dongryeol Ryu[2], Hyun Joo Lee[9], Jongkil Joo[9*] and Ki-Tae Ha[3,4*]

[1]Department of Molecular Biology and Immunology, Kosin University College of Medicine, Busan, South Korea, [2]Department of Molecular Cell Biology, Sungkyunkwan University School of Medicine, Suwon, South Korea, [3]Korean Medical Research Center for Healthy Aging, Pusan National University, Yangsan, South Korea, [4]Department of Korean Medical Science, School of Korean Medicine, Pusan National University, Yangsan, South Korea, [5]Department of Biochemistry, Kosin University College of Medicine, Busan, South Korea, [6]Department of Anatomy, School of Medicine, Pusan National University, Yangsan, South Korea, [7]Department of Biomedical Informatics, School of Medicine, Pusan National University, Yangsan, South Korea, [8]Department of Korean Medicine Obstetrics and Gynecology, Pusan National University Korean Medicine Hospital, Yangsan, South Korea, [9]Department of Obstetrics and Gynecology, Pusan National University Hospital, Busan, South Korea

Endometriosis is a gynecological disease prevalent in women of reproductive age, and it is characterized by the ectopic presence and growth of the eutopic endometrium. The pathophysiology and diagnostic biomarkers of endometriosis have not yet been comprehensively determined. To discover molecular markers and pathways underlying the pathogenesis of endometriosis, we identified differentially expressed genes (DEGs) in three Gene Expression Omnibus microarray datasets (GSE11691, GSE23339, and GSE7305) and performed gene set enrichment analysis (GSEA) and protein–protein interaction (PPI) network analyses. We also validated the identified genes *via* immunohistochemical analysis of tissues obtained from patients with endometriosis or healthy volunteers. A total of 118 DEGs (79 upregulated and 39 downregulated) were detected in each dataset with a lower (fold change) FC cutoff ($\log2|FC| > 1$), and 17 DEGs (11 upregulated and six downregulated) with a higher FC cutoff ($\log2|FC| > 2$). KEGG and GO functional analyses revealed enrichment of signaling pathways associated with inflammation, complement activation, cell adhesion, and extracellular matrix in endometriotic tissues. Upregulation of seven genes (*C7*, *CFH*, *FZD7*, *LY96*, *PDLIM3*, *PTGIS*, and *WISP2*) out of 17 was validated *via* comparison with external gene sets, and protein expression of four genes (*LY96*, *PDLIM3*, *PTGIS*, and *WISP2*) was further analyzed by immunohistochemistry and western blot analysis. Based on these results, we suggest that TLR4/NF-κB and Wnt/frizzled signaling pathways, as

well as estrogen receptors, regulate the progression of endometriosis. These pathways may be therapeutic and diagnostic targets for endometriosis.

KEYWORDS

endometriosis, LY96, PDLIM3, PTGIS, TLR4/NF-κB, Wnt/frizzled, estrogen receptor

Introduction

Endometriosis is a common gynecological disorder in which endometrial tissue grows outside the uterus (1). Endometriosis affects around 10% of women of reproductive age and often causes dysmenorrhea, chronic pelvic pain, and infertility (1, 2). Despite the widespread acceptance of the retrograde menstruation theory proposed by Sampson in 1927, the pathogenesis of endometriosis remains poorly understood (3). Thus, various other factors, including genetic, epigenetic, stem cell, inflammatory, angiogenic, and immunological factors, should be considered to better understand the complex pathophysiology of endometriosis (3, 4). Gynecological surgery is the major therapeutic option for endometriosis treatment. Oral contraceptives, progestins, nonsteroidal anti-inflammatory drugs, and gonadotropin-releasing hormone agonists are alternative treatment options (2, 5). However, the efficacy of these treatment strategies, whether surgical or non-surgical, is still limited due to the high recurrence rate of the disease.

Despite its high prevalence, the diagnosis of endometriosis is often delayed as it has no symptoms distinct from those of ordinary menstrual cramps (6, 7). Histopathological examination by invasive laparoscopy or surgery is the gold standard for diagnosis of endometriosis (2). The identification of diagnostic biomarkers is thus urgently required to improve the diagnosis and treatment of patients with endometriosis. Previous reviews on this topic have focused on identification of potential biomarker candidates from specimens such as peritoneal fluid, blood, urine, and endometrial biopsies (6–9), highlighting several factors as noninvasive diagnostic biomarkers, including growth factors, hormones, cytokines, complements, glycoproteins, and antibodies. However, these biomarkers are merely used to supplement diagnosis of endometriosis, as none of them has demonstrated sufficient sensitivity and specificity (10).

Integration of different types of omics data is routinely utilized to discover and validate novel disease biomarkers (11, 12). Potential diagnostic biomarkers and therapeutic targets of endometriosis have been proposed in such integrative bioinformatics studies based on the identification of differentially expressed genes (DEGs) (13–16). However, identification of common biomarkers that are consistently detected in all datasets is difficult due to the heterogeneity among independent datasets. Here, three microarray datasets (GSE11691, GSE23339, and GSE7305), which include gene expression data from normal endometrial and endometriosis tissues, were obtained from the Gene Expression Omnibus (GEO) database. Non-biased bioinformatics analyses, including identification of DEGs, gene set enrichment analysis (GSEA), and protein–protein interaction (PPI) network analysis, were conducted, and the findings were further validated by analyzing immunohistochemistry (IHC) and western blot analysis of tissue specimens obtained from patients with endometriosis or healthy volunteers. Based on the obtained results, we propose six biomarkers as potential targets for the diagnosis and treatment of endometriosis.

Materials and methods

Data collection

The datasets for analysis in this study were chosen according to the inclusion and exclusion criteria summarized in Supplementary Figure 1. Briefly, the gene sets were obtained by searching the Gene Expression Omnibus (GEO; https://www.ncbi.nlm.nih.gov/geo) database and filtered by three different criteria, including characteristics of data, experiment, and sample. Among six datasets filtered by three criteria, GSE11691, GSE23339, and GSE7305 were applied for DEG and GSEA analysis. Whereas GSE135485 and GSE25628 were used as validation datasets due to their imbalanced and small sample sizes, GSE6364 was filtered out as it contained the data from normal endometrium but not endometriosis. The gene expression data used in this study (GSE11691, GSE23339, GSE7305, GSE135485, and GSE25628) were downloaded from the GEO database, and a total of 128 samples were collected. GSE11691 using the GPL96 platform includes data obtained from nine endometriosis and normal uterine endometrium samples, respectively (17). GSE23339 includes data derived from 10 endometriosis and nine normal uterine endometrium samples using the GPL6102 platform I (18). GSE7305 using the GPL570 platform includes data collected from 10 endometriosis and normal uterine endometrium samples, respectively (19).

GSE135485 includes data collected from 54 endometriosis and four normal uterine endometrium samples using the GPL21290 platform. GSE25628 includes data from seven endometriosis and six normal uterine endometrium samples using the GPL571 platform (20). Detailed information on the datasets is summarized in Table 1.

Data processing and identification of DEGs

Transcriptome analysis was conducted using R (version 4.1.1) *via* RStudio (Desktop version, 1.4.1717). The three datasets selected for DEG identification (GSE11691, GSE23339, and GSE7305) were downloaded from the GEO database of the National Center for Biotechnology Information using the GEOquery R package (17–19, 21). Multiple probes related to the same gene were reduced to one, and summarized as median values for further analysis. Since gene expression profiles differed between samples included in GSE11691, quantile normalization was applied using the preprocessCore R package (https://github.com/bmbolstad/preprocessCore). DEGs were defined as genes with adjusted *p*-values and Log2|FoldChange| (Log|FC|) less and greater than 0.05 and 1, respectively. We adjusted *p*-value to correct the false positive error caused by the multiple tests and calculated it by the Benjamini & Hochberg method (22), which is one of the popular tools to minimize the false discovery rate. The cutoff criteria that we applied was 0.1. For analysis of the pathway and PPI of DEGs, we adopted Log|FC| > 1 and used Log|FC| > 2 for heatmap and network analysis.

Pathway enrichment analysis

Analysis of Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) biological processes of DEGs were performed using the JEPETTO plugin (version 1.3.1) of Cytoscape (version 3.8.2). Visualization was performed by constructing a scatter plot with XD-score and *q*-value as axes. *q*-value < 0.25 was used as the cutoff criterion, according to GSEA guidance (https://software.broadinstitute.org/cancer/software/gsea/wiki/index.php/FAQ).

PPI network analysis

The interactions of proteins encoded by the DEGs were identified using the STRING plugin (version 1.7.0) of Cytoscape by using "*Homo sapiens*" as search keyword, and a confidence score cutoff higher than 0.4. GeneMANIA plugin (version 3.5.2) was used to identify physical interactions. Visualization of the network was performed using Cytoscape.

Heatmap construction and network analysis of DEGs

A heatmap of the top 17 DEGs identified in each dataset was generated using Morpheus, a versatile matrix visualization and analysis software from the Broad Institute at the Massachusetts Institute of Technology (https://software.broadinstitute.org/morpheus/).

Network analysis was performed as previously described (23). Briefly, the pattern of co-expression between the normal and endometriosis groups was visualized based on Spearman's correlation. For this purpose, dplyr, stringr, ggpubr, ggplot2, igraph, ggraph, corrr, corrplot, tidyverse, and reshape2 R packages were applied.

GSEA

GSEA of each gene expression dataset was performed using the GSEA software (version 4.1.0) from the Broad Institute at the Massachusetts Institute of Technology (https://www.gsea-msigdb.org/gsea/index.jsp). The absolute value of the normalized enrichment score (NES), the enrichment score for the gene set after it had been normalized across analyzed gene sets, was set to > 1.5 as the cutoff criteria. The false discovery rate (FDR) *q*-value, which represents the estimated probability that the normalized enrichment score constitutes a false-positive

TABLE 1 Details of endometriosis GEO data used in this study.

GEO	Author (year)	Platform	Method	Samples	Normal	Endometriosis	PMID
GSE11691	Hull et al (2008)	GPL96	Microarray	Endometrium	9	9	18688027
GSE23339	Hawkins et al (2011)	GPL6102	Microarray	Endometrium	9	10	21436257
GSE7305	Hever et al. (2007)	GPL570	Microarray	Endometrium	10	10	17640886
GSE135485	Yana et al. (2019)	GPL21290	NGS	Endometrium	4	53	–
GSE25628	Crispi et al. (2013)	GPL571	Microarray	Endometrium	6	7	23460397

finding, was set to < 0.02. GO network analyses were performed on each GSEA by utilizing Cytoscape to visualize enrichment maps.

Clinical sample collection

Laparoscopic surgeries were performed at the Department of Obstetrics and Gynecology of Pusan National University Hospital (Busan, Korea) between June 2019 and December 2020. The surgical process was performed during the early follicular phase to rule out the potential early pregnancy and the possibility of the ovarian cyst being functional (24, 25). To obtain normal uterine endometrial tissue as the control group, we gathered the patients with male-factor infertility with normal gynecologic anatomy who underwent endometrial scratch prior to proceeding to programmed or natural embryo-transfer cycle, and the patients with hormone-independent ovarian cysts such as mature teratoma or cystadenoma who underwent laparoscopy and presented no endometriotic lesion in the pelvic cavity. The exclusion criteria were irregular menstrual periods, the presence of endocrine disorders such as hyperprolactinemia or thyroid dysfunction, and medication history with dysmenorrhea management such as GnRH analogs, oral contraceptives, or progestins during the past three months to the recruitment. Eligible patients were further examined and proven morphologically free from possible asymptomatic endometriosis and other hormone-dependent gynecological pathologies, such as uterine adenomyosis and leiomyoma, by undergoing imaging studies including gynecological ultrasonography, abdominal computed tomography scans and/or pelvic magnetic resonance imaging. All specimens from the control group were further histologically assessed and confirmed as normal endometrial tissue by pathologists. Endometriosis was pathologically diagnosed in tissue specimens derived from 32 patients. Endometriosis was classified into stages I-IV according to the revised American Society for Reproductive Medicine (r-ASRM) classification system. The average stage detected in the samples was approximately III. The average age of the patients was 33.33 ± 7.53 years, whereas the average BMI was 21.51 ± 3.52. Samples of normal endometrial tissue were obtained from ten healthy volunteers who have not been diagnosed with endometriosis. The average age of the volunteers was 30.3 ± 4.9 years, whereas the average BMI was 22.06 ± 2.06. There was no statistically significant difference between the average age and BMI values of the two groups (with *p*-values of 0.16 and 0.56). Tissue specimens were deposited in the Biobank of the Pusan National University Hospital. Detailed information on the patient and normal volunteer is summarized in Supplementary Table 1.

This study was approved by the Institutional Review Board (IRB) of Pusan National University Hospital (2104-009-101). All patients signed informed consents for the study protocol. All procedures were conducted in accordance with IRB guidelines.

IHC analysis

Fresh tissues collected by the laparoscopic surgeries were rinsed with normal saline to remove blood and impurities, and rapidly frozen and stored in the Biobank of Pusan National University Hospital. The donated specimens were fixed with 4% formaldehyde solution, and processed to obtain paraffin embedded tissue blocks. Five–µm thick sections of tissue blocks were deparaffinized by soaking in xylene and gradient ethanol solution. The sections were then incubated with primary antibodies, including anti-lymphocyte antigen 96 (LY96; 1:200; ab22048, Abcam), anti-PDZ And LIM Domain 3 (PDLIM3; 1:200; HPA004749, Atlas Antibodies, Bromma, Sweden), anti-prostaglandin I2 synthase (PTGIS; 1:200; ab23668, Abcam), and anti-WNT1-inducible-signaling pathway protein 2 (WISP2; 1:200; ab28317, Abcam) at 4°C overnight. The sections were then incubated with Dako REAL EnVision Detection System (K5007; Dako, Jena, Germany) for 1 h. After rinsing with phosphate-buffered saline (PBS), immunostaining was visualized using DAB+ chromogen buffer (K5007, Dako). The slides were counterstained with hematoxylin solution, and representative images were taken using an optical microscope (Axio Scope A1; Carl Zeiss, Oberkochen, Germany). Histopathological scoring was performed through examination under a light microscope by a pathologist. Staining intensity was classified as follows: 0, negative immunostaining; 1, weak expression level; 2, moderate expression level; 3, strong expression level; and 4, very strong expression level.

Western blot analysis

Total proteins were extracted from frozen tissue specimens using protein lysis buffer containing 10 mM HEPES pH 7.45, 150 mM sodium chloride, 1% (*w*/*v*) NP–40, 5 mM sodium pyrophosphate, 5 mM sodium fluoride, and 2 mM sodium vanadate with a protease inhibitor cocktail (Roche Applied Science, Penzburg, Germany). 30 µg of protein lysates was electrophoresed by sodium dodecyl sulfate–polyacrylamide gel and transferred onto nitrocellulose membranes (0.45 µm; ThermoFisher Scientific, Waltham, MA). The membranes were blocked with 5% (*w*/*v*) non-fat dry milk and incubated with primary antibodies against target proteins, including LY96, PDLIM3, PTGIS, and WISP2 at 4°C overnight. The membranes

were washed and incubated with proper secondary antibodies conjugated with horseradish peroxidase. The bands of interesting proteins were detected with the enhanced chemiluminescence Plus kit (ThermoFisher Scientific) using ImageQuant LAS 4000 imaging system (GE healthcare, Chicago, IL).

Identification of potential druggable genes

The Drug-Gene Interaction Database (DGIdb, http://www.dgidb.org) is an online database that facilitates interpretation of the results of genome-wide studies, and generation of hypotheses in the context of druggable genome (26). DGIbd was used to identify potentially druggable genes from an input list of genes including the statistically significant DEGs.

Statistical analysis

To evaluate the statistical difference between the two groups, Student's *t*-test was performed using GraphPad Prism (version 5.01; GraphPad Software, San Diego, CA, USA). Statistical significance was set at $p < 0.05$.

Results

Identification of DEGs

We first determined the distributions of gene expression levels in three human endometrial transcriptome datasets, GSE11691, GSE23339, and GSE7305 (17–19). The transcriptome profiles of GSE11691 showed the highest level of variation among the three transcriptomes. Thus, we conducted quantile normalization to avoid artifacts in subsequent analyses. The gene expression data included in GSE23339 and GSE7305 datasets were converted to Log2 scale (Figure 1A), and DEGs were then identified using these three datasets. As highlighted in the colored volcano plots (Figure 1B), a total of 536 (361 upregulated and 175 downregulated), 1,042 (562 upregulated and 480 downregulated), and 1,515 (882 upregulated and 633 downregulated) DEGs were identified in GSE11691, GSE23339, and GSE7305, respectively (Figure 1C). Moreover, integration of DEGs shared across the datasets revealed 118 (79 upregulated and 39 downregulated; Figure 1C) and 17 (11 upregulated and six downregulated; Figure 1D) common DEGs with a lower (fold change) FC cutoff (log2|FC| > 1) and a higher FC cutoff (log2|FC| > 2), respectively (Table 2).

Pathway enrichment analysis

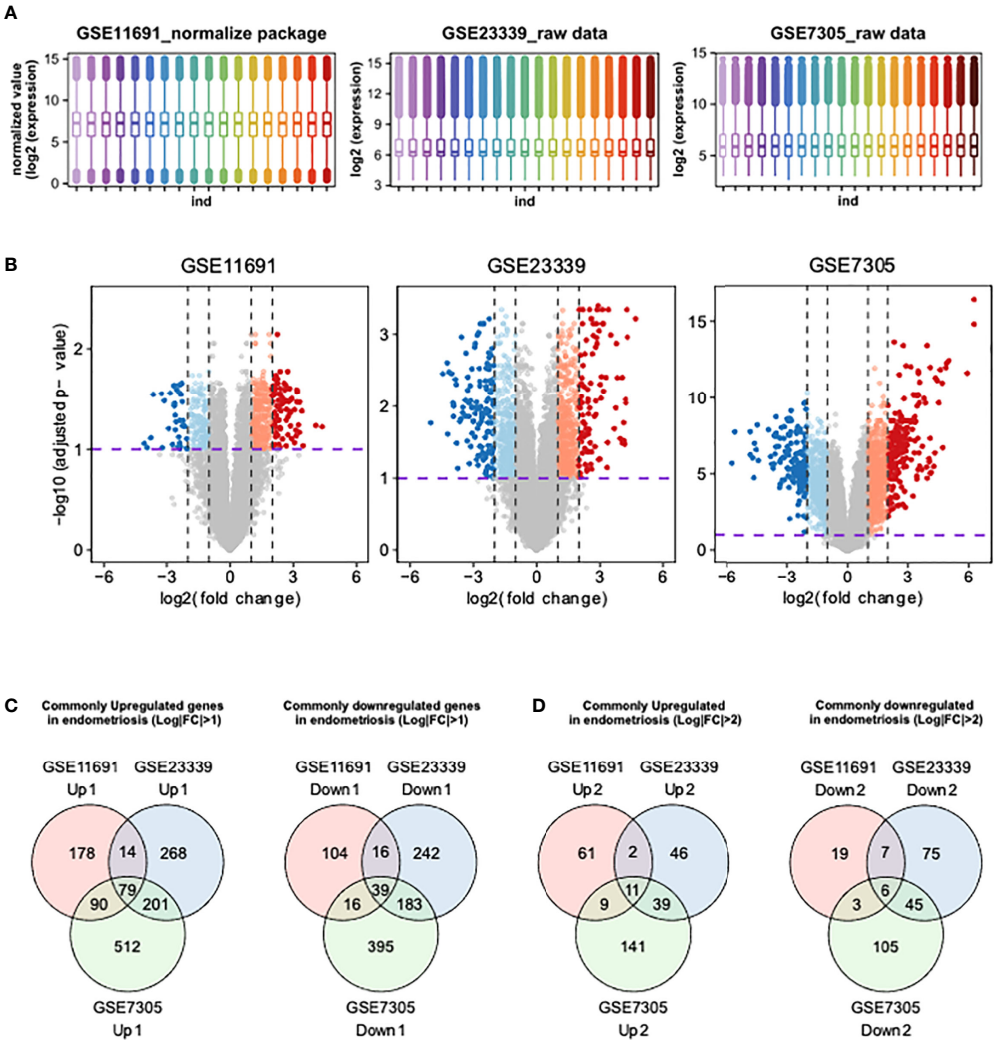
To elucidate the pathways and molecular functions related to the 118 common DEGs identified above, KEGG and GO enrichment analyses were conducted. The analysis results revealed the involvement of the identified DEGs in the extracellular matrix, cell adhesion, complement activation, immune response, and inflammation processes (Figure 2A).

PPI network analysis

Interactions between proteins encoded by 118 DEGs identified above were analyzed using the STRING and GeneMANIA plugins of Cytoscape. STRING analysis resulted in a network comprising 118 nodes (genes) and 197 edges (interactions). A total of 37 nodes did not have any edges, and four nodes had only few edges. The rest of the network, including 77 genes and 195 interactions is shown in Figure 2B. The genes *AP1M2*, *BGN*, *C3*, *C3AR1*, *CCL2*, *CD14*, *CD163*, *COL14A1*, *FCGR2A*, *FGL2*, *FN1*, *LYZ*, *MS4A4A*, *RNASE6*, *TYROBP*, and *VCAM1* had relatively higher number of interactions (over four edges). GeneMANIA analysis resulted in a network consisting of 138 nodes and 72 edges. Among these nodes, 77 genes did not have any physical or pathway interactions, and 24 genes had merely simple interactions. The main interaction network harboring 37 nodes and 57 edges is shown in Figure 2C. The genes showing relatively higher number of interactions (over three edges) in the network were identified to be *C3, CFH, CNDP2, COL11A1, COL14A1, FABP4, FN1, HLA-DPA1, HLA-DPB1, HLA-DQA1, HLA-DRA, ITGB8, LAMA4, LY96, and TAGLN*. STRING analysis revealed *CCL2* as significant (with over three edges), based on 15 common DEGs with a cutoff value of Log2|FC| > 2. On the other hand, GeneMANIA revealed that three proteins encoded by *AGR2, FZD7*, and *LY96* were in physical or pathway contact with each other (Supplementary Figure 2).

Network analysis of DEGs

The heatmaps (Figure 3A) depicted the expression levels of the 17 frequent DEGs with *p*-values less than 0.05 and Log2|FC| more than 2, clearly illustrating the differential expression patterns of those selected genes in endometriosis compared to



FIGURES 1
Identification of DEGs using integrative bioinformatics analysis. **(A)** The distribution of transcriptome obtained from GSE11691, GSE23339, and GSE7305 were shown. **(B)** The volcano plots show the DEGs from GSE11691, GSE23339, and GSE7305. Red indicates relative upregulated genes with *p*-value < 0.05 and Log2|FC| > 2, orange indicates relative upregulated genes with *p*-value < 0.05 and 1 < Log2|FC| ≤ 2, blue indicates relative downregulated genes with *p* < 0.05 and Log2|FC| > 2, and light cyan indicates relative downregulated genes with *p* < 0.05 and 1 < Log2|FC| ≤ 2. **(C, D)** Venn diagrams of upregulated and downregulated genes DEGs from three indicated datasets. The cutoff values of Log2|FC| were set to 1 **(C)** and 2 **(D)**.

TABLE 2 The list of identified DEGs in three endometriosis datasets, GSE11691, GSE23339, and GSE7305.

DEGs	Gene names
Up-regulated (27)	*AEBP1, AGTR1, BGN, C3, C3AR1, C7, CCL2, CD14, CD163, CFH, CHL1, COL11A1, COL14A1, COLEC12, CPE, CPVL, CSTA, CXCL12, DKK3, DPYD, DPYSL3, DSE, ELMO1, EVI2B, FABP4, FAM129A, FCGR2A, FCHSD2, FGL2, FMO1, FMO2, FMOD, FN1, FRZB, FZD7, GAS1, HEG1, HLA_DPA1, HLA_DPB1, HLA_DQA1, HLA_DRA, IGJ, IL4R, ITM2A, KCTD12, LAMA4, LHFP, LTBP2, LY96, LYZ, MEIS2, MMP23A, MN1, MNDA, MS4A4A, MS4A6A, NUAK1, OLFML1, PDE1A, PDGFRL, PDLIM3, PLSCR4, PLXDC2, PRELP, PTGER4, PTGIS, RNASE1, RNASE6, ROBO3, SGCE, SULF1, TAGLN, TCEAL2, THBS2, TNFSF14, TYROBP, VCAM1, VSIG4, WISP2*
Down-regulated (28)	*ACSL5, AGR2, AP1M2, BTBD3, CDS1, CLDN10, CLDN3, CNDP2, DEFB1, EDN3, ELF3, GALNT4, GRAMD1C, GRHL2, HMGCR, HOOK1, HOXB6, HPN, HSD17B2, IL20RA, IRF6, ITGB8, KIAA1324, MME, OSR2, PAPSS1, PEMT, PERP, PLS1, PPM1H, PRSS16, PRSS8, PTPN3, RAB25, SH3YL1, SLC3A1, SPINT2, TPD52L1, TSPAN1*

Red and blue characters indicate genes enriched in endometriosis and normal endometrial tissues, respectively (log2|FC|>2.0).

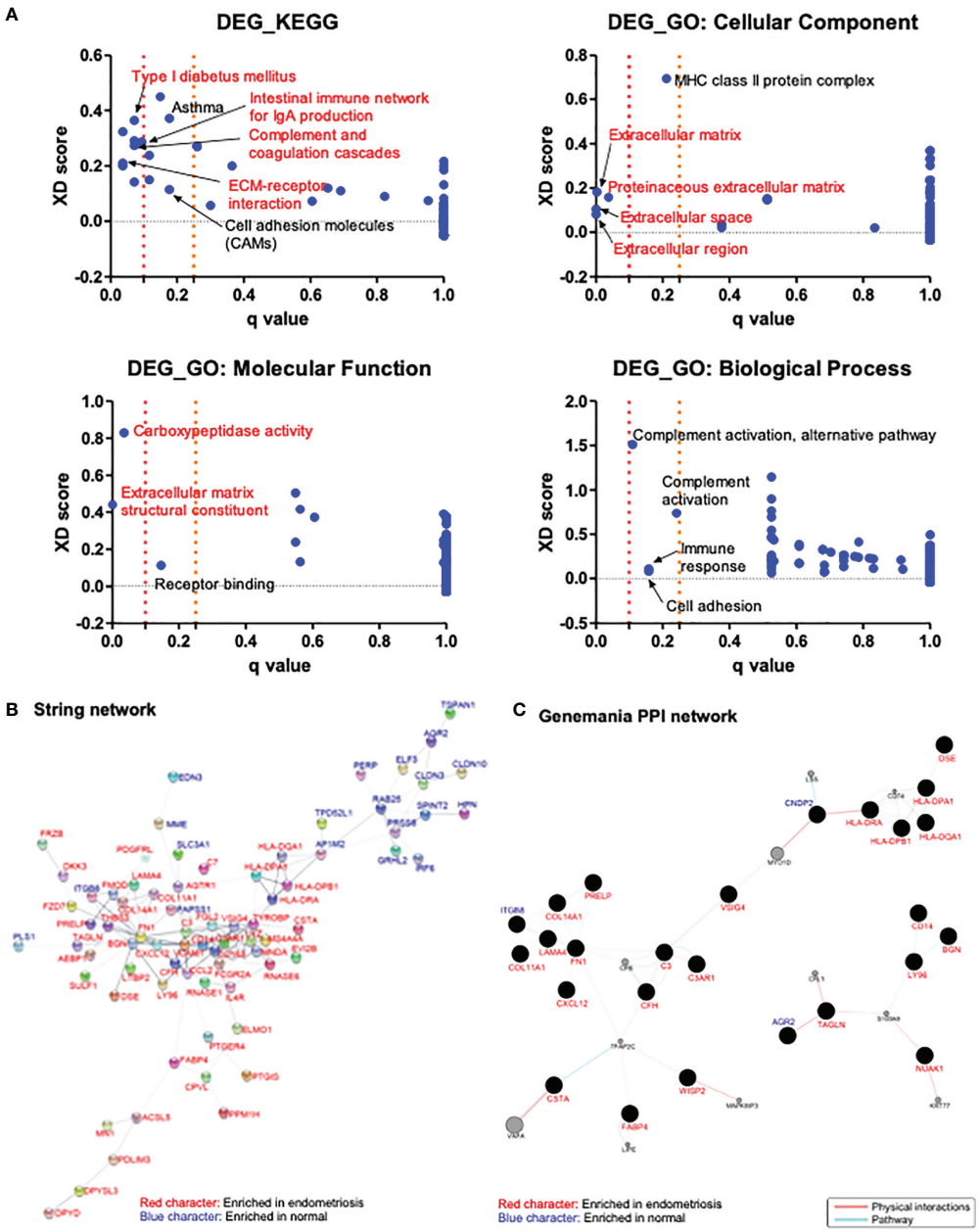


FIGURE 2
Analysis of the enriched pathways and interaction networks of DEGs in endometriosis. **(A)** KEGG and GO pathway analysis of DEGs from GSE11691, GSE23339, and GSE7305 datasets with Log2|FC| > 1 were performed with JEPETTO plugin of Cytoscape, and scatter plot were constructed with *q*-value and XD-score as *x*- and y-axis, respectively. Red character indicates the pathways with *q*-value < 0.1 and black character indicates the pathways with 0.1 < *q*-value < 0.25. **(B, C)** Protein–protein interaction network of DEGs from three datasets with Log2|FC| > 1 were analyzed in Cytoscape with plugins of STRING **(B)** and GeneMANIA **(C)**. Red character indicates genes enriched in endometriosis and blue character indicates genes enriched in normal.

normal control across three independent datasets. In addition, gene networks showing the co-expression patterns of the selected genes were constructed based on Spearman's correlation. The 17 DEGs were highly associated with one another in three independent datasets from normal tissues, and thereby generated a massive co-expression gene network (Figure 3B). On the other hand, the number of correlations, shown as edges, was drastically reduced in ectopic lesions of

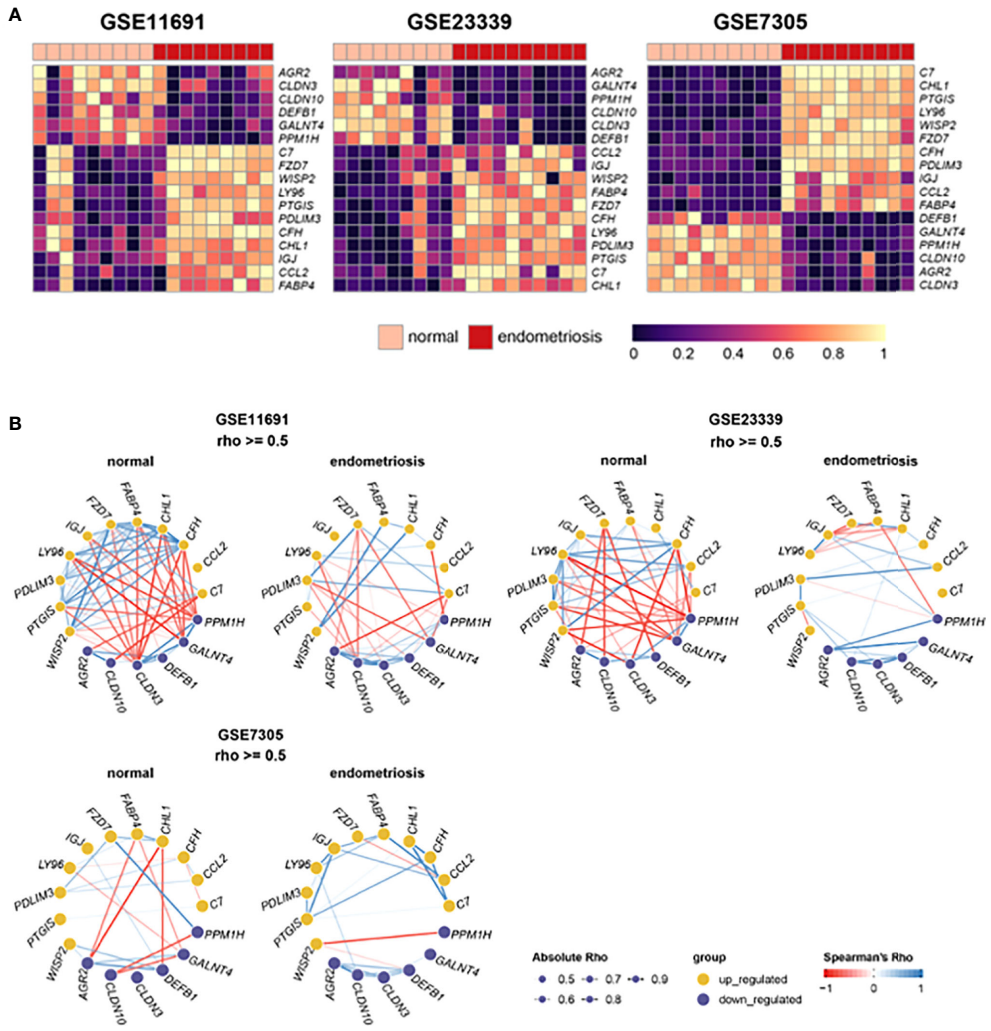


FIGURE 3
Expression and correlation of 22 DEGs with Log2|FC| > 2. **(A)** Heat maps show the expression levels of 22 DEGs in datasets, GSE11691, GSE23339, and GSE7305. **(B)** Gene networks display correlations of 22 DEGs in the normal and endometrial tissues of each dataset. The depth of edges indicates absolute Rho ranging from 0.5 to 1. The color of edges indicates co-expression ranging from -1 (red) to 1 (blue) by Spearman's Rho.

endometriosis patients compared to normal sites. Interestingly, these patterns of gene networks with decreased correlations under endometriosis were comparable across the three independent datasets. Hence, the decrease in correlations between these 17 DEGs may contribute to the pathophysiology of endometriosis.

Identification of functional pathways by GSEA

GSEA was carried out to identify functional pathways in endometriosis that are shared across the three datasets analyzed (GSE11691, GSE23339, and GSE7305). A total of 11 common pathways were identified in the hallmark analysis, including 9 upregulated and 2 downregulated pathways (Figure 4A). Inflammation-related pathways such as IL6-JAK/STAT3 signaling, inflammatory response, interferon α response, interferon γ response, and TNFα signaling *via* NF-κB, were found to be significantly upregulated in patients with endometriosis. In addition, nine common pathways were found to be upregulated in KEGG analysis (Figure 4B). Further, inflammation-related pathways including chemokine signaling pathway and NOD-like receptor signaling pathway, cytokine-cytokine receptor interaction, viral myocarditis, Leishmania infection, and asthma were found to be upregulated in patients with endometriosis. In GO network analysis, inflammation-related pathways, such as immune

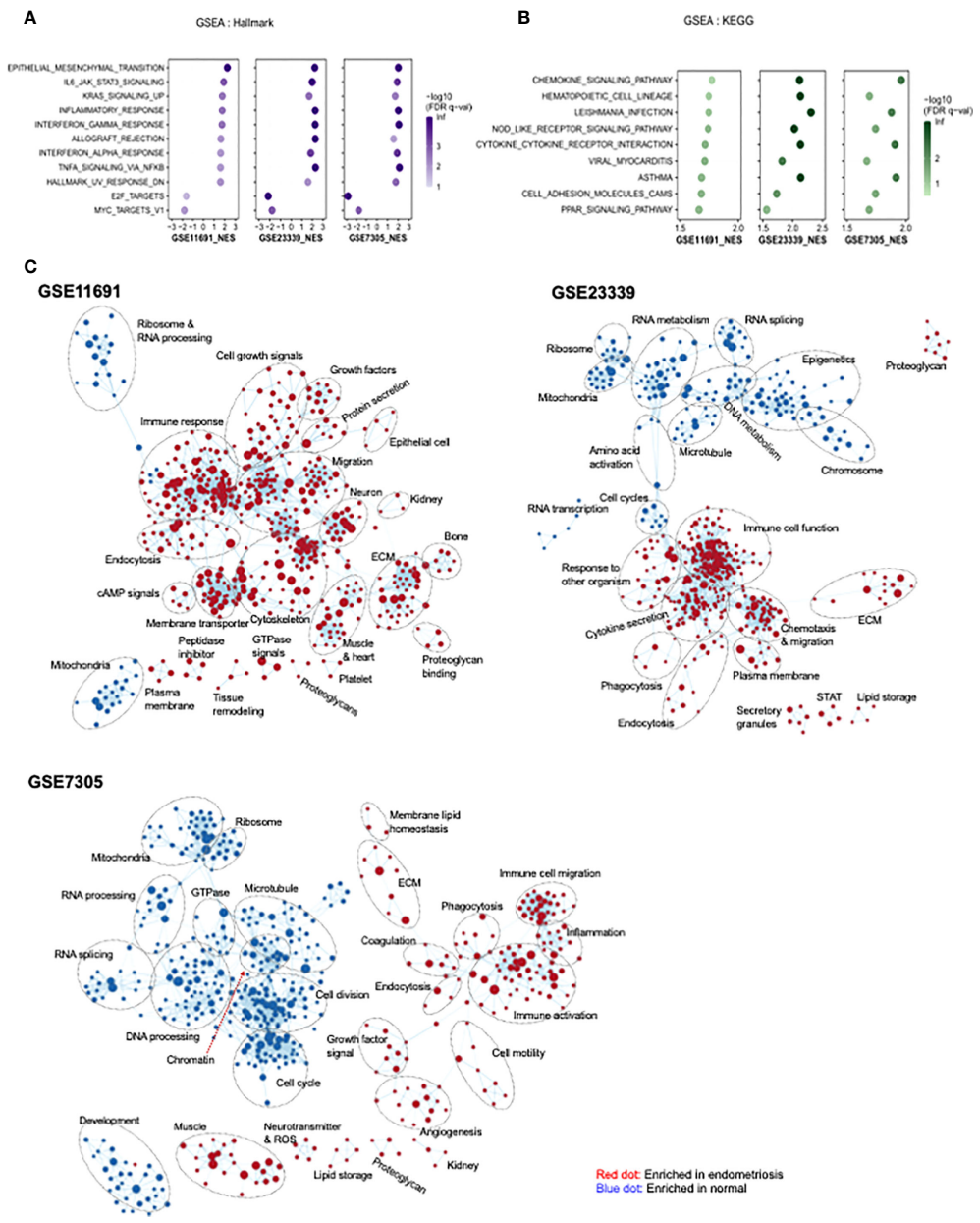


FIGURE 4
Identification of pathway networks by GSEA. **(A, B)** Common pathway categories identified by GSEA with gene set database of Hallmark **(A)** and KEGG **(B)** are presented. **(C)** GSEA was performed to obtain enriched GO-terms and visualized using Enrichment Map plugin of Cytoscape. The size of each node indicates the size of gene set. Red and blue indicate the node enriched in endometriosis and normal tissue, respectively.

response, chemotaxis, and immune cell migration, and cell-cell interaction-related pathways, such as extracellular matrix, proteoglycan, and endocytosis, were commonly enriched for genes of three datasets with endometriosis (Figure 4C). Phagocytosis-related pathways were upregulated in endometriosis in two datasets, GSE23339 and GSE7305. The small portions of pathways related to platelets and coagulation were upregulated in endometriosis of the two gene sets, GSE11691 and GSE7305. GO pathways, including mitochondria, ribosomes, and RNA splicing and/or processing, were commonly enriched in three normal datasets. Finally, the GO pathways involved in the cell cycle and microtubules were downregulated in two datasets, GSE23339 and GSE7305.

Protein expression of identified DEGs in endometriosis lesions

To further evaluate the reliability of the results from integrative analysis of GSE11691, GSE23339, and GSE7305, the expression levels of 17 common DEGs in two external datasets, GSE135485 and GSE25628, were determined. The expression levels of 10 genes in GSE135485, including *C7, CFH, CHL1, CLDN3, FZD7, IGJ, LY96, PDLIM3, PTGIS*, and *WISP2* were correlated with those in the validation sets (Supplementary Table 2). However, the expression levels of other genes did not correlate with those in the integrated data. In the GSE25628 dataset, expression levels of 11 genes, including *AGR2, C7, CFH, FABP4, FZD7, GALNT4, LY96, PDLIM3, PPM1H*, *PTGIS*, and *WISP2* were in line with integrative analysis results (Supplementary Table 3). According to results from two external datasets, GSE135485 and GSE25628, the expression levels of seven genes, including *C7*, *CFH*, *FZD7*, *LY96*, *PDLIM3*, *PTGIS*, and *WISP2* were correlated with training sets listed in Table 2.

The roles of *C7*, *CFH*, and *FZD7* in endometriosis have been reported previously (8, 29). These studies revealed higher expression of C7, CFH, and FZD7 proteins in tissues with endometriosis compared to normal endometrium by IHC analysis. High expression of *PDLIM3* in endometriosis has also been reported in several previous bioinformatics studies with little supportive experimental evidence (15, 30, 31). Thus, we conducted IHC and western blot analysis on tissues from normal endometrium and endometriosis foci to further evaluate the expression of proteins encoded by the identified genes, including *LY96*, *PDLIM3*, *PTGIS*, and *WISP2*. None of these genes have yet been evaluated in terms of their expression profiles in endometriosis tissue. Quantitative assessments of histological images clearly demonstrated higher expression levels of LY96, PDLIM3, PTGIS, and WISP2, proteins in tissues from patients with endometriosis, in line with integrative analysis results (Figure 5). According to western blot analysis, the protein levels of LY96, PDLIM3, and PTGIS were higher in the tissues of endometriosis patients. However, the expression of WISP2 was not correlated with the IHC analysis (Supplementary Figure 3).

Identification of potent druggable genes

To further identify relevant drug-gene interactions and potential druggable target genes, we utilized the DGIdb by applying *C7*, *CFH*, *FZD7*, *LY96*, *PDLIM3*, and *PTGIS* as queries. Three genes, *FZD7*, *LY96*, and *PTGIS*, were found to be associated with drugs vantictumab, eritoran tetrasodium, and phenylbutazone, respectively (Table 3). *CFH* was predicted to be a target gene for three antibody drugs: bevacizumab, eculizumab, and ranibizumab. No potential gene-drug interactions were identified for *C7* and *PDLIM3*.

Discussion

Endometriosis is a chronic inflammatory disease. The infiltration of immune cells and secretion of inflammatory mediators in the peritoneal microenvironment cause symptoms and signs observed in endometriosis patients (32). Endometriosis is characterized by inflammation and subsequent fibrosis, which eventually lead to pelvic discomfort, bowel and urinary problems, and infertility (32, 33). In contrast to normal endometrium, accumulated hemorrhage and tissue injury are key steps for initiating inflammation in the endometrial lesion and peritoneal cavity (33, 34). Hormonal alterations, particularly cyclic estrogen fluctuations, also contribute to an inflammatory imbalance in endometriosis (35). In contrast to other inflammatory diseases, increased estradiol production and estrogen receptor β (ERβ) expression in ectopic endometriosis lesions jointly activate nuclear factor-κB (NF-κB), a key inflammatory regulator (36, 37). Thus, the combination of oral contraceptives and non-steroidal anti-inflammatory drugs (NSAIDs) has been advised as the initial medical treatment option (10, 38). However, the clinical efficacy of this combination is suboptimal due to its low potency and side effects.

In this study, we identified six genes that may be utilized as diagnostic and/or therapeutic targets in endometriosis. STRING and GeneMANIA analyses revealed no evidence of any potent protein–protein interaction between these genes. Complement 7 (C7) and complement factor H (CFH), both of which are components of the complement system, have previously been identified as potent indicators of complement activation in endometriosis and endometriosis-associated ovarian cancer (29). C3, another member of the complement system, has been reported to be upregulated in endometriosis (39) and was also identified in our study as a common upregulated gene. Other members of the complement activation pathway, such as complement C3a receptor 1 (C3AR1) and V-set and immunoglobulin domain containing 4 (VSIG4), are also known in endometriosis, and upregulation of complement and coagulation pathways in endometriosis has been well-reported in several studies (15, 29, 39, 40). These studies suggested that autoimmune response in endometriosis is associated with the complement system (29, 39). In addition, the membrane attack complex in the complement system, which is composed of several complement proteins including C5b, C6, C7, C8, and C9, may also cause tissue damage and thereby induce inflammation in endometrial lesions.

Lymphocyte antigen 96 (LY96), also known as myeloid differentiation protein 2 (MD-2), has been reported its

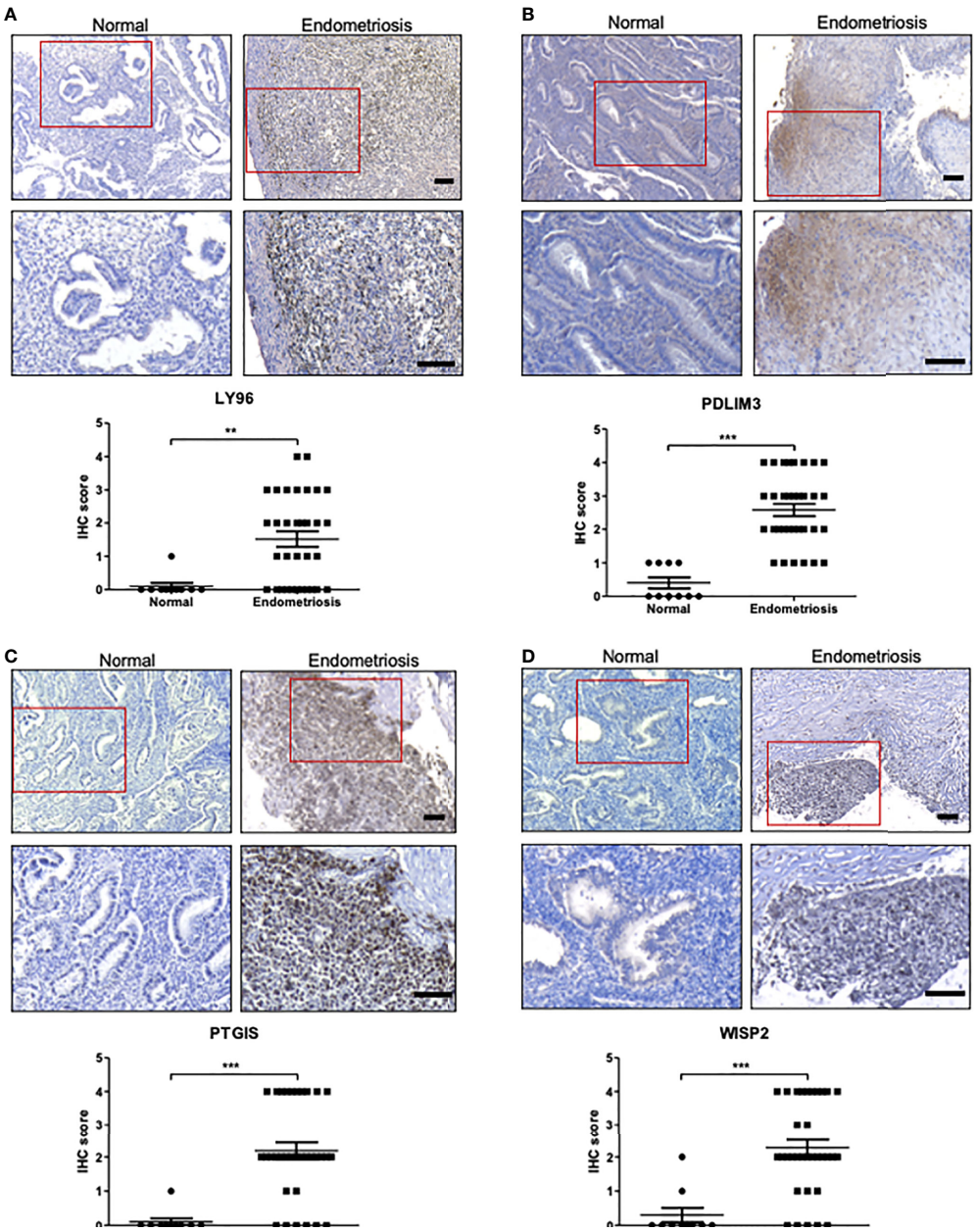


FIGURE 5
The protein expression of LY96, PDLIM3, PTGIS, and WISP2 in endometriosis tissue. **(A–D)** The protein expression of **(A)** LY96, **(B)** PDLIM3, **(C)** PTGIS, and **(D)** WISP2 was measured by IHC analysis of endometrial tissues from healthy volunteers or endometriosis patients. The representative IHC images are shown in upper panel (bar = 100 μm). The graphs in lower panel represent IHC score. $^{**}p < 0.01$ and $^{***}p < 0.001$.

expression in the uterine endometrium (28, 41) and is a coreceptor of Toll-like receptor 4 (TLR4) (42). The TLR4 system is crucial for pathogen recognition and the activation of innate immunity. The complex of bacterial lipopolysaccharide (LPS) and LPS-binding protein interacts with cluster of differentiation 14 (CD14), a glycosylphosphatidylinositol-anchored membrane protein, and transfers LPS to LY96, consequently facilitating the dimerization of TLR4 (43). Damage-associated molecular patterns (DAMPs) secreted by most types of damaged tissues also bind to the TLR4 system and activate downstream pro-inflammatory signals similar to pathogen-associated molecular patterns, including bacterial LPS (44). Prostaglandin I2 synthase (PTGIS), also known as prostacyclin synthase (PGIS) or cytochrome P450 isomerase

10.3389/fendo.2022.942368

TABLE 3 Potent drugs identified in DGIdb corresponding to consistently upregulated six DEGs in endometriosis.

Gene	Drug	Approved	Interaction score	Interaction types	Drug class	PMID
C7	–	–	–	–	–	–
CFH	ECULIZUMAB	Yes	14.14	na	Antibody	19854549, 27799617
	BEVACIZUMAB	Yes	7.53	na	Antibody	26439641
	RANIBIZUMAB	Yes	58.35	na	Antibody	21558292, 22840423
FZD7	VANTICTUMAB	No (Clinical trial)	27.28	Antagonist	Antibody	22753465
LY96	ERITORAN TETRASODIUM	No (Clinical trial)	31.83	Antagonist	–	23512062
PDLIM3	–	–	–	–	–	–
PTGIS	PHENYLBUTAZONE	Yes (For animal use)	37.13	Inhibitor	Non-steroidal anti-inflammatory agent	3917545, 6434940

8A1 (CYP8A1), is an enzyme that converts prostaglandin H2 to prostaglandin I2, and thereby modulates the inflammatory response (45). The role of PTGIS in inflammatory diseases is controversial, as it promotes progression of rheumatoid arthritis yet suppresses progression of pulmonary vascular disease and atherosclerosis (46). Although PTGIS was identified as a transcriptional target gene of NF-κB (47), the regulation of PTGIS mRNA expression does not exactly correlate to those of typical NF-κB targets (48, 49). In the vascular system and uterine endometrium, estrogen receptors work in concert with NF-κB to regulate the activity of prostaglandin-synthesizing enzymes, including COX-2 and PTGIS (50, 51). Furthermore, high levels of prostacyclin and its derivative, 6-keto-prostaglandin F1α, have been detected in the peritoneal fluid of endometriosis patients (52, 53). PTGIS expression was shown to be downregulated in a murine model of implanted endometrium (54); however, proteomics analyses demonstrated upregulated PTGIS expression in human ovarian endometrioma samples (55). In this study, we demonstrated the increased expression of *LY96* and *PTGIS via* integrative transcriptome analysis, and further validated by IHC analysis of human-derived tissues.

Frizzled 7 (FZD7) is a member of the frizzled family and an atypical G protein-coupled receptor for Wnt proteins. FZD7 interacts with Dishevelled (Dvl) and lipoprotein receptor-related proteins (LRPs) in the presence of canonical Wnt signaling, and thereby promotes β-catenin signaling (56). This signaling pathway is closely related to embryonic development, cell proliferation, epithelial-to-mesenchymal transition, and carcinogenesis (57, 58). Wnt signaling is also involved in the production of enzymes related to prostaglandin metabolism in bone and skin, including COX-2 and PTGIS (59, 60). The crosstalk between the Wnt/frizzled and TLR4/NF-κB signaling pathways is well-established in chronic inflammation, development, and tumorigenesis (61, 62). PDZ and LIM domain 3 (PDLIM3) are involved in cytoskeleton assembly, in particular the formation of Z-disks in skeletal muscles (63). Although several microarray and proteome analyses have already revealed high PDLIM3 expression in this regard (15, 30, 31, 55), the exact role of PDLIM3 in endometriosis remains to be elucidated. The expression of Wnt1-inducible signaling pathway protein 2 (WISP2) might be another evidence of the connection between Wnt/frizzled and TLR4/NF-κB signaling pathway. The protein, also known as cellular communication network factor 5 (CCN5), is a secretory protein and a member of the connective tissue growth factor family (64). The expression of WISP2 is induced by Wnt signaling, and restricts cell growth, migration, adhesion, and differentiation, particularly in the vascular system and cancer cells (65–67). Secreted WISP2 is also involved in the activation of the canonical Wnt signaling pathway (68). However, our examination of WISP2 expression was not consistent between IHC and Western blot analysis. the delicate role of the WISP2 in the endometriosis still remains ambiguous and further extensive studies are required.

In endometriosis, chronic inflammation is co-regulated by ERβ-related signaling and the classical NF-κB signaling pathway (36, 37). Estrogen stimulation increases the expression of PDLIM3 in human prostate cancer (69). However, even if the expression level of PTGIS does not perfectly match that of the ERβ, it may be negatively influenced by ERβ (70, 71). We thus propose here the existence of signaling interactions between the validated DEGs including *C7, CFH, FZD7, LY96, PDLIM3*, and *PTGIS*, and pathways including TLR4/NF-κB, Wnt/frizzled, and estrogen receptors (Figure 6). Further research is needed to elucidate the precise roles of the identified genes in endometriosis.

We also found four druggable genes: *FZD7*, *LY96*, *PTGIS*, and *CFH*. *FZD7* is a direct target of vantictumab, a neutralizing antibody currently being developed as an anticancer agent, particularly for triple-negative breast cancer (72, 73). In

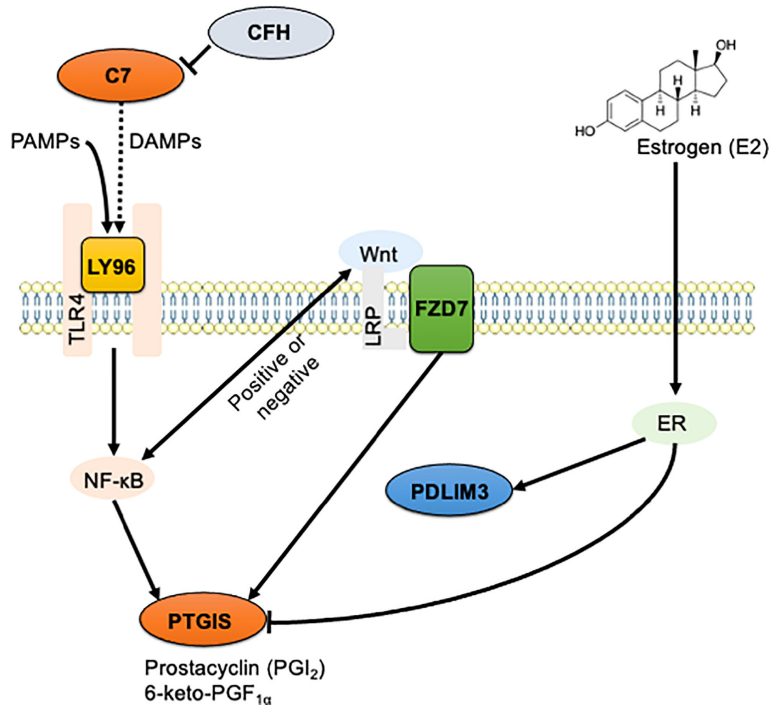


FIGURE 6
The schematic illustration of the hypothesized signaling network in endometriosis. Signaling interaction between six identified DEGs including *C7*, *CFH*, *FZD7*, *LY96*, *PDLIM3*, and *PTGIS*, and three enriched pathways including TLR4/NF-κB, Wnt/frizzled, and estrogen receptors were hypothesized and schematically illustrated. Solid line indicates direct interaction, and dashed line indicates indirect and/or proposed interaction.

addition to vantictumab, an oligopeptide Fz7-21 and a small molecule SRI37892 have been evaluated as a new Fzd7-targeting agent to disrupt the Wnt signaling pathway for inhibiting intestinal stem cell function and cancer progression (74, 75). We suspect that these agents may have been identified for candidate drugs if registration in DGIdb. *LY96* has been identified as a target of eritoran tetrasodium, a TLR4 antagonist drug that has been indicated for the treatment of sepsis in several clinical studies (76). However, eritoran tetrasodium was not successful due to its similar mortality compared to that of placebo (42). Instead of eritoran tetrasodium, we suggest that MD2-IN-1, isofraxidin, and L48H37, studied in the inflammation and cancer research field at the preclinical level, might be applied to treat endometriosis as LY96-targeting agents (77–79). Phenylbutazone is a non-steroidal anti-inflammatory drug (NSAID) that inhibits prostaglandin H synthases (PTGS1 and PTGS2) and PTGIS through peroxide-mediated deactivation (27). Although it has been approved for the treatment of backache and ankylosing spondylitis, phenylbutazone is currently withdrawn from human medicine, as it can cause severe adverse effects such as suppression of white blood cell production and aplastic anemia (80). For CFH-targeting drugs, DGIdb has suggested two VEGF-neutralizing antibodies, including ranibizumab and bevacizumab, and one C5-antagonizing antibody, eculizumab. As CFH is a non-specific off-target for these antibodies, its clinical application may result in significant adverse effects, particularly in patients with a mutated CFH gene (81–85). None of these drugs for targeting identified genes have yet been used to target the identified genes for endometriosis treatment. Additional *in vivo* and clinical studies are needed to determine the efficacy of these drugs in endometriosis treatment.

The datasets should ideally be comparable in terms of sample collection, underlying disease, menstrual cycle, and experiment types for the analysis of the collection of public gene sets. These elements of the datasets used for DEG analysis varied, particularly with regard to the menstrual cycle and underlying diseases such leiomyoma. We tried to select datasets using a filter of three objective criteria despite the restriction of only being able to access the datasets from public databases. In addition, we meticulously gathered normal control samples during the early follicular phase from healthy volunteers who were free of any gynecological diseases. However, there is almost no significant chance of finding genes that follow the menstrual cycle or underlying disease. The results clearly indicate that the genes discovered by DEG analysis may be important for conditions beyond than underlying disease and/or the menstrual cycle.

In conclusion, we identified 118 DEGs (79 upregulated and 39 downregulated) that may be involved in endometriosis pathogenesis. KEGG and GO functional analyses revealed enrichment of inflammation, complement activation, cell adhesion, and extracellular matrix pathways in endometriosis. Six genes, *C7, CFH, FZD7, LY96, PDLIM3*, and *PTGIS* were verified as upregulated DEGs by comparison to external gene sets, IHC and western blot analyses further confirmed the elevated protein expression levels of LY96, PDLIM3, and PTGIS in human endometrial lesions. We further analyzed the involvement of these genes in signaling pathways including TLR4/NF-κB, Wnt/frizzled, and estrogen receptors. We expect that subsequent studies will confirm the genes identified here as essential biomarkers for endometriosis diagnosis and treatment.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

Ethics statement

The studies involving human participants were reviewed and approved by Institutional Review Board (IRB) of Pusan National University Hospital (2104-009-101). The patients/participants provided their written informed consent to participate in this study.

Author contributions

YJ analyzed informatics data. MC and J-SJ conducted IHC experiments. S-JB, J-YK, and JS analyzed the data. YK and DR validated and visualized the results from the informatics analysis. J-KP, HL, and JJ collected human samples and analyzed the clinical signatures and statistics. S-JB and YJ wrote the draft of this manuscript. S-JB, J-YK, and JS revised the manuscript. JJ and K-TH conceptualized this study and reviewed the manuscript. All authors read and approved the final manuscript.

Funding

This research was supported by a grant from Kosin University College of Medicine (2021) and by the National Research Foundation of Korea (NRF) grants funded by the Korean government (MIST: NRF-2020R1C1C1003703 to S-JB, NRF-2022R1I1A1A01063460 and NRF-2021R1A5A8029876 to YJ, NRF-2020R1F1A1067185 to JJ, and NRF-2021R1A4A1025662 to S-JB and K-TH).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.942368/full#supplementary-material

References

1. Taylor HS, Kotlyar AM, Flores VA. Endometriosis is a chronic systemic disease: Clinical challenges and novel innovations. *Lancet* (2021) 397(10276):839–52. doi: 10.1016/S0140-6736(21)00389-5

2. Parasar P, Ozcan P, Terry KL. Endometriosis: Epidemiology, diagnosis and clinical management. *Curr Obstet Gynecol Rep* (2017) 6(1):34–41. doi: 10.1007/s13669-017-0187-1

3. Kapoor R, Stratopoulou CA, Dolmans MM. Pathogenesis of endometriosis: New insights into prospective therapies. *Int J Mol Sci* (2021) 22(21):11700. doi: 10.3390/ijms222111700

4. Augoulea A, Alexandrou A, Creatsa M, Vrachnis N, Lambrinoudaki I. Pathogenesis of endometriosis: The role of genetics, inflammation and oxidative stress. *Arch Gynecol Obstet* (2012) 286(1):99–103. doi: 10.1007/s00404-012-2357-8

5. Taylor HS, Kotlyar AM, Flores VA. Endometriosis is a chronic systemic disease: Clinical challenge and novel innovations. *Lancet* (2021) 397(10276):839–52. doi: 10.1016/S0140-6736(21)00389-5

6. Ahn SH, Singh V, Tayade C. Biomarkers in endometriosis: Challenges and opportunities. *Fertil Steril* (2017) 107(3):523–32. doi: 10.1016/j.fertnstert.2017.01.009

7. Anastasiu CV, Moga MA, Elena Neculau A, Balan A, Scarneciu I, Dragomir RM, et al. Biomarkers for the noninvasive diagnosis of endometriosis: State of the art and future perspectives. *Int J Mol Sci* (2020) 21(5):1750. doi: 10.3390/ijms21051750

8. MDH T, Fassbender A, F O D, Vanhie A. Endometriosis biomarkers: Will codevelopment in academia-industry partnerships result in new and robust

noninvasive diagnostic tests? *Biol Reprod* (2019) 101(6):1140–5. doi: 10.1093/biolre/ioz016

9. Tian Z, Chang XH, Zhao Y, Zhu HL. Current biomarkers for the detection of endometriosis. *Chin Med J (Engl)* (2020) 133(19):2346–52. doi: 10.1097/CM9.0000000000001063

10. Burghaus S, Schafer SD, Beckmann MW, Brandes I, Brunahl C, Chvatal R, et al. Diagnosis and treatment of endometriosis. guideline of the dggg, sggg and oeggg (S2k level, awmf registry number 015/045, august 2020). *Geburtshilfe Frauenheilkd* (2021) 81(4):422–46. doi: 10.1055/a-1380-3693

11. Goulielmos GN, Matalliotakis M, Matalliotaki C, Eliopoulos E, Matalliotakis I, Zervou MI. Endometriosis research in the -omics era. *Gene* (2020) 741:144545. doi: 10.1016/j.gene.2020.144545

12. Sun YV, Hu YJ. Integrative analysis of multi-omics data for discovery and functional studies of complex human diseases. *Adv Genet* (2016) 93:147–90. doi: 10.1016/bs.adgen.2015.11.004

13. Cui D, Liu Y, Ma J, Lin K, Xu K, Lin J. Identification of key genes and pathways in endometriosis by integrated expression profiles analysis. *PeerJ* (2020) 8:e10171. doi: 10.7717/peerj.10171

14. Lu ZZ, Gao Y. Screening differentially expressed genes between endometriosis and ovarian cancer to find new biomarkers for endometriosis. *Ann Med* (2021) 53(1):1377–89. doi: 10.1080/07853890.2021.1966087

15. Chen M, Zhou Y, Xu H, Hill C, Ewing RM, He D, et al. Bioinformatic analysis reveals the importance of epithelial-mesenchymal transition in the development of endometriosis. *Sci Rep* (2020) 10(1):8442. doi: 10.1038/s41598-020-65606-9

16. Dai FF, Bao AY, Luo B, Zeng ZH, Pu XL, Wang YQ, et al. Identification of differentially expressed genes and signaling pathways involved in endometriosis by integrated bioinformatics analysis. *Exp Ther Med* (2020) 19(1):264–72. doi: 10.3892/etm.2019.8214

17. Hull ML, Escareno CR, Godsland JM, Doig JR, Johnson CM, Phillips SC, et al. Endometrial-peritoneal interactions during endometriotic lesion establishment. *Am J Pathol* (2008) 173(3):700–15. doi: 10.2353/ajpath.2008.071128

18. Hawkins SM, Creighton CJ, Han DY, Zariff A, Anderson ML, Gunaratne PH, et al. Functional microrna involved in endometriosis. *Mol Endocrinol* (2011) 25(5):821–32. doi: 10.1210/me.2010-0371

19. Hever A, Roth RB, Hevezi P, Marin ME, Acosta JA, Acosta H, et al. Human endometriosis is associated with plasma cells and overexpression of b lymphocyte stimulator. *Proc Natl Acad Sci U.S.A.* (2007) 104(30):12451–6. doi: 10.1073/pnas.0703451104

20. Crispi S, Piccolo MT, D'Avino A, Donizetti A, Viceconte R, Spyrou M, et al. Transcriptional profiling of endometriosis tissues identifies genes related to organogenesis defects. *J Cell Physiol* (2013) 228(9):1927–34. doi: 10.1002/jcp.24358

21. Davis S, Meltzer PS. Geoquery: A bridge between the gene expression omnibus (Geo) and bioconductor. *Bioinformatics* (2007) 23(14):1846–7. doi: 10.1093/bioinformatics/btm254

22. Benjamini Y, Hochberg Y. Controlling the false discovery rate - a practical and powerful approach to multiple testing. *J R Stat Soc B* (1995) 57(1):289–300. doi: 10.1111/j.2517-6161.1995.tb02031.x

23. Kim J, Lee H, Jin EJ, Jo Y, Kang BE, Ryu D, et al. A microfluidic device to fabricate one-step cell bead-laden hydrogel struts for tissue engineering. *Small* (2022) 18(1):e2106487. doi: 10.1002/smll.202106487

24. Hwu YM, Wu FS, Li SH, Sun FJ, Lin MH, Lee RK. The impact of endometrioma and laparoscopic cystectomy on serum anti-mullerian hormone levels. *Reprod Biol Endocrinol* (2011) 9:80. doi: 10.1186/1477-7827-9-80

25. Schweppe KW, Ring D. Peritoneal defects and the development of endometriosis in relation to the timing of endoscopic surgery during the menstrual cycle. *Fertil Steril* (2002) 78(4):763–6. doi: 10.1016/s0015-0282(02)03431-3

26. Griffith M, Griffith OL, Coffman AC, Weible JV, McMichael JF, Spies NC, et al. Dgidb: Mining the druggable genome. *Nat Methods* (2013) 10(12):1209–10. doi: 10.1038/nmeth.2689

27. Reed GA, Griffin IO, Eling TE. Inactivation of prostaglandin h synthase and prostacyclin synthase by phenylbutazone. requirement for peroxidative metabolism. *Mol Pharmacol* (1985) 27(1):109–14.

28. Sheldon IM, Cronin J, Goetze L, Donofrio G, Schuberth HJ. Defining postpartum uterine disease and the mechanisms of infection and immunity in the female reproductive tract in cattle. *Biol Reprod* (2009) 81(6):1025–32. doi: 10.1095/biolreprod.109.077370

29. Suryawanshi S, Huang X, Elishaev E, Budiu RA, Zhang L, Kim S, et al. Complement pathway is frequently altered in endometriosis and endometriosis-associated ovarian cancer. *Clin Cancer Res* (2014) 20(23):6163–74. doi: 10.1158/1078-0432.Ccr-14-1338

30. Kobayashi H, Yamashita Y, Iwase A, Yoshikawa Y, Yasui H, Kawai Y, et al. The ferroimmunomodulatory role of ectopic endometriotic stromal cells in ovarian endometriosis. *Fertil Steril* (2012) 98(2):415–22.e1-12. doi: 10.1016/j.fertnstert.2012.04.047

31. Logan PC, Yango P, Tran ND. Endometrial stromal and epithelial cells exhibit unique aberrant molecular defects in patients with endometriosis. *Reprod Sci* (2018) 25(1):140–59. doi: 10.1177/1933719117704905

32. Lin YH, Chen YH, Chang HY, Au HK, Tzeng CR, Huang YH. Chronic niche inflammation in endometriosis-associated infertility: Current understanding and future therapeutic strategies. *Int J Mol Sci* (2018) 19(8):2385. doi: 10.3390/ijms19082385

33. Wang Y, Nicholes K, Shih IM. The origin and pathogenesis of endometriosis. *Annu Rev Pathol* (2020) 15:71–95. doi: 10.1146/annurev-pathmechdis-012419-032654

34. Lu J, Wang Z, Cao J, Chen Y, Dong Y. A novel and compact review on the role of oxidative stress in female reproduction. *Reprod Biol Endocrinol* (2018) 16(1):80. doi: 10.1186/s12958-018-0391-5

35. Garcia-Gomez E, Vazquez-Martinez ER, Reyes-Mayoral C, Cruz-Orozco OP, Camacho-Arroyo I, Cerbon M. Regulation of inflammation pathways and inflammasome by sex steroid hormones in endometriosis. *Front Endocrinol (Lausanne)* (2019) 10:935. doi: 10.3389/fendo.2019.00935

36. Gou Y, Li X, Li P, Zhang H, Xu T, Wang H, et al. Estrogen receptor beta upregulates Ccl2 *Via* nf-kappab signaling in endometriotic stromal cells and recruits macrophages to promote the pathogenesis of endometriosis. *Hum Reprod* (2019) 34(4):646–58. doi: 10.1093/humrep/dez019

37. Han SJ, Lee JE, Cho YJ, Park MJ, O'Malley BW. Genomic function of estrogen receptor beta in endometriosis. *Endocrinology* (2019) 160(11):2495–516. doi: 10.1210/en.2019-00442

38. Koninckx PR, Fernandes R, Ussia A, Schindler L, Wattiez A, Al-Suwaidi S, et al. Pathogenesis based diagnosis and treatment of endometriosis. *Front Endocrinol (Lausanne)* (2021) 12:745548. doi: 10.3389/fendo.2021.745548

39. Rekker K, Saare M, Eriste E, Tasa T, Kukuskina V, Roost AM, et al. High-throughput mrna sequencing of stromal cells from endometriomas and endometrium. *Reproduction* (2017) 154(1):93–100. doi: 10.1530/REP-17-0092

40. Jiang L, Zhang MM, Wang SX, Han YY, Fang XL. Common and specific gene signatures among three different endometriosis subtypes. *Peerj* (2020) 8:e8730. doi: 10.7717/peerj.8730

41. Hirata T, Osuga Y, Hirota Y, Koga K, Yoshino O, Harada M, et al. Evidence for the presence of toll-like receptor 4 system in the human endometrium. *J Clin Endocrinol Metab* (2005) 90(1):548–56. doi: 10.1210/jc.2004-0241

42. Opal SM, Laterre PF, Francois B, LaRosa SP, Angus DC, Mira JP, et al. Effect of eritoran, an antagonist of Md2-Tlr4, on mortality in patients with severe sepsis: The access randomized trial. *JAMA* (2013) 309(11):1154–62. doi: 10.1001/jama.2013.2194

43. Lu YC, Yeh WC, Ohashi PS. Lps/Tlr4 signal transduction pathway. *Cytokine* (2008) 42(2):145–51. doi: 10.1016/j.cyto.2008.01.006

44. Lee KM. Seong SY. partial role of Tlr4 as a receptor responding to damage-associated molecular pattern. *Immunol Lett* (2009) 125(1):31–9. doi: 10.1016/j.imlet.2009.05.006

45. Wang B, Wu L, Chen J, Dong L, Chen C, Wen Z, et al. Metabolism pathways of arachidonic acids: Mechanisms and potential therapeutic targets. *Signal Transduct Target Ther* (2021) 6(1):94. doi: 10.1038/s41392-020-00443-w

46. Stitham J, Midgett C, Martin KA, Hwa J. Prostacyclin: An inflammatory paradox. *Front Pharmacol* (2011) 2:24. doi: 10.3389/fphar.2011.00024

47. Pahl HL. Activators and target genes of Rel/Nf-kappab transcription factors. *Oncogene* (1999) 18(49):6853–66. doi: 10.1038/sj.onc.1203239

48. Zhao H, Hiroi T, Hansen BS, Rade JJ. Cyclic stretch induces cyclooxygenase-2 gene expression in vascular endothelial cells *Via* activation of nuclear factor kappa-beta. *Biochem Biophys Res Commun* (2009) 389(4):599–601. doi: 10.1016/j.bbrc.2009.09.028

49. Gurgul-Convey E, Lenzen S. Protection against cytokine toxicity through endoplasmic reticulum and mitochondrial stress prevention by prostacyclin synthase overexpression in insulin-producing cells. *J Biol Chem* (2010) 285(15):11121–8. doi: 10.1074/jbc.M109.054775

50. Shah BH. Estrogen stimulation of cox-2-Derived Pgi2 confers atheroprotection. *Trends Endocrinol Metab* (2005) 16(5):199–201. doi: 10.1016/j.tem.2005.05.008

51. King AE, Collins F, Klonisch T, Sallenave JM, Critchley HO, Saunders PT. An additive interaction between the nfkappab and estrogen receptor signalling pathways in human endometrial epithelial cells. *Hum Reprod* (2010) 25(2):510–8. doi: 10.1093/humrep/dep421

52. Drake TS, O'Brien WF, Ramwell PW, Metz SA. Peritoneal fluid thromboxane B2 and 6-Keto-Prostaglandin F1 alpha in endometriosis. *Am J Obstet Gynecol* (1981) 140(4):401–4. doi: 10.1016/0002-9378(81)90033-8

53. Ylikorkala O, Makila UM. Prostacyclin and thromboxane in gynecology and obstetrics. *Am J Obstet Gynecol* (1985) 152(3):318–29. doi: 10.1016/s0002-9378(85)80221-0

54. Kusakabe KT, Abe H, Kondo T, Kato K, Okada T, Otsuki Y. DNA Microarray analysis in a mouse model for endometriosis and validation of candidate factors with human adenomyosis. *J Reprod Immunol* (2010) 85(2):149–60. doi: 10.1016/j.jri.2010.02.008

55. Vehmas AP, Muth-Pawlak D, Huhtinen K, Saloniemi-Heinonen T, Jaakkola K, Laajala TD, et al. Ovarian endometriosis signatures established through discovery and directed mass spectrometry analysis. *J Proteome Res* (2014) 13(11):4983–94. doi: 10.1021/pr500384n

56. Yang L, Wu X, Wang Y, Zhang K, Wu J, Yuan YC, et al. Fzd7 has a critical role in cell proliferation in triple negative breast cancer. *Oncogene* (2011) 30(43):4437–46. doi: 10.1038/onc.2011.145

57. Yang Y. Wnt signaling in development and disease. *Cell Biosci* (2012) 2(1):14. doi: 10.1186/2045-3701-2-14

58. Sompel K, Elango A, Smith AJ, Tennis MA. Cancer chemoprevention through frizzled receptors and emt. *Discovery Oncol* (2021) 12(1):32. doi: 10.1007/s12672-021-00429-2

59. Robinson JA, Chatterjee-Kishore M, Yaworsky PJ, Cullen DM, Zhao W, Li C, et al. Wnt/Beta-catenin signaling is a normal physiological response to mechanical loading in bone. *J Biol Chem* (2006) 281(42):31720–8. doi: 10.1074/jbc.M602308200

60. Lombardi A, Arseni L, Carriero R, Compe E, Botta E, Ferri D, et al. Reduced levels of prostaglandin I2 synthase: A distinctive feature of the cancer-free trichothiodystrophy. *Proc Natl Acad Sci USA* (2021) 118(26):e2024502118. doi: 10.1073/pnas.2024502118

61. Du Q, Geller DA. Cross-regulation between wnt and nf-kappab signaling pathways. *For Immunopathol Dis Therap* (2010) 1(3):155–81. doi: 10.1615/ForumImmunDisTher.v1.i3

62. Ma B, Hottiger MO. Crosstalk between Wnt/Beta-catenin and nf-kappab signaling pathway during inflammation. *Front Immunol* (2016) 7:378. doi: 10.3389/fimmu.2016.00378

63. Pashmforoush M, Pomies P, Peterson KL, Kubalak S, Ross JJr., Hefti A, et al. Adult mice deficient in actinin-associated lim-domain protein reveal a developmental pathway for right ventricular cardiomyopathy. *Nat Med* (2001) 7(5):591–7. doi: 10.1038/87920

64. Sabbah M, Prunier C, Ferrand N, Megalophonos V, Lambein K, De Wever O, et al. Ccn5, a novel transcriptional repressor of the transforming growth factor beta signaling pathway. *Mol Cell Biol* (2011) 31(7):1459–69. doi: 10.1128/MCB.01316-10

65. Russo JW, Castellot JJ. Ccn5: Biology and pathophysiology. *J Cell Commun Signal* (2010) 4(3):119–30. doi: 10.1007/s12079-010-0098-7

66. Yoon PO, Lee MA, Cha H, Jeong MH, Kim J, Jang SP, et al. The opposing effects of Ccn2 and Ccn5 on the development of cardiac hypertrophy and fibrosis. *J Mol Cell Cardiol* (2010) 49(2):294–303. doi: 10.1016/j.yjmcc.2010.04.010

67. Fritah A, Saucier C, De Wever O, Bracke M, Bieche I, Lidereau R, et al. Role of wisp-2/Ccn5 in the maintenance of a differentiated and noninvasive phenotype in human breast cancer cells. *Mol Cell Biol* (2008) 28(3):1114–23. doi: 10.1128/MCB.01335-07

68. Grunberg JR, Hammarstedt A, Hedjazifar S, Smith U. The novel secreted adipokine Wnt1-inducible signaling pathway protein 2 (Wisp2) is a mesenchymal cell activator of canonical wnt. *J Biol Chem* (2014) 289(10):6899–907. doi: 10.1074/jbc.M113.511964

69. Chakravarty D, Sboner A, Nair SS, Giannopoulou E, Li R, Hennig S, et al. The oestrogen receptor alpha-regulated lncrna Neat1 is a critical modulator of prostate cancer. *Nat Commun* (2014) 5:5383. doi: 10.1038/ncomms6383

70. Rebordao MR, Galvao A, Pinto-Bravo P, Pinheiro J, Gamboa S, Silva E, et al. Endometrial prostaglandin synthases, ovarian steroids, and oxytocin receptors in mares with oxytocin-induced luteal maintenance. *Theriogenology* (2017) 87:193–204. doi: 10.1016/j.theriogenology.2016.08.028

71. Ospina JA, Duckles SP, Krause DN. 17beta-estradiol decreases vascular tone in cerebral arteries by shifting cox-dependent vasoconstriction to vasodilation. *Am J Physiol Heart Circ Physiol* (2003) 285(1):H241–50. doi: 10.1152/ajpheart.00018.2003

72. Diamond JR, Becerra C, Richards D, Mita A, Osborne C, O'Shaughnessy J, et al. Phase ib clinical trial of the anti-frizzled antibody vantictumab (Omp-18r5) plus paclitaxel in patients with locally advanced or metastatic Her2-negative breast cancer. *Breast Cancer Res Treat* (2020) 184(1):53–62. doi: 10.1007/s10549-020-05817-w

73. Gurney A, Axelrod F, Bond CJ, Cain J, Chartier C, Donigan L, et al. Wnt pathway inhibition *Via* the targeting of frizzled receptors results in decreased growth and tumorigenicity of human tumors. *Proc Natl Acad Sci U.S.A.* (2012) 109(29):11717–22. doi: 10.1073/pnas.1120068109

74. Nile AH, de Sousa EMF, Mukund S, Piskol R, Hansen S, Zhou L, et al. A selective peptide inhibitor of frizzled 7 receptors disrupts intestinal stem cells. *Nat Chem Biol* (2018) 14(6):582–90. doi: 10.1038/s41589-018-0035-2

75. Zhang W, Lu W, Ananthan S, Suto MJ, Li Y. Discovery of novel frizzled-7 inhibitors by targeting the receptor's transmembrane domain. *Oncotarget* (2017) 8(53):91459–70. doi: 10.18632/oncotarget.20665

76. Barochia A, Solomon S, Cui X, Natanson C, Eichacker PQ. Eritoran tetrasodium (E5564) treatment for sepsis: Review of preclinical and clinical studies. *Expert Opin Drug Metab Toxicol* (2011) 7(4):479–94. doi: 10.1517/17425255.2011.558190

77. Jin J, Yu X, Hu Z, Tang S, Zhong X, Xu J, et al. Isofraxidin targets the Tlr4/Md-2 axis to prevent osteoarthritis development. *Food Funct* (2018) 9(11):5641–52. doi: 10.1039/c8fo01445k

78. Wang Y, Shan X, Dai Y, Jiang L, Chen G, Zhang Y, et al. Curcumin analog L48h37 prevents lipopolysaccharide-induced Tlr4 signaling pathway activation and sepsis *Via* targeting Md2. *J Pharmacol Exp Ther* (2015) 353(3):539–50. doi: 10.1124/jpet.115.222570

79. Zhang Y, Wu J, Ying S, Chen G, Wu B, Xu T, et al. Discovery of new Md2 inhibitor from chalcone derivatives with anti-inflammatory effects in lps-induced acute lung injury. *Sci Rep* (2016) 6:25130. doi: 10.1038/srep25130

80. Worboys M, Toon E. Phenylbutazone (Bute, pbz, epz): One drug across two species. *Hist Philos Life Sci* (2018) 40(2):27. doi: 10.1007/s40656-018-0191-4

81. Medina FM, Alves Lopes da Motta A, Takahashi WY, Carricondo PC, Dos Santos Motta MM, Melo MB, et al. Pharmacogenetic effect of complement factor h gene polymorphism in response to the initial intravitreal injection of bevacizumab for wet age-related macular degeneration. *Ophthalmic Res* (2015) 54(4):169–74. doi: 10.1159/000439172

82. Smailhodzic D, Muether PS, Chen J, Kwestro A, Zhang AY, Omar A, et al. Cumulative effect of risk alleles in cfh, Arms2, and vegfa on the response to ranibizumab treatment in age-related macular degeneration. *Ophthalmology* (2012) 119(11):2304–11. doi: 10.1016/j.ophtha.2012.05.040

83. McKibbin M, Ali M, Bansal S, Baxter PD, West K, Williams G, et al. Cfh, vegf and Htra1 promoter genotype may influence the response to intravitreal ranibizumab therapy for neovascular age-related macular degeneration. *Br J Ophthalmol* (2012) 96(2):208–12. doi: 10.1136/bjo.2010.193680

84. Fakhouri F, Fila M, Provot F, Delmas Y, Barbet C, Chatelet V, et al. Pathogenic variants in complement genes and risk of atypical hemolytic uremic syndrome relapse after eculizumab discontinuation. *Clin J Am Soc Nephrol* (2017) 12(1):50–9. doi: 10.2215/CJN.06440616

85. Davin JC, Gracchi V, Bouts A, Groothoff J, Strain L, Goodship T. Maintenance of kidney function following treatment with eculizumab and discontinuation of plasma exchange after a third kidney transplant for atypical hemolytic uremic syndrome associated with a cfh mutation. *Am J Kidney Dis* (2010) 55(4):708–11. doi: 10.1053/j.ajkd.2009.08.011

frontiers | Frontiers in Physiology

TYPE Review
PUBLISHED 13 December 2022
DOI 10.3389/fphys.2022.937988

OPEN ACCESS

EDITED BY
Mainak Dutta,
Birla Institute of Technology and Science, United Arab Emirates

REVIEWED BY
Lin Zhang,
Shandong University, China
Sangam Jha,
All India Institute of Medical Sciences, India

*CORRESPONDENCE
Muhammad Dain Yazid,
dain@ukm.edu.my

SPECIALTY SECTION
This article was submitted to Reproduction, a section of the journal Frontiers in Physiology

RECEIVED 14 June 2022
ACCEPTED 21 November 2022
PUBLISHED 13 December 2022

CITATION
Lih Yuan T, Sulaiman N, Nur Azurah AG, Maarof M, Rabiatul AR and Yazid MD (2022), Oestrogen-induced epithelial-mesenchymal transition (EMT) in endometriosis: Aetiology of vaginal agenesis in Mayer-Rokitansky-Küster-Hauser (MRKH) syndrome. *Front. Physiol.* 13:937988. doi: 10.3389/fphys.2022.937988

Oestrogen-induced epithelial-mesenchymal transition (EMT) in endometriosis: Aetiology of vaginal agenesis in Mayer-Rokitansky-Küster-Hauser (MRKH) syndrome

Too Lih Yuan[1], Nadiah Sulaiman[1], Abdul Ghani Nur Azurah[2], Manira Maarof[1], Rabiatul Adawiyah Razali[1] and Muhammad Dain Yazid[1]*

[1]Centre for Tissue Engineering and Regenerative Medicine, Faculty of Medicine, Universiti Kebangsaan Malaysia Medical Centre, Cheras, Malaysia, [2]Department of Obstetrics and Gynaecology, Faculty of Medicine, Universiti Kebangsaan Malaysia Medical Centre, Cheras, Malaysia

Endometriosis occurs when endometrial-like tissue forms and grows outside the uterus due to oestrogen-induced epithelial-mesenchymal transition in the female reproductive tract. Factors that suppress this event could become potential therapeutic agents against disease occurrence and progression. However, an overview of these studies is still lacking. This review assessed the impact of a number factors on oestrogen-mediated epithelial-mesenchymal transition in the emergence of several diseases in the female reproductive tract, primarily endometriosis. The association between epithelial-mesenchymal transition and Mayer-Rokitansky-Küster-Hauser (MRKH) syndrome was also investigated. Oestrogen, Wnt4 and epithelial-mesenchymal transition were chosen as keywords in Scopus, PubMed, and Web of Science searches performed on 28th June 2021. Study selection was refined to cancer-irrelevant, English, original articles published between years 2011–2021. The full-text assessment was carried out for topic-related articles after title and abstract screening. Included studies were summarised and assessed for their risk of bias using the Office of Health Assessment and Translation tool. In this review, 10 articles investigating oestrogen and epithelial-mesenchymal transition in the female reproductive tract were summarised and classified into two groups: seven studies under 'factor'-modulated epithelial-mesenchymal transition and three studies under

Abbreviations: E2, Oestrogen; EM, Endometriosis; EMT, Epithelial-mesenchymal transition; ERα, Oestrogen receptor α; ERβ, Oestrogen receptor β; IUA, Intrauterine adhesion; LXA_4, Lipoxin A_4; MALAT1, Metastasis associated lung adenocarcinoma transcript 1; MRKH, Mayer-Rokitansky-Küster-Hauser syndrome; NRP1, Neurophilin 1; PCOS, Polycystic ovary syndrome; RhoA, Transforming protein RhoA; Snail, Zinc finger protein SNAI1; TGF-β1, Transforming growth factor beta 1; TWIST1, Twist-related protein 1; Wnt4, Wnt family member 4; ZEB, Zinc finger E-box-binding homeobox; α-SMA, Alpha-smooth muscle actin.

'factor'-manipulated oestrogen-induced epithelial-mesenchymal transition. The current evidence proposes that epithelial-mesenchymal transition is one of the prime causes of reproductive-related disease. This event could be mediated by distinct stimuli, specifically oestrogen and Wnt4 aberration. The results of this review suggest that oestrogen and Wnt4 participate in epithelial-mesenchymal transition in vaginal epithelial cells in MRKH syndrome, adopting from the theories of endometriosis development, which could therefore serve as a foundation for novel target treatment, specifically related to vaginal epithelialisation, to ensure better surgical outcomes.

KEYWORDS

endometriosis, epithelial-mesenchymal transition, Mayer-Rokitansky-Küster-Hauser syndrome, oestrogen, Wnt4

1 Introduction

Endometriosis (EM) is a benign gynaecological disease affecting approximately 6–10% of reproductive age women worldwide (Li et al., 2021). It is a condition where the tissue that usually lines the uterus grows outside of the uterine cavity and appears as endometrial glands and stroma-like lesions (Pontikaki et al., 2016; Yang and Yang, 2017). EM is also defined as an oestrogen-dependent inflammatory disorder, and studies have been continuously executed to develop suitable treatments for EM in response to oestrogen (E2) (Mai et al., 2019). In a study conducted by Pellegrini and others, the mRNA expression of oestrogen receptors (ERα and Erβ) in human ectopic endometrial tissue was found to be significantly upregulated compared to normal and eutopic endometrial tissues (Pellegrini et al., 2012). Similarly, Matsuzaki and others demonstrated that both eutopic endometrium and endometriotic tissues obtained from patients displayed high mRNA levels of ERα and Erβ (Matsuzaki et al., 2001). These findings suggest that E2 is likely to exert a promoting effect during EM development by binding to its receptors (Trukhacheva et al., 2009).

Epithelial-mesenchymal transition (EMT) is a reversible transition of cell features from an epithelial to mesenchymal phenotype, which provokes cellular changes in morphology, inflammation (Sisto et al., 2019), invasion, migration (Furuya et al., 2017) and proliferation (Meng et al., 2016). Epithelial cells undergoing the transition process often transform from a cobblestone shape to a spindle shape (Chen et al., 2017). Importantly, the transition may not be permanent, as transformed mesenchymal cells could convert back to epithelial derivatives *via* mesenchymal-epithelial transition (MET) (Kalluri and Weinberg, 2009). Generally, EMT is known to be crucial for embryogenesis, wound healing and cancer, and thus has been categorised into three subtypes accordingly (Kalluri and Weinberg, 2009). Many studies have correlated EMT with the pathogenesis and development of EM (Matsuzaki and Darcha, 2012; Xiong et al., 2016; Lin et al., 2019; Zhang et al., 2019; Wang et al., 2020), inferring that the establishment of an endometriotic lesion would present the features of EMT, such as loss of cell polarity, disintegration of cell-cell junctions, increased in cell mobility, gain of N-cadherin expression and concomitant loss of E-cadherin expression (Bartley et al., 2014). As such, Proestling and others, who conducted a study using ectopic endometrial lesions from patients with EM, found that cadherin-1 mRNA expression was clearly downregulated while Twist-related protein 1 (TWIST1) mRNA expression was significantly increased (Proestling et al., 2015). Chen et al. revealed a significant decrease in E-cadherin expression and a significant increase in C-X-C motif chemokine 12 in the endometrial tissue of EM patients (Chen et al., 2020). Another finding presented by Yu et al. (2021) showed strong staining of N-cadherin and E-cadherin in endometriotic lesions and control endometria, respectively. These observations show that EMT participates in the development of EM, and is a workable indicator in tracing the aetiology of the disease.

To date, E2 has been extensively studied for its inductive roles in the occurrence of EMT in multiple diseases, particularly within the cancer field (Ding et al., 2006; Huang et al., 2007; Park et al., 2008; Mishra et al., 2015; Yoriki et al., 2019). Some studies have also suggested that external stimuli such as intracellular molecules and signalling pathways are partly involved in E2-induced EMT in cancer. As an example, Das and others overexpressed nuclear respiratory factor 1, a transcription factor in E2-treated MCF10 A cells, which resulted in the generation of highly invasive mesenchymal breast cancer stem cells *via* EMT (Das et al., 2018). However, studies investigating the influence of these stimuli on E2-induced EMT in the non-cancer field are still lacking. Hence, this review assessed how the EMT process can be modulated by these stimuli, including E2, mostly in EM development. This review also shows that E2 has possible interactions with Wnt4 in inducing EMT in vaginal epithelial cells, adopting the theories of EM pathogenesis. This further obstructs the process of vaginal

epithelialisation in Mayer-Rokitansky-Küster-Hauser (MRKH) syndrome patients. The outcomes of this review are urgently needed as they might have clinical implications not only in terms of establishing new therapeutic agents against respective diseases, but also to foreseeably clarify the genesis of a number of conditions.

2 Materials and methods

2.1 Search methods

On 28 June 2021, a comprehensive search was conducted electronically in three databases, specifically PubMed, Scopus and Web of Science, to obtain relevant studies. The databases were searched for articles with the central keywords 'oestrogen', 'epithelial-mesenchymal transition' and 'Wnt4'. Wnt family member 4 (Wnt4) was included to relate the EMT in MRKH as it was previously found to be mutated in an animal model and in MRKH patients. These keywords were decided on after a thorough discussion between the review authors, and several attempts were made with the modification of keywords and Boolean operators. The finalised and utilised terms were 'Wnt4' OR 'oestrogen' AND 'epithelial to mesenchymal transition' OR 'EMT'.

2.2 Eligibility criteria

In addition to the application of the selected terms, the search was further restricted to a few criteria. The search was refined to 10-year limit for studies published from 2011 to 2021. Only English original articles were considered during the search. In contrast, articles respective to cancer research were unselected by adding the term 'NOT cancer' during the search. After the search was performed accordingly, the results were extracted to carefully screen the titles and abstracts for topic-related articles. As this review aimed for EMT regulation by E2 and/or distinct stimuli, articles unassociated to this aim were eliminated.

2.3 Selection of reviews

Three review authors worked separately to screen each retrieved study. Initially, the articles with titles irrelevant to the topic were removed, likewise when reviewing abstracts. Duplicates were all eliminated after the title assessment. The reviewers then explored the full texts of the selected articles to ensure data applicability for inclusion and to remove unrelated studies. Disagreement during the selection process was resolved through discussion among the review team.

2.4 Data extraction and synthesis

Three independently working reviewers extracted pertinent data from every chosen study. The request for full-text papers was conducted *via* ResearchGate for articles that were not publicly accessible. Data obtained from the chosen studies were summarised and thematically tabulated based on author, aim, disease/event, type of cell/tissue, treatment, findings and conclusions.

2.5 Risk bias assessment

Applying an adapted version of risk of bias tool which was the Office of Health Assessment and Translation (OHAT), three review authors independently assessed the risk of bias of the included studies (Ruszymah et al., 2020). This analysis tool entails the risk of bias in several domains: 1) selection bias; 2) performance bias; 3) detection bias; 4) attrition bias; 5) reporting bias. Studies were deemed as exhibiting a low risk of bias (+), high risk of bias (−), unclear risk of bias (?) or not applicable (NA) (Supplementary Table S1). Consensus was achieved *via* discussion between the review authors for any variance in the risk of bias analysis.

3 Results

3.1 Search results

A total of 150 articles were yielded from the search of three databases mainly: 59 articles from PubMed, 47 articles from Scopus and 44 articles from Web of Science. Three review authors worked separately to screen each study retrieved to minimise bias while enhancing the quality of the search results. A total of 73 articles were eliminated based on title screening and 38 articles were found to be duplicated and thus removed. A further evaluation of the abstracts showed that 10 articles were irrelevant to either E2, Wnt4 or EMT. The remaining 29 articles were obtained and underwent a full-text assessment. The assessment resulted in 10 articles being selected for this review; the unselected studies were not focused on EMT. Figure 1 presents the flow chart of the selection process.

3.2 Study characteristics

All included studies in this review were published from 2011 to 2021 and were performed as *in vitro*-based research. Intriguingly, these studies are corresponded to female reproductive system which could be implying that Wnt4, E2 and EMT have imperceptible functions in female reproductive system. The data obtained from these studies

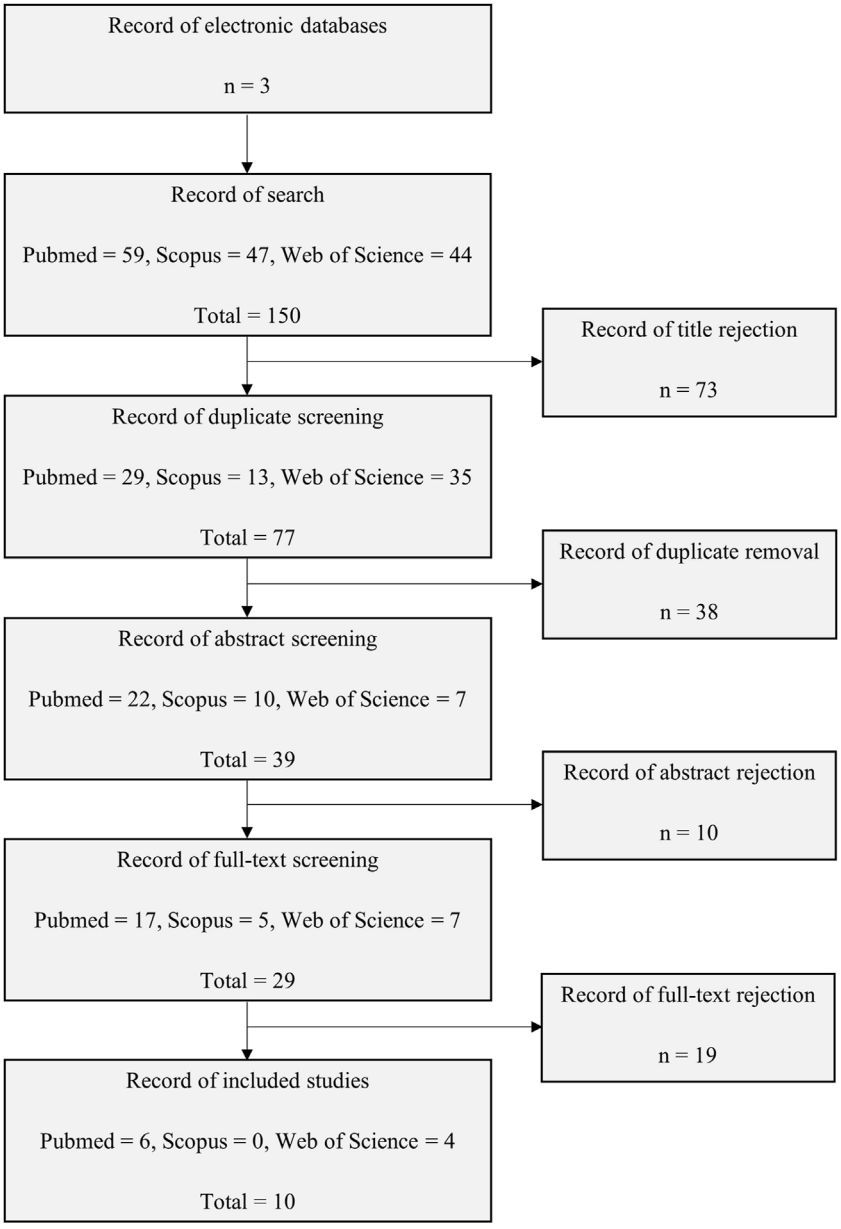


FIGURE 1
Flow chart of the study selection process from electronic databases of PubMed, Scopus, and Web of Science.

were summarised and classified into two groups: seven studies under 'factor'-modulating EMT and three studies under 'factor'-manipulating E2-induced EMT. Collectively, the main disease investigated was EM, while three studies investigated intrauterine adhesion (IUA), polycystic ovary syndrome (PCOS) with/without endometrial hyperplasia and adenomyosis. Furthermore, the human cell type that was generally used in analysing the mechanisms of these diseases was endometrial cells. Only one study employed endometrial tissue. An overview of the selected studies is displayed in Table 1 and Table 2.

4 Discussion

The full search and complete assessment of numerous studies provided 10 articles correlated to E2 and EMT in the female reproductive system. A further assessment of these articles divulged the feasibility of varying stimuli in mediating EMT activities to initiate, support or hamper respective diseases. E2 is widely known for its contributions in the development and regulation of female reproductive functions (Findlay et al., 2010; Tang et al., 2019), yet little is known about its capacity to modulate

TABLE 1 Summary and classification of the articles on EMT modulation.

Type of Induction	Aim	Disease/ Event	Type of Cell/ Tissue	Treatment	Findings	Conclusion	References
Factor promoting EMT	1. To verify the role and expression of Rho family on proliferation and EMT in EM 2. To assess the correlation and molecular mechanism between Rho family and E2 pathway	EM	Human eutopic endometrial epithelial cells	Cells were co-cultured with RhoA and siRhoA lentiviral supernatants	1. Western blot analysis showed that RhoA enhancement attenuated E-cadherin expression and increased vimentin and N-cadherin expressions 2. Transwell, EdU labelling, and CCK-8 assays revealed that RhoA promoted migration, cell division and proliferation 3. RhoA enhancement strengthened the effect of E2-promoting EMT in Western blot analysis	RhoA/ROCK pathway was upregulated by E2/ERα/ERK pathway to promote EMT and proliferation, resulting in EM development	Huang et al. (2020)
	To investigate the function of E2 in cellular transition, migratory, and inflammatory responses of endocervical epithelial cells (EECs) and cervical stromal cells (CSCs)	Uterine contractions	1. Human endocervical epithelial cells 2. Human cervical stromal cells	Cells were treated with 5,000 pg/mL E2 and 100 ng/ml LPS (an infection stimulus)	EECs and CSCs which were under untreated and LPS-treated conditions, E2 unaltered: 1. Immunocytochemical staining of CK-18 and vimentin expressions 2. Cell shape index based on cell shape analysis 3. Levels of EMT markers exhibited in Western blot analysis	E2 probably supported EMT and localized inflammation in the presence of infection stimulus in migrating and remodelling cervical cells	Tantengco et al. (2021)
	1. To assess the effects of ZEB1 knockdown on growth, mobility, and invasion 2. To identify the effects of E2 on ZEB1 expression and promoter activity	EM	Ishikawa cells	Cells were transfected with recombinant shZEB1 lentivirus and ZEB1 promoter reporter gene recombinant vectors	1. ZEB1 downregulation suppressed cell proliferation, migration and invasion as shown in transwell and MTS analyses 2. Knockdown of ZEB1 induced E-cadherin while inhibited vimentin protein expressions as per Western blot results	E2 induced ZEB1 expression and contributed to EMT phenomenon in EM.	Wu et al. (2018a)
Factor inhibiting EMT	1. To study the effects of E2 on EMT in normal and fibrotic endometrium	IUA	1. Normal human endometrial epithelial cells.	1. Cells were cultured with 10, 20, 30 and 50 nM E2 for 24, 48 and 72 h	1. Induction of TGF-β1 in IUA cell model decreased epithelial markers and increased mesenchymal markers as confirmed by Western blotting. The effects were reversed in TGF-β1-induced fibrosis cells pre-treated with 30 nM E2	E2 inhibited TGF-β1-induced EMT by activating Wnt/β-catenin signalling pathway to prevent IUA occurrence and development	Cao et al. (2020)
	2. To investigate the function of E2 on TGF-β1-induced EMT and Wnt/β-catenin signalling in an IUA cell model		2. IUA cell model	2. Cells were exposed to 10, 30 and 50 ng/ml TGF-β1 for 24 h	2. RT-PCR analysis showed that mRNA expression levels of Wnt/β-catenin signalling molecules were increased in TGF-β1-induced fibrosis cells when E2 was added		
	1. To examine the effects of melatonin on migration, invasion	EM	1. Normal endometrial	1. Cells were treated with 1 mM melatonin	1. CCK-8 assay showed that melatonin decreased growth and E2-induced	By upregulating Numb expression and decreasing	Qi et al. (2018)

(Continued on following page)

TABLE 1 (*Continued*) Summary and classification of the articles on EMT modulation.

Type of Induction	Aim	Disease/ Event	Type of Cell/ Tissue	Treatment	Findings	Conclusion	References
	and EMT in normal and endometriotic epithelial cells		epithelial cells (NECs)		cell growth in EuECs and NEC.	activity of Notch signalling pathway, melatonin might impede E2-induced migration, invasion and EMT in NECs and EuECs	
	2. To study possible signalling pathway involved		2. Endometriotic eutopic epithelial cells (EuECs)	2. Cell were cultured with/without 10 nM E2 for 24, 48 and 72 h	2. Migration and invasion assays revealed that melatonin significantly decreased migration and invasion in both cells 3. In Western blot, melatonin eliminated E2-induced vimentin and E2-downregulated Numb expressions in NEC.		
Factor altering EMT	1. To examine the expression and localization of MAPK signalling and EMT components in endometrium of non-PCOS and PCOS patients with and without EH 2. To identify the effects of metformin on EMT *in vitro*	PCOS with and without EH	Endometrial tissue	Tissues were treated with 10 nM E2 and/or 20 mM metformin for 24 h	Immunoblot results showed treatment of metformin alone or in combination with E2 altered protein expressions of CK 8, Claudin 1, ZO-1, N-cadherin, Slug, Snail and α-SMA in endometrial tissues of PCOS patients	Abnormal regulation of EMT and MAPK signalling components impaired function and homeostasis of endometrial cells. Metformin regulated endometrial EMT protein expression in an E2-dependent manner.	Hu M et al. (2020)
	1. To investigate the roles of miR200s and MALAT1 in ectopic endometrium	EM	1. Human endometrial epithelial cells	Cell were transfected with siRNA-MALAT1 and mir200c mimics	1. MiR200c reversed E2-mediated EMT.	E2-mediated MALAT1/miR200s expression to promote EMT in EM, which MALAT1 and miR200s exhibited significant reciprocal inhibition	Du et al. (2019)
	2. To examine the effects of E2 on EMT and MALAT1/ miR200s in both endometrial epithelial cells and Ishikawa cells		2. Ishikawa cells		2. MALAT1 knockdown attenuated E2-induced EMT. 3. E2 decreased E-cadherin expression and increased vimentin expression in a dose-dependent manner		

E2, oestrogen; EMT, epithelial-mesenchymal transition; EM, endometriosis; RhoA, transforming protein RhoA; EdU labelling, 5-ethynyl-2′-deoxyuridine labelling; CCK-8 assay, cell counting kit-8 assay; ERα, oestrogen receptor α; LPS, lipopolysaccharide; CK-18, cytokeratin-18; ZEB1, zinc finger E-box-binding homeobox 1; TGF-β, transforming growth factor beta 1; IUA, intrauterine adhesion; RT-PCR, real-time polymerase chain reaction; MAPK, mitogen-activated protein kinase; PCOS, polycystic ovary syndrome; EH, endometrial hyperplasia; CK 8, cytokeratin 8; ZO-1, zonula occludens-1; Slug, zinc finger protein SNAI2; Snail, zinc finger protein SNAI1; α-SMA, alpha-smooth muscle actin; MALAT1, metastasis associated lung adenocarcinoma transcript 1.

EMT in the female reproductive tract. Together, this review assessed the roles of external stimuli related to E2 in mediating EMT in the development of diseases that ordinarily arise in the female reproductive system, typically EM (Figure 2).

4.1 Epithelial-to-mesenchymal transition in endometriosis

4.1.1 EMT activation

EMT upregulation is one of the essential molecular mechanisms in disease development. It is defined as an increase in the expression of mesenchymal traits in cells undergoing plastic transformation from an epithelial state (Eades et al., 2011). This process is characterized by the disintegration of epithelial cell-cell junctions, loss of apical-basal polarity, rearrangement of cytoskeletal composition, changes in cell shape and morphology, increased cell protrusions and motility, loss of epithelial markers, and the activation of mesenchymal phenotype-inducing genes (Lamouille et al., 2014). In addition to signalling pathways and membrane receptors, the upregulation of EMT necessitates activation from external stimuli (Thiery et al., 2009). The Rho family, part of the Ras superfamily, encompasses small signalling proteins such as guanosine triphosphatases (Heo and Meyer, 2003). Huang *et al.*

TABLE 2 Summary and classification of the articles on manipulation of E2-induced EMT.

	Aim	Disease/ Event	Type of Cell/ Tissue	Treatment	Findings	Conclusion	References
1	To assess the expression pattern and function of NRP1 in E2-induced EMT in endometrial cells of adenomyosis	Adenomyosis	Human endometrial cells (HEC-1-A)	1. Cells were infected with NRP1 and shRNA NRP1 retroviruses for 72 h	1. From RT-PCR results, NRP1 retrovirus infection decreased epithelial markers of CDH1 and occludin mRNA expression while increased mesenchymal markers of ACTA2 and N-cadherin mRNA expression in endometrial cells. The results were similar in Western blot analysis whereby expression of E-cadherin was reduced and α-SMA was enhanced	NRP1 participated in E2-induced EMT in promoting adenomyosis development	Hu R et al. (2020)
				2. Cells were treated with 0.1, 1 and 10 μM E2 for 24 h	2. Detected in both RT-PCR and Western blot, E2 treatment in endometrial cells promoted NRP1 protein and mRNA expressions in a dose-dependent manner		
					3. NRP1 shRNA obviously restored expressions of epithelial markers while lowered expressions of mesenchymal markers in E2-induced EMT in endometrial cells as presented in the results of RT-PCR and Western blot		
2	To inspect the potential roles of circ_0004712 in E2-induced EMT in EM development	EM	1. Ishikawa cells	1. Cells were treated with 10^{-8} mol/L E2 for 48 h	1. Circ_0004712 knockdown repressed E2-induced cell migration activity in transwell assay	Upregulating circ_0004712 resulted in E2-induced cell migration *via* EMT event and the molecular mechanism might be correlated with β-catenin pathway in EM development	He et al. (2020)
			2. End1/ E6E7 cells	2. Cells were transfected with siRNA of circ_0004712 and miR-148a-3p mimics	2. In dual-luciferase assay, direct binding sites between circ_0004712 and miR-148a-3p as well as miR-148a-3p and SOS2 were presented, suggesting miR-148a-3p targeted SOS2 and bound by circ_0004712 to regulate E2-induced EMT in endometrial epithelial cells		
					3. Transwell assay demonstrated that impeding circ_0004712 or enhancing miR-148a-3p expression significantly inhibiting E2-induced migration activity in cells		
3	To explore the molecular mechanism of LXA_4 in inhibiting E2-induced EMT in EM	EM	Eutopic endometrial epithelial cells	Cells were treated with 200 nM E2 and 100 nM LXA_4 for 48 h	1. Cells appeared spindle-shape and fibroblast-like after E2 treatment. Co-treatment of E2 and LXA_4 reversed the morphological appearance change as observed in phase-contrast microscopy	LXA_4 markedly repressed E2-enhanced EMT progress through ALXR-dependent manner, causing downregulation of ERK and p38 MAPK phosphorylation, thus suppressing EM development	Wu et al. (2018b)
					2. LXA_4 abrogated the effects in mRNA and protein expressions of E2-induced of vimentin, N-cadherin and ZEB1 as well as E2-reduced E-cadherin as shown in qRT-PCR and Western blot		
					3. Cell migration and invasion assays revealed that LXA_4 reduced E2-stimulated invasion and migration in cells		

E2, oestrogen; EMT, epithelial-mesenchymal transition; EM, endometriosis; NRP1, neurophilin 1, RT-PCR, real-time polymerase chain reaction; CDH1, cadherin-1; ACTA2, actin alpha 2; α-SMA, alpha-smooth muscle actin; SOS2, son of sevenless homolog 2; LXA_4, lipoxin A_4; ZEB1, zinc finger E-box-binding homeobox 1; qRT-PCR, quantitative real-time polymerase chain reaction; ALXR, lipoxin A_4 receptor; p38 MAPK, p38 mitogen-activated protein kinase.

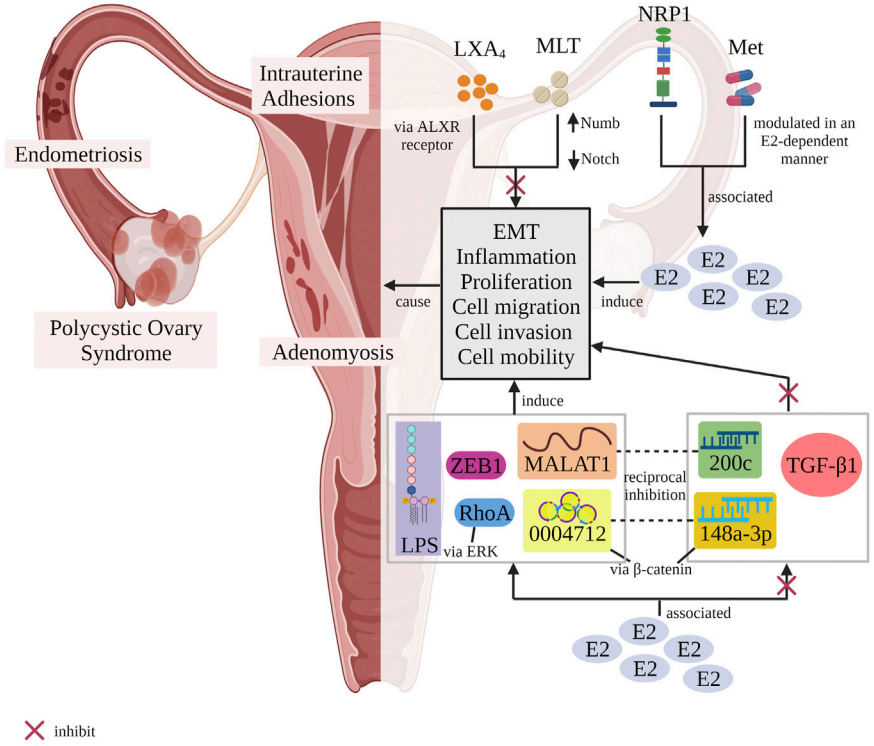


FIGURE 2
Schematic illustrating the potentiality of different stimuli in regulating EMT process with or without the association of E2 resulting in diseases-related female reproductive tract. LXA_4 inhibited EMT *via* ALXR-dependent manner. Melatonin upregulated Numb expression and decreased the activity of Notch1 signalling pathway to block EMT occurrence. In contrast, both NRP1 and metformin were associated to E2 in regulating EMT. Metformin modulated EMT in an E2-dependent manner. Besides that, E2 was correlated to TGF-β1, miR200c and miR-148a-3p in suppressing EMT event while upregulated MALAT1, circ_0004712, ZEB1, LPS and RhoA (*via* activating ERK signalling pathway) to induce EMT process. There were two pairs of stimuli, MALAT1/miR200c as well as circ_0004712/miR-148a-3p which possessed an antagonism effect between them. An example, the antagonism effect happened when circ_0004712 sponged miR-148a-3p to activate β-catenin signalling pathway, thus promoting EMT event. E2, oestrogen; EMT, epithelial-mesenchymal transition; LXA_4, lipoxin A_4; MLT, melatonin; NRP1, neurophilin 1; Met, metformin; Numb and Notch, Notch1/Numb signalling pathway; ALXR, lipoxin A_4 receptor; 200c, miR200c; 0004712, circ_0004712; 148a-3p, miR-148a-3p; LPS, lipopolysaccharide; β-catenin, β-catenin signalling pathway; ERK, ERK signalling pathway; TGF-β1, transforming growth factor-β1; ZEB1, zinc finger E-box-binding homeobox 1; RhoA, transforming protein RhoA; MALAT1, metastasis-associated lung adenocarcinoma transcript 1 (Designed with BioRender: https://biorender.com/).

attempted to demonstrate the role and molecular mechanism of the Rho family on the E2 pathway, as well as proliferation and EMT in EM (Huang et al., 2020). They demonstrated the attenuation of E-cadherin and increased vimentin as well as N-cadherin protein expression in transforming protein RhoA (RhoA)-overexpressed eutopic endometrial epithelial cells. Moreover, other assays used in their study confirmed that the induction of RhoA resulted in increased cell migration, cell division and proliferation. In contrast, loss of RhoA function by utilising siRNA suppressed all these effects. In addition, RhoA has been shown to have an effect of E2 in terms of promoting EMT in EM. Likewise, Tantengco and others deduced that E2 apparently supports EMT in the migration and remodelling of cervical cells in the presence of an infection stimulus such as lipopolysaccharide (Tantengco et al., 2021). E2 was also shown to induce zinc finger E-box-binding homeobox 1 (ZEB1), a transcription factor related to EMT, in a study conducted by Wu *et al.* (Wu R.-F. et al., 2018). This evidence shows that EMT could be upregulated in EM, not only by the modulation of transcription factors, but also by the induction of external stimuli.

4.1.2 Manipulation of EMT activation

Apart from the possible EMT inducers and EMT inhibitors, there are other stimuli that alter EMT in endometrial cells depending on the manipulated variables. An example of these manipulated variables is the co-treatment of cells with E2. Hu and others investigated treatment with metformin (a medication used to treat PCOS) alone or in combination with E2 in cultured

human PCOS endometrial tissues and found inconsistently altered epithelial and mesenchymal markers (Hu M et al., 2020). For instance, there was an increase in the protein level of zinc finger protein SNAI1 (Snail) and decreased protein levels of claudin 1, N-cadherin and alpha-smooth muscle actin (α-SMA) when endometrial tissues were treated with metformin only. In combination with E2 treatment, the protein levels of cytokeratin 8 and Snail increased, whereas the protein levels of claudin 1, zonula occludens-1, zinc finger protein SNAI2 (Slug) and α-SMA decreased, suggesting that metformin might regulate endometrial EMT in an E2-dependent manner.

A similar study by Du et al. incorporated E2 treatment to examine endometrial EMT in both human endometrial epithelial cells and Ishikawa cells using metastasis associated lung adenocarcinoma transcript 1 (MALAT1), which is another class of long non-coding RNAs and miR200 family, which consists of non-coding microRNA members. Their study showed that the RNA expression levels of miR200s were downregulated while MALAT1 was upregulated in endometrial epithelial cells treated with E2 (Du et al., 2019). Also, they found that MALAT1/miR200s were associated to EMT, cell proliferation, growth, apoptosis, migration and invasion. This evidence not only demonstrates that E2 could alter MALAT1/miR200s expression to differentially regulate EMT in EM, but also implicates the suitability of MALAT1/miR200s as a diagnostic and prognostic biomarker of diseases. This is due to an antagonism effect between MALAT1 and miR200c found in Ishikawa cells when MALAT1 inhibition attenuated miR200c inhibitor-enhanced overexpression of EMT-associated markers (ZEB1, ZEB2 and vimentin) while miR200c inhibition increased the expression levels of siRNA-MALAT1-downregulated EMT-associated markers. The sponge effect of MALAT1 on miR200c has been a research subject in investigating the pathogenesis and progression of multiple diseases, including EM (Li et al., 2016; Liang et al., 2017; Pa et al., 2017), indicating a new therapeutic strategy.

4.1.3 Targeting EMT inhibition in halting disease progression

It has been demonstrated that EMT instigates pathological processes in the normal epithelial cells of reproductive organs, leading to EM, adenomyosis and metastasis (Bilyk et al., 2017). Thus, extensive research has been performed to ameliorate related diseases by means of targeting EMT (Chen et al., 2020; Roy et al., 2021). However, EMT is connected to a vast number of processes and many signalling pathways may overlap (Cieślik et al., 2013). Therefore, targeting a specific orchestrator of EMT could improve the sensitivity in terms of halting disease progression. Transforming growth factor beta 1 (TGF-β1) is a secreted polypeptide cytokine. It is a prominent target in disease research as it controls myriad cellular responses during human development. Additionally, the TGF-β1 signalling pathway has been the most attentively defined pathway in inducing EMT (Gonzalez and Medici, 2014), as it activates the transcription of EMT-targeted genes, for example Snail, ZEB, forkhead box protein C2, high-mobility group AT-hook 2, paired related homeobox 1 and TWIST1 (Lamouille et al., 2014). Cao *et al.* found that treatment with TGF-β1 in an IUA cell model decreased the expression of epithelial markers and increased the expression of mesenchymal markers; interestingly, the effects were reversed when cells were pre-treated with E2 (Cao et al., 2020).

Previously, melatonin, a hormone that controls sleep-wake cycle, was shown to inhibit TGF-β1-induced EMT in A549 cells, which are adenocarcinomic human alveolar epithelial cells (Yu et al., 2016). This study found that melatonin is associated with TGF-β1 in inhibiting EMT. Moreover, melatonin abolished E2-induced cell migration, cell invasion and EMT by modulating Notch1/Numb expression in EM epithelial cells (Qi et al., 2018). This study futher verified that melatonin might be an ideal therapeutic in attenuating EMT to restrain the development of EM. Olive extracts from *Olea europaea* have also been shown to prevent EMT in human nasal respiratory epithelial cells by retaining a cuboidal cell shape, with higher expression of E-cadherin and lower expression of vimentin (Razali et al., 2018). Ginsenoside Rh2, a bioactive component in ginseng, has shown unexpected abilities in apoptosis induction and EMT inhibition in two types of human endometrial cell lines, HEC1A and Ishikawa cells (Kim et al., 2017). The results show that there were significant increases in the number of apoptotic cells and the E-cadherin/tubulin ratio as well as notable decreases in the vimentin/tubulin ratio, cell invasion rate and cell migration ability. These studies attest that even natural bioactive compounds can inhibit EMT in diseased cells.

4.2 Modulation and regulation of E2-induced EMT

It is compelling that E2 promotes EMT in a number of E2-dependent diseases like EM and adenomyosis. Hence, researchers tend to induce EMT activities by treating targeted cells with E2 prior to further analysis of a disease. An essential mechanistic link between E2 and EMT is circular RNA (circRNA), which is a long non-coding RNA (lncRNA) capable of regulating genes at the transcriptional or post-transcriptional level (Qu et al., 2015); these molecules act as an miRNA 'sponge' to inhibit the activity of one or multiple miRNAs (Yu and Kuo, 2019). Evidence of circRNA regulated E2-induced EMT was presented in a study by He *et al.* using Transwell assays, where knockdown of cir_0004712 coupled with overexpression of miR-48a-3p significantly repressed cell migration in E2-treated endometrial epithelial cells; this effect was recovered with the inhibition of miR-48a-3p (He et al., 2020). This result shows that circ_0004712 could bind to miR-148a-3p to mediate E2-induced EMT in EM.

Another stimulus that could modulate E2-induced EMT is the protein receptor neurophilin 1 (NRP1). Hu and others showed that silencing NRP1 clearly restored the expression of E-cadherin, abolished the expression of α-SMA and impaired cell migration in E2-induced endometrial cells (Hu R et al., 2020). This result indicates that NRP1 might be a potential therapeutic for adenomyosis patients as it could modulate E2-induced EMT in endometrial cells. Lipoxin A_4 (LXA_4), a pro-resolving and anti-inflammatory molecule, was investigated for its effects on EMT in E2-induced eutopic endometrial epithelial cells. Based on a study by Wu *et al.*, combined treatment with E2 and LXA_4 reversed the morphological change in cells that initially appeared spindle-shape and fibroblast-like when treated with E2 alone (Wu R. F. et al., 2018). Also, LXA_4 has been shown to reduce the effect of E2-induced migration and invasion in these cells. Since LXA_4 competes with E2 to bind to ER (Russell et al., 2011), these results suggest that LXA_4 could repress EM development by occupying ER to inhibit E2 signalling, thereby averting EMT in endometrial cells.

4.3 Crosstalk between Wnt, E2 and EMT: Is this related to MRKH syndrome ?

Vaginal agenesis is a congenital disorder whereby the vagina does not develop. This rare disorder is commonly seen in young girls with cervical agenesis and MRKH syndrome (Nakhal and Creighton, 2012). In some cases, the signs and symptoms are identified when patients are evaluated for primary amenorrhea with otherwise typical growth and pubertal development (Fontana et al., 2017). To treat MRKH syndrome, a two-step procedure is used: creation of a vagina (neovagina) and subsequent anastomosis of the uterus with the newly created vagina. The use of *in vitro* cultured vaginal mucosa has emerged as a novel technique to epithelise the new vaginal wall in recent years. In this review, we assessed the involvement of Wnt4 and E2 on EMT in the female reproductive tract, specifically EM. Since information on these factors on vaginal agenesis is still lacking, we postulate that EMT, with the involvement of Wnt4 and E2, also occurs in the vagina as the endometrium extends downwards to vaginal tract.

Wnt4 has been shown to be crucial for female sexual development. Wnt4 protein suppresses male sexual differentiation by repressing the biosynthesis of gonadal androgen. Inhibition of Wnt4 has been studied by knocking out the Wnt4 gene in mice, resulting in failed Müllerian duct formation (Vainio et al., 1999). Wnt4 has also been studied with regard to its function in the development of the Müllerian duct, as mutations in or the absence of Wnt4 in the Müllerian mesenchyme results in vaginal agenesis (Biason-Lauber et al., 2004; Biason-Lauber et al., 2007; Prunskaite-Hyyryläinen et al., 2015; St-Jean et al., 2019), a deformity that is usually diagnosed in MRKH patients. One case study discussed a woman without structures derived from the Müllerian ducts (uterus and fallopian tubes), with unilateral renal agenesis and clinical signs of androgen excess (Biason-Lauber et al., 2007). In contrast, earlier studies reported that they detected no Wnt4 abnormalities in the DNA extracted from women with MRKH syndrome (Drummond et al., 2008; Ravel et al., 2009; Philibert et al., 2011), considering that the function of Wnt4 gene and the genotypic variability of MRKH are not well comprehended yet.

Numerous signalling pathways can initiate EMT in response to the presence of stimuli such as E2. One of the leading pathways associated with EMT is the Wnt signalling pathway (Zmarzły et al., 2020). Existing research on the effects of Wnt signalling in E2-induced EMT is scarce. From the assessment of the studies included in this review, very few studies were found to evaluate the relationships between Wnt proteins and EMT-induced cells, specifically in relation to diseases associated with the female reproductive tract. Despite that, there was one study conducted by Guo et al. (2014), who successfully showed the overexpression of Wnt4 gene upregulated the markers of EMT by the decreased expression of E-cadherin and increased expression of α-SMA. Previous study has demonstrated that the expression of Wnt4 is directly regulated by E2, in an ERα-dependent manner in growth-hormone (GH)-producing cells (Miyakoshi et al., 2009). Another study showed that the number of Wnt4 mRNA copies increased with the injection of E2 in mice's uteri. Also, a proposed model in the study suggested that E2 could lead to the activation of Wnt signalling by induction of Wnt4 expression in uterine epithelial cells. (Hou et al., 2004). These studies indicated that E2 and Wnt4 associate to each other and their interactions could result in the activation of EMT.

As reported, the role of EMT in EM development is possible *via* E2 induction but the association with vaginal agenesis in MRKH patients remains unrevealed. Figure 3 showed possible relations between EM and vaginal agenesis as those affected organs are descended from the primitive Mullerian duct and speculated to involve Wnt4-expressing Mullerian cells from the Mullerian development process. Many theories have been came up in explaining the pathogenesis of EM but remain to be proven. In particular, coelomic metaplasia: Specialized cells of peritoneum share common embryological lineage (from coelomic epithelium) as endometrial cells, develop into endometriotic lesions by metaplastic transformation (Gruenwald, 1942; Dhesi and Morelli, 2015). Mullerian remnants: These cells misplaced the primitive endometrial cells during organogenesis and implanted in their migratory path across the pelvic floor due to aberrant differentiation and migration, later induced into endometrial cells by specific stimuli (e.g., E2) (Mai et al., 1998). Those theories revealed that EMT involves in endometrial-like tissue formation as the Mullerian cells were found proliferated, migrated subsequently progressed into endometriotic lesions.

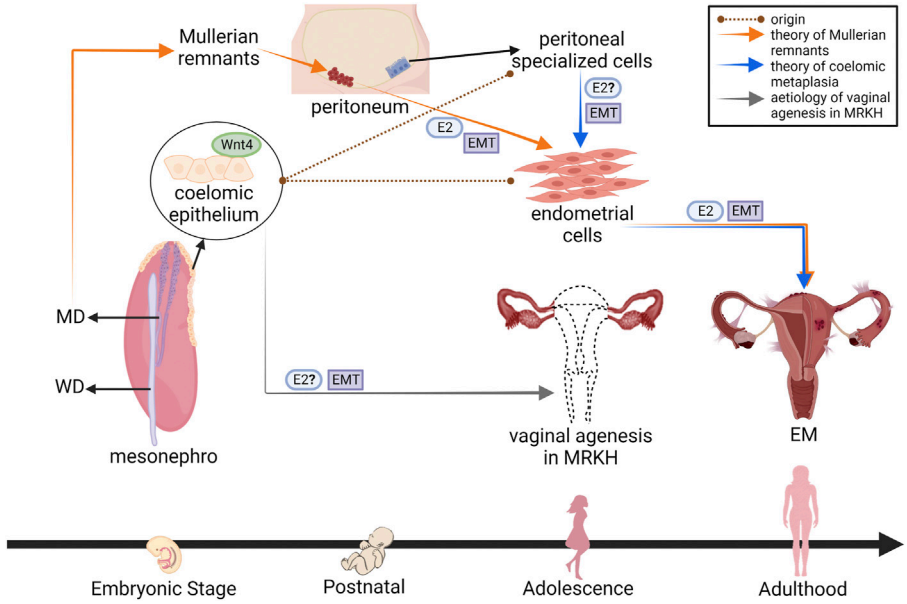


FIGURE 3
Crosstalk between E2, Wnt4 and EMT in the development of EM: Aetiology of vaginal agenesis in MRKH. Pathogenesis of EM has been classically defined to originate from Mullerian-related cells expressing Wnt4 in early human development. Under the influence of E2 and other appropriate stimuli, the cells exhibit EMT and progress into endometriotic lesions in adults. Corresponding to MRKH patients which also initiated from Mullerian cells, the disorder develops when Wnt4 is mutated in coelomic epithelium of embryo. This could then trigger EMT which leads to female reproductive organ formation failure, such as vagina and uterus. E2, oestrogen; EMT, epithelial-mesenchymal transition; Wnt4, Wnt family member 4; MD, Mullerian duct; WD, Wolffian duct; MRKH, Mayer-Rokitansky-Küster-Hauser syndrome; EM, endometriosis (Designed with BioRender: https://biorender.com/).

EMT induction factors that are closely related to EM have been discussed such as E2; involves in hormonal regulation, and Wnt4; Mullerian-expressing genes. Bilyk et al. (2017) and Baranov et al. (2018) stated that EM in adults might be associated to the early life of Mullerian-expressing genes in uterine endometrium. On the other hand, vaginal agenesis is developed when the Mullerian duct fails to form as a result of Wnt4 anomalies and other factors, potentially E2 and EMT as adopted from the theories of EM development. The Mullerian progenitors, coelomic epithelial cells, express Wnt4 for regulating Mullerian invagination and elongation. These Mullerian stages require Wnt4-expressing progenitor cells to undergo EMT and differentiate into Mullerian mesenchymal cells, enabling cranial-caudal cell migration of Mullerian duct in close proximity to Wolffian duct. Once Mullerian ducts fuse with urogenital sinus, the cells undergo MET and specialize into epithelial subtypes, giving rise to female reproductive tract constituting fallopian tubes, uterus, cervix and upper vagina (Bilyk et al., 2017; Santana Gonzalez et al., 2021). This supports earlier studies reporting vaginal agenesis occurs when there is deregulation of Wnt4 (Wnt4 mutation or deficiency) as well as the speculation of EMT (cells remain mesenchymal and not forming epithelial) during the development of Mullerian duct, though the role of E2 in this event is still elusive.

Thus, it could be assumed that E2 and Wnt4 associate in an undefined manner to induce EMT in the development of diseases in female reproductive tract such as EM as well as vaginal aplasia in MRKH patients. Still, further research has to be done to strongly validate how E2 regulate Wnt4 or Wnt signalling pathway to potentiate EMT in disease development. Collectively, this review will provide new insights into the mechanisms related to E2 and Wnt4 in regulating vaginal epithelialisation to ensure better surgical outcomes. We anticipate that this will become a key aspect in the surgical procedure for creating a new vagina (neovagina) in the near future.

5 Conclusion

In relation to the overall results discussed in this review, there is a constraint whereby the paucity of EMT in the non-cancer field limits complete understanding on the regulation of EMT

processes in non-cancer related diseases. Hence, this review may provide a new perspective for non-cancer research, especially regarding reproductive-related studies. This review presents the potentials to influence EMT through various stimuli in diseases associated with the female reproductive system, chiefly EM. Additionally, E2 could also be an EMT regulator and is prone to be mediated by other EMT orchestrators to cause EMT, principally in endometrial epithelial cells. This review also highlighted the possible participation of Wnt and E2 in EMT in vaginal epithelial cells, which may result in the complete absence of vaginal structure, an abnormality observed in women with MRKH syndrome. Collectively, this overview could serve as a foundation to pursue ideal or novel targets for the treatment of diseases associated with the female reproductive system, namely vaginal agenesis, by regulating vaginal epithelialisation to ensure better surgical outcomes.

Author contributions

MDY, TLY, NS, and AGNA conceived of the presented idea. MDY, TLY, and NS developed the theory followed by performing the data extractions. MDY and TLY verified the data extraction methods and extracted data. MDY, NS, MM, and RAR supervised the findings of this work. All authors discussed the results and contributed to the final manuscript. All authors read and approved the final manuscript.

Funding

This work was supported by the Fundamental Research Grant Scheme from The Ministry of Higher Education Malaysia (FRGS/1/2020/SKK0/UKM/02/25).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2022.937988/full#supplementary-material

References

Baranov, V., Malysheva, O., and Yarmolinskaya, M. (2018). Pathogenomics of endometriosis development. *Int. J. Mol. Sci.* 19, E1852. doi:10.3390/ijms19071852

Bartley, J., Jülicher, A., Hotz, B., Mechsner, S., Hotz, H., and Julicher, A. (2014). Epithelial to mesenchymal transition (EMT) seems to be regulated differently in endometriosis and the endometrium. *Arch. Gynecol. Obstet.* 289, 871–881. doi:10.1007/s00404-013-3040-4

Biason-Lauber, A., Konrad, D., Navratil, F., and Schoenle, E. J. (2004). A WNT4 mutation associated with Müllerian-duct regression and virilization in a 46, XX woman. *N. Engl. J. Med.* 351, 792–798. doi:10.1056/NEJMOA040533

Biason-Lauber, A., De Filippo, G., Konrad, D., Scarano, G., Nazzaro, A., Schoenle, E. J., et al. (2007). WNT4 deficiency—a clinical phenotype distinct from the classic mayer–rokitansky–kuster–hauser syndrome: A case report. *Hum. Reprod.* 22, 224–229. doi:10.1093/HUMREP/DEL360

Bilyk, O., Coatham, M., Jewer, M., and Postovit, L.-M. (2017). Epithelial-to-Mesenchymal transition in the female reproductive tract: From normal functioning to disease pathology. *Front. Oncol.* 0, 145. doi:10.3389/FONC.2017.00145

Cao, J., Liu, D., Zhao, S., Yuan, L., Huang, Y., Ma, J., et al. (2020). Estrogen attenuates TGF-β1-induced EMT in intrauterine adhesion by activating Wnt/β-catenin signaling pathway. *Braz. J. Med. Biol. Res.* 53, 9794. doi:10.1590/1414-431x20209794

Chen, M., Zhou, Y., Xu, H., Hill, C., Ewing, R. M., He, D., et al. (2020). Bioinformatic analysis reveals the importance of epithelial-mesenchymal transition in the development of endometriosis. *Sci. Rep.* 101 (10), 8442–8512. doi:10.1038/s41598-020-65606-9

Chen, Q., Yang, W., Wang, X., Li, X., Qi, S., Zhang, Y., et al. (2017). TGF-β1 induces EMT in bovine mammary epithelial cells through the TGFβ1/smad signaling pathway. *Cell. Physiol. biochem.* 43, 82–93. doi:10.1159/000480321

Cieślik, M., Hoang, S. A., Baranova, N., Chodaparambil, S., Kumar, M., Allison, D. F., et al. (2013). Epigenetic coordination of signaling pathways during the epithelial-mesenchymal transition. *Epigenetics Chromatin* 6, 28. doi:10.1186/1756-8935-6-28

Das, J. K., Felty, Q., Poppiti, R., Jackson, R. M., and Roy, D. (2018). Nuclear respiratory factor 1 acting as an oncoprotein drives estrogen-induced breast carcinogenesis. *Cells* 7, 234. doi:10.3390/CELLS7120234

Dhesi, A. S., and Morelli, S. S. (2015). Endometriosis: A role for stem cells. *Womens Health* 11, 35–49. doi:10.2217/whe.14.57

Ding, J. X., Feng, Y. J., Yao, L. Q., Yu, M., Jin, H. Y., and Yin, L. H. (2006). The reinforcement of invasion in epithelial ovarian cancer cells by 17β-Estradiol is associated with up-regulation of Snail. *Gynecol. Oncol.* 103, 623–630. doi:10.1016/j.ygyno.2006.04.023

Drummond, J. B., Reis, F. M., Boson, W. L. M., Silveira, L. F. G., Bicalho, M. A. C., and Marco, L. De (2008). Molecular analysis of the WNT4 gene in 6 patients with Mayer-Rokitansky-Küster-Hauser syndrome. *Fertil. Steril.* 90, 857–859. doi:10.1016/J.FERTNSTERT.2007.07.1319

Du, Y., Zhang, Z., Xiong, W., Li, N., Liu, H., He, H., et al. (2019). Estradiol promotes EMT in endometriosis via MALAT1/ miR200s sponge function. *Reproduction* 157, 179–188. doi:10.1530/REP-18-0424

Eades, G., Yao, Y., Yang, M., Zhang, Y., Chumsri, S., and Zhou, Q. (2011). miR-200a regulates SIRT1 expression and epithelial to mesenchymal transition (EMT)-like transformation in mammary epithelial cells. *J. Biol. Chem.* 286, 25992–26002. doi:10.1074/JBC.M111.229401

Findlay, J. K., Liew, S. H., Simpson, E. R., and Korach, K. S. (2010). Estrogen signaling in the regulation of female reproductive functions. *Handb. Exp. Pharmacol.* 198, 29–35. doi:10.1007/978-3-642-02062-9_2

Fontana, L., Gentilin, B., Fedele, L., Gervasini, C., and Miozzo, M. (2017). Genetics of mayer–rokitansky–küster–hauser (MRKH) syndrome. *Clin. Genet.* 91, 233–246. doi:10.1111/CGE.12883

Furuya, M., Masuda, H., Hara, K., Uchida, H., Sato, K., Sato, S., et al. (2017). ZEB1 expression is a potential indicator of invasive endometriosis. *Acta Obstet. Gynecol. Scand.* 96, 1128–1135. doi:10.1111/AOGS.13179

Gonzalez, D. M., and Medici, D. (2014). Signaling mechanisms of the epithelial-mesenchymal transition. *Sci. Signal.* 7, re8. doi:10.1126/scisignal.2005189

Gruenwald, P. (1942). Origin of endometriosis from the mesenchyme of the celomic walls. *Am. J. Obstetrics Gynecol.* 44, 470–474. doi:10.1016/S0002-9378(42)90484-8

Guo, Y., Li, Z., Ding, R., Li, H., Zhang, L., Yuan, W., et al. (2014). Parathyroid hormone induces epithelial-to-mesenchymal transition via the Wnt/β-catenin signaling pathway in human renal proximal tubular cells. *Int. J. Clin. Exp. Pathol.* 7, 5978–5987.

He, X., Liu, N., Mu, T., Lu, D., Jia, C., Wang, S., et al. (2020). Oestrogen induces epithelial-mesenchymal transition in endometriosis via circ_0004712/miR-148a-3p sponge function. *J. Cell. Mol. Med.* 24, 9658–9666. doi:10.1111/jcmm.15495

Heo, W. D., and Meyer, T. (2003). Switch-of-Function mutants based on morphology classification of Ras superfamily small GTPases. *Cell* 113, 315–328. doi:10.1016/S0092-8674(03)00315-5

Hou, X., Tan, Y., Li, M., Dey, S. K., and Das, S. K. (2004). Canonical Wnt signaling is critical to estrogen-mediated uterine growth. *Mol. Endocrinol.* 18, 3035–3049. doi:10.1210/ME.2004-0259

Hu, M., Zhang, Y., Li, X., Cui, P., Li, J., Brännström, M., et al. (2020). Alterations of endometrial epithelial-mesenchymal transition and MAPK signalling components in women with PCOS are partially modulated by metformin *in vitro*. *Mol. Hum. Reprod.* 26, 312–326. doi:10.1093/molehr/gaaa023

Hu, R., Peng, G. Q., Ban, D. Y., Zhang, C., Zhang, X. Q., and Li, Y. P. (2020). High-expression of neuropilin 1 correlates to estrogen-induced epithelial-mesenchymal transition of endometrial cells in adenomyosis. *Reprod. Sci.* 27, 395–403. doi:10.1007/s43032-019-00035-2

Huang, Y., Fernandez, S. V., Goodwin, S., Russo, P. A., Russo, I. H., Sutter, T. R., et al. (2007). Epithelial to mesenchymal transition in human breast epithelial cells transformed by 17beta-estradiol. *Cancer Res.* 67, 11147–11157. doi:10.1158/0008-5472.CAN-07-1371

Huang, Z. X., Mao, X. M., Wu, R. F., Huang, S. M., Ding, X. Y., Chen, Q. H., et al. (2020). RhoA/ROCK pathway mediates the effect of oestrogen on regulating epithelial-mesenchymal transition and proliferation in endometriosis. *J. Cell. Mol. Med.* 24, 10693–10704. doi:10.1111/jcmm.15689

Kalluri, R., and Weinberg, R. A. (2009). The basics of epithelial-mesenchymal transition. *J. Clin. Invest.* 119, 1420–1428. doi:10.1172/JCI39104

Kim, J. H., Kim, M., Yun, S. M., Lee, S., No, J. H., Suh, D. H., et al. (2017). Ginsenoside Rh2 induces apoptosis and inhibits epithelial-mesenchymal transition in HEC1A and Ishikawa endometrial cancer cells. *Biomed. Pharmacother.* 96, 871–876. doi:10.1016/j.biopha.2017.09.033

Lamouille, S., Xu, J., and Derynck, R. (2014). Molecular mechanisms of epithelial–mesenchymal transition. *Nat. Rev. Mol. Cell Biol.* 15, 178–196. doi:10.1038/NRM3758

Li, Q., Zhang, C., Chen, R., Xiong, H., Qiu, F., Liu, S., et al. (2016). Disrupting MALAT1/miR-200c sponge decreases invasion and migration in endometrioid endometrial carcinoma. *Cancer Lett.* 383, 28–40. doi:10.1016/j.canlet.2016.09.019

Li, W.-N., Wu, M.-H., and Tsai, S.-J. (2021). HYPOXIA AND REPRODUCTIVE HEALTH: The role of hypoxia in the development and progression of endometriosis. *Reproduction* 161, F19–F31. doi:10.1530/REP-20-0267

Liang, Z., Chen, Y., Zhao, Y., Xu, C., Zhang, A., Zhang, Q., et al. (2017). miR-200c suppresses endometriosis by targeting MALAT1 *in vitro* and *in vivo*. *Stem Cell Res. Ther.* 8, 251. doi:10.1186/S13287-017-0706-Z

Lin, D., Huang, Q., Wu, R., Dai, S., Huang, Z., Ren, L., et al. (2019). Long non-coding RNA AFAP1-AS1 promoting epithelial-mesenchymal transition of endometriosis is correlated with transcription factor ZEB1. *Am. J. Reprod. Immunol.* 81, 13074. doi:10.1111/aji.13074

Mai, H., Wei, Y., Yin, Y., Huang, S., Lin, H., Liao, Y., et al. (2019). LINC01541 overexpression attenuates the 17β-Estradiol-induced migration and invasion capabilities of endometrial stromal cells. *Syst. Biol. Reprod. Med.* 65, 214–222. doi:10.1080/19396368.2018.1549290

Mai, K. T., Yazdi, H. M., Perkins, D. G., and Parks, W. (1998). Development of endometriosis from embryonic duct remnants. *Hum. Pathol.* 29, 319–322. doi:10.1016/S0046-8177(98)90110-1

Matsuzaki, S., and Darcha, C. (2012). Epithelial to mesenchymal transition-like and mesenchymal to epithelial transition-like processes might be involved in the pathogenesis of pelvic endometriosis. *Hum. Reprod.* 27, 712–721. doi:10.1093/HUMREP/DER442

Matsuzaki, S., Murakami, T., Uehara, S., Canis, M., Sasano, H., and Okamura, K. (2001). Expression of estrogen receptor alpha and beta in peritoneal and ovarian endometriosis. *Fertil. Steril.* 75, 1198–1205. doi:10.1016/S0015-0282(01)01783-6

Meng, J., Zhang, X.-T., Liu, X.-L., Fan, L., Li, C., Sun, Y., et al. (2016). WSTF promotes proliferation and invasion of lung cancer cells by inducing EMT via PI3K/Akt and IL-6/STAT3 signaling pathways. *Cell Signal* 28, 1673. doi:10.1016/j.cellsig.2016.07.008

Mishra, S., Tai, Q., Gu, X., Schmitz, J., Poullard, A., Fajardo, R. J., et al. (2015). Estrogen and estrogen receptor alpha promotes malignancy and osteoblastic tumorigenesis in prostate cancer. *Oncotarget* 6, 44388–44402. doi:10.18632/ONCOTARGET.6317

Miyakoshi, T., Kajiya, H., Miyajima, K., Takei, M., Tobita, M., Takekoshi, S., et al. (2009). The expression of Wnt4 is regulated by estrogen via an estrogen receptor alpha-dependent pathway in rat pituitary growth hormone-producing cells. *Acta histochem. cytochem.* 42, 205–213. doi:10.1267/AHC.09033

Nakhal, R. S., and Creighton, S. M. (2012). Management of vaginal agenesis. *J. Pediatr. Adolesc. Gynecol.* 25, 352–357. doi:10.1016/j.jpag.2011.06.003

Pa, M., Naizaer, G., Seyiti, A., and Kuerbang, G. (2017). Long noncoding RNA MALAT1 functions as a sponge of MiR-200c in ovarian cancer. *Oncol. Res.* doi:10.3727/096504017x15049198963076

Park, S.-H., Cheung, L. W. T., Wong, A. S. T., and Leung, P. C. K. (2008). Estrogen regulates Snail and Slug in the down-regulation of E-cadherin and induces metastatic potential of ovarian cancer cells through estrogen receptor alpha. *Mol. Endocrinol.* 22, 2085–2098. doi:10.1210/ME.2007-0512

Pellegrini, C., Gori, I., Achtari, C., Hornung, D., Chardonnens, E., Wunder, D., et al. (2012). The expression of estrogen receptors as well as GREB1, c-MYC, and cyclin D1, estrogen-regulated genes implicated in proliferation, is increased in peritoneal endometriosis. *Fertil. Steril.* 98, 1200–1208. doi:10.1016/j.fertnstert.2012.06.056

Philibert, P., Biason-Lauber, A., Gueorguieva, I., Stuckens, C., Pienkowski, C., Eatrice Lebon-Labich, B., et al. (2011). Molecular analysis of WNT4 gene in four adolescent girls with mullerian duct abnormality and hyperandrogenism (atypical Mayer-Rokitansky-K € uster-Hauser syndrome). *Fertil. Steril.* 95, 2683–2686. doi:10.1016/j.fertnstert.2011.01.152

Pontikaki, A., Sifakis, S., and Spandidos, D. A. (2016). Endometriosis and breast cancer: A survey of the epidemiological studies. *Oncol. Lett.* 11, 23–30. doi:10.3892/OL.2015.3895

Proestling, K., Birner, P., Gamperl, S., Nirtl, N., Marton, E., Yerlikaya, G., et al. (2015). Enhanced epithelial to mesenchymal transition (EMT) and upregulated MYC in ectopic lesions contribute independently to endometriosis. *Reprod. Biol. Endocrinol.* 13, 75. doi:10.1186/S12958-015-0063-7

Prunskaite-Hyyryläinen, R., Skovorodkin, I., Xu, Q., Miinalainen, I., Shan, J., and Vainio, S. J. (2015). Wnt4 coordinates directional cell migration and extension of the müllerian duct essential for ontogenesis of the female reproductive tract. *Hum. Mol. Genet.* 25, 1059–1073. doi:10.1093/hmg/ddv621

Qi, S., Yan, L., Liu, Z., Mu, Y., Li, M., Zhao, X., et al. (2018). Melatonin inhibits 17β-estradiol-induced migration, invasion and epithelial-mesenchymal transition in normal and endometriotic endometrial epithelial cells. *Reprod. Biol. Endocrinol.* 16, 62. doi:10.1186/s12958-018-0375-5

Qu, S., Yang, X., Li, X., Wang, J., Gao, Y., Shang, R., et al. (2015). Circular RNA: A new star of noncoding RNAs. *Cancer Lett.* 365, 141–148. doi:10.1016/j.canlet.2015.06.003

Ravel, C., LorenCo, D., Dessolle, L., Mandelbaum, J., McElreavey, K., Darai, E., et al. (2009). Mutational analysis of the WNT gene family in women with Mayer-Rokitansky-Kuster-Hauser syndrome. *Fertil. Steril.* 91, 1604–1607. doi:10.1016/j.fertnstert.2008.12.006

Razali, R. A., Nik Ahmad Eid, N. A. H., Jayaraman, T., Amir Hassan, M. A., Azlan, N. Q., Ismail, N. F., et al. (2018). The potential of Olea europaea extracts to prevent TGFβ1-induced epithelial to mesenchymal transition in human nasal respiratory epithelial cells. *BMC Complement. Altern. Med.* 18, 197. doi:10.1186/S12906-018-2250-5

Roy, S., Sunkara, R. R., Parmar, M. Y., Shaikh, S., and Waghmare, S. K. (2021). EMT imparts cancer stemness and plasticity: new perspectives and therapeutic potential. *Front. Biosci.* 26, 238–265. doi:10.2741/4893

Russell, R., Gori, I., Pellegrini, C., Kumar, R., Achtari, C., and Canny, G. O. (2011). Lipoxin A 4 is a novel estrogen receptor modulator. *FASEB J.* 25, 4326–4337. doi:10.1096/FJ.11-187658

Ruszymah, B. H. I., Sainik, N. Q. A. V., Nordin, A., Saim, A. B., and Sulaiman, N. (2020). Cardioprotective effects of honey and its constituent: An evidence-based review of laboratory studies and clinical trials. *Int. J. Environ. Res. Public Health* 17, E3613. doi:10.3390/IJERPH17103613

Santana Gonzalez, L., Rota, I. A., Artibani, M., Morotti, M., Hu, Z., Wietek, N., et al. (2021). Mechanistic drivers of müllerian duct development and differentiation into the oviduct. *Front. Cell Dev. Biol.* 9, 605301–605319. doi:10.3389/fcell.2021.605301

Sisto, M., Lorusso, L., Tamma, R., Ingravallo, G., Ribatti, D., and Lisi, S. (2019). Interleukin-17 and -22 synergy linking inflammation and EMT-dependent fibrosis in Sjögren's syndrome. *Clin. Exp. Immunol.* 198, 261–272. doi:10.1111/CEI.13337

St-Jean, G., Boyer, A., Zamberlam, G., Godin, P., Paquet, M., and Boerboom, D. (2019). Targeted ablation of Wnt4 and Wnt5a in Müllerian duct mesenchyme impedes endometrial gland development and causes partial Müllerian agenesis. *Biol. Reprod.* 100, 49–60. doi:10.1093/BIOLRE/IOY160

Tang, Z.-R., Zhang, R., Lian, Z.-X., Deng, S.-L., and Yu, K. (2019). Estrogen-receptor expression and function in female reproductive disease. *Cells* 8, 1123. doi:10.3390/CELLS8101123

Tantengco, O. A. G., Richardson, L. S., and Menon, R. (2021). Effects of a gestational level of estradiol on cellular transition, migration, and inflammation in cervical epithelial and stromal cells. *Am. J. Reprod. Immunol.* 85, e13370–e13372. doi:10.1111/aji.13370

Thiery, J. P., Acloque, H., Huang, R. Y. J., and Nieto, M. A. (2009). Epithelial-mesenchymal transitions in development and disease. *Cell* 139, 871–890. doi:10.1016/J.CELL.2009.11.007

Trukhacheva, E., Lin, Z., Reierstad, S., Cheng, Y.-H., Milad, M., and Bulun, S. E. (2009). Estrogen receptor (ER) beta regulates ERalpha expression in stromal cells derived from ovarian endometriosis. *J. Clin. Endocrinol. Metab.* 94, 615–622. doi:10.1210/JC.2008-1466

Vainio, S., Heikkilä, M., Kispert, A., Chin, N., and McMahon, A. P. (1999). Female development in mammals is regulated by Wnt-4 signalling. *Nature* 397, 405–409. doi:10.1038/17068

Wang, D., Luo, Y., Wang, G., and Yang, Q. (2020). CircATRNL1 promotes epithelial–mesenchymal transition in endometriosis by upregulating Yes-associated protein 1 *in vitro*. *Cell Death Dis.* 117 (11), 594–613. doi:10.1038/s41419-020-02784-4

Wu, R.-F., Chen, Z.-X., Zhou, W.-D., Li, Y.-Z., Huang, Z.-X., Lin, D.-C., et al. (2018a). High expression of ZEB1 in endometriosis and its role in 17β-estradiol-induced epithelial-mesenchymal transition. *Int. J. Clin. Exp. Pathol.* 11, 4744–4758. Available at: http://www.ncbi.nlm.nih.gov/pubmed/31949550%0A.

Wu, R. F., Huang, Z. X., Ran, J., Dai, S. J., Lin, D. C., Ng, T. W., et al. (2018b). Lipoxin A4 suppresses estrogen-induced epithelial–mesenchymal transition via ALXR-dependent manner in endometriosis. *Reprod. Sci.* 25, 566–578. doi:10.1177/1933719117718271

Xiong, Y., Liu, Y., Xiong, W., Zhang, L., Liu, H., Du, Y., et al. (2016). Hypoxia-inducible factor 1α-induced epithelial–mesenchymal transition of endometrial epithelial cells may contribute to the development of endometriosis. *Hum. Reprod.* 31, 1327–1338. doi:10.1093/HUMREP/DEW081

Yang, Y.-M., and Yang, W.-X. (2017). Epithelial-to-mesenchymal transition in the development of endometriosis. *Oncotarget* 8, 41679–41689. doi:10.18632/ONCOTARGET.16472

Yoriki, K., Mori, T., Kokabu, T., Matsushima, H., Umemura, S., Tarumi, Y., et al. (2019). Estrogen-related receptor alpha induces epithelial-mesenchymal transition through cancer-stromal interactions in endometrial cancer. *Sci. Rep.* 9, 6697–6699. doi:10.1038/s41598-019-43261-z

Yu, C.-Y., and Kuo, H.-C. (2019). The emerging roles and functions of circular RNAs and their generation. *J. Biomed. Sci.* 26, 29–12. doi:10.1186/S12929-019-0523-Z

Yu, N., Sun, Y-T., Su, X-M., He, M., Dai, B., and Kang, J. (2016). Melatonin attenuates TGFβ1-induced epithelial-mesenchymal transition in lung alveolar epithelial cells. *Mol. Med. Rep.* 14, 5567–5572. doi:10.3892/MMR.2016.5950

Yu, Q., Wang, J., Li, T., Guo, X., Ding, S., Che, X., et al. (2021). Recepteur d'origine nantais contributes to the development of endometriosis via promoting epithelial-mesenchymal transition of a endometrial epithelial cells. *J. Cell. Mol. Med.* 25, 1601–1612. doi:10.1111/JCMM.16261

Zhang, M., Wang, S., Tang, L., Wang, X., Zhang, T., Xia, X., et al. (2019). Downregulated circular RNA hsa_circ_0067301 regulates epithelial-mesenchymal transition in endometriosis via the miR-141/Notch signaling pathway. *Biochem. Biophys. Res. Commun.* 514, 71–77. doi:10.1016/J.BBRC.2019.04.109

Zmarzły, N., Hermyt, E., Kruszniewska-Rajs, C., Gola, J., Witek, A., Mazurek, U., et al. (2020). Expression profile of EMT-related genes and miRNAs involved in signal transduction via the Wnt pathway and cadherins in endometrial cancer. *Curr. Pharm. Biotechnol.* 21, 1663–1671. doi:10.2174/1389201021666201218125900

frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 18 January 2023
DOI 10.3389/fendo.2022.1034614

OPEN ACCESS

EDITED BY
Abdel Halim Harrath,
King Saud University, Saudi Arabia

REVIEWED BY
Silvia Vannuccini,
University of Florence, Italy
Carla Piccinato,
Independent Researcher, Brazil
Antonio Simone Laganà,
University of Palermo, Italy

*CORRESPONDENCE
Chantal Guillemette
chantal.guillemette@crchudequebec.ulaval.ca
Tea Lanišnik Rižner
tea.lanisnik-rizner@mf.uni-lj.si

†These authors have contributed equally to this work and share first authorship

‡These authors share senior authorship

SPECIALTY SECTION
This article was submitted to Reproduction, a section of the journal Frontiers in Endocrinology

RECEIVED 01 September 2022
ACCEPTED 19 December 2022
PUBLISHED 18 January 2023

CITATION
Emond J-P, Caron P, Pušić M, Turcotte V, Simonyan D, Vogler A, Osredkar J, Rižner TL and Guillemette C (2023) Circulating estradiol and its biologically active metabolites in endometriosis and in relation to pain symptoms. *Front. Endocrinol.* 13:1034614. doi: 10.3389/fendo.2022.1034614

Circulating estradiol and its biologically active metabolites in endometriosis and in relation to pain symptoms

Jean-Philippe Emond[1†], Patrick Caron[1†], Maja Pušić[2], Véronique Turcotte[1], David Simonyan[3], Andrej Vogler[4], Joško Osredkar[5], Tea Lanišnik Rižner[2*‡] and Chantal Guillemette[1,6*‡]

[1]Pharmacogenomics Laboratory, Centre Hospitalier Universitaire (CHU) de Québec – Université Laval Research Center and Faculty of Pharmacy, Université Laval, Québec City, QC, Canada, [2]Laboratory for Molecular Basis and Biomarkers of Hormone Dependent Diseases, Institute of Biochemistry, Medical Faculty, University of Ljubljana, Ljubljana, Slovenia, [3]Statistical and Clinical Research Platform, CHU de Québec – Université Laval Research Center, Québec City, QC, Canada, [4]Department of Obstetrics & Gynaecology, University Medical Centre Ljubljana, Ljubljana, Slovenia, [5]Clinical Institute of Clinical Chemistry and Biochemistry, University Medical Centre Ljubljana, Ljubljana, Slovenia, [6]Canada Research Chair in Pharmacogenomics, Université Laval, Québec City, QC, Canada

Objectives: Endometriosis (EM) is an estrogen-dominant inflammatory disease linked to infertility that affects women of reproductive age. EM lesions respond to hormonal signals that regulate uterine tissue growth and trigger inflammation and pain. The objective of this study was to evaluate whether estradiol (E_2) and its biologically active metabolites are differentially associated with EM given their estrogenic and non-estrogenic actions including proliferative and inflammatory properties.

Design: We performed a retrospective study of 209 EM cases and 115 women without EM.

Methods: Pain-related outcomes were assessed using surveys with validated scales. Preoperative serum levels of estradiol (E_2) and estrone (E_1), their 2-, 4- and 16- hydroxylated (OH) and methylated (MeO) derivatives (n=16) were measured by mass spectrometry. We evaluated the associations between estrogen levels and EM anatomic sites, surgical stage, risk of EM, and symptoms reported by women. Spearman correlations established the relationships between circulating steroids.

Results: Of the sixteen estrogens profiled, eleven were detected above quantification limits in most individuals. Steroids were positively correlated, except 2-hydroxy 3MeO-E_1 (2OH-3MeO-E_1). Higher 2OH-3MeO-E_1 was linked to an increased risk of EM (Odd ratio (OR)=1.91 (95%CI 1.09-3.34); P=0.025). Ovarian EM cases displayed enhanced 2-hydroxylation with higher 2MeO-E_1 and 2OH-E_1 levels (P< 0.009). Abdominal, pelvic and back pain symptoms were also linked to higher 2OH-3MeO-E_1 levels (OR=1.86; 95%CI 1.06-3.27; P=0.032).

10.3389/fendo.2022.1034614

Conclusions: The 2-hydroxylation pathway emerges as an unfavorable feature of EM, and is associated with ovarian EM and pain related outcomes.

KEYWORDS

endometriosis, catechol estrogens, steroids, mass spectrometry, pain symptoms

Introduction

Endometriosis (EM) affects approximately 10% of women of reproductive age (1) and is defined by the presence of endometrial glands or stoma outside the uterine cavity. It is a non-malignant disease nonetheless associated with dysmenorrhea, dyspareunia, pelvic pain and infertility due to the presence of ectopic tissue and inflammation (2–4). Endometriotic lesions can be superficial peritoneal, ovarian or deeply infiltrating (5, 6). The etiology of endometriosis is complex (7) and multifactorial thus several theories have been proposed to explain the clinical manifestation of endometriosis; Sampson's theory of retrograde menstruation, coelomic metaplasia theory, Mullerian rests theory, stem cell theory, impaired immune system theory, and others (8). Endometriosis is considered a chronic inflammatory disease with altered peritoneal environment in patients with endometriosis. The ectopic lesions recruit immune cells which leads to production of pro-inflammatory molecules and cytokines and also promote angiogenesis and innervation and thus contribute to survival of these lesions (9).

EM is an estrogen-dependent disease with molecular hallmarks of genetic predisposition, altered hormonal milieu (estrogen dependence and progesterone resistance) and inflammation (10, 11). Changes in steroid biotransformation pathways have been reported leading to an increased local production of estrogens in endometriosis lesions (12). EM lesions respond to hormonal signals such as estradiol (E_2) that regulate uterine tissue growth and triggers inflammation, and that are linked to pain symptoms (13). Excessive inflammation also leads to changes in sex steroid receptors (ERα and ERβ) expression and enhanced estrogen biosynthesis in endometriotic lesions, involving aromatase, sulfatase and other pathways (14–21). Treatment of pelvic pain in EM thus includes the use of nonsteroidal anti-inflammatory drugs, oral contraceptives and progestins.

Estrogens comprise a vast array of hydroxylated (OH) and methoxylated (MeO) catechol estrogen (CE) metabolites with diverse biological activities. The synthesis of CE metabolites from E_2 and estrone (E_1) involves various metabolic routes, namely the 2-hydroxylation (2OH), 4-hydroxylation (4OH) and 16-hydroxylation (16OH) pathways and the action of the catechol-O-methyltransferase (COMT) to form 2- and 4-MeOCEs (22, 23). Besides acting as ligands of ERα and ERβ, CEs also present non-estrogenic properties (22, 24). MeOCEs have antiangiogenic and antiproliferative actions whereas 4-OHCEs have procarcinogenic properties (22, 25–27). Both the 2OH and 4OH CE derivatives generally have reduced estrogenic effects (24, 28) as opposed to the 16OH pathway that retains most of its estrogenic properties, with a preferential action on the ERβ (24). In addition, E_2 was previously found to be associated with pain due to its effects on nerves and inflammation (13). Some CEs present estrogenic activities resembling E_2 and they may be prone to cause pain.

Given the suspected biological roles of catechol estrogens, and their associations with several hormone-sensitive diseases including endometrial cancer (29, 30), we hypothesized that circulating levels of E_2 and/or its biologically active metabolites were associated with an altered risk of EM (primary objective) and severity of pain symptoms (secondary objective).

Material and methods

Study cohort

The study design corresponded to a retrospective case-control study comprising cases and controls from the same type of population (31). Part of this cohort was described previously (32–34). Patients' enrolment took place from March 2008 to June 2018 at the Departments of Obstetrics and Gynecology at the University Medical Centre Ljubljana, Slovenia. The study comprised patients who visited gynecologist with problems/symptoms that are indicative for laparoscopy surgery. The inclusion criteria were an indication for a diagnostic laparoscopy for symptoms suggestive of EM such as pain, infertility, ovarian cysts, other gynecological pathologies such as myomas and tubal sterilization. The exclusion criteria were pregnancy, age below 18 years, menopausal status, gynecological malignancies, cancelled surgery, previous hysterectomy, drug abuse and HIV infection (32). Of the 341 women, 17 participants were excluded to manage confounders: six because of missing data on mandatory age and/or BMI, three

due to unknown case or control status, four with polycystic ovary syndrome (PCOS) that could impact hormone levels, one due to a prior unidentified menopausal state, and three with significant anomalies of the menstrual cycle of unknown etiology. The remaining cohort of 324 women underwent either diagnostic laparoscopy or laparoscopic tubal sterilization and were divided according to presence (n=209, cases) or absence (n=115, controls) of EM. Controls were further divided into two groups (patients with benign pathologies (n=79) and healthy controls (n=35). Patients with benign pathologies had symptoms suggestive for EM (infertility and/or pain) or other gynecological pathologies. Healthy patients underwent laparoscopic tubal sterilization and had no symptoms suggestive for EM (Figure 1). A post-hoc ANOVA power analysis test (power package and R Statistical Software v4.1.2; R Core Team 2021) estimated that a sample size per group of 36 was suffcient). The clinical characteristics presented in Table 1 included age, body mass index (BMI), type of EM (ovarian, ovarian and peritoneal, peritoneal, and deep infiltrating), rASRM stage of disease (35), smoking status (current, former or never), use of hormonal therapy (last three months), use of oral contraception (last three months), and endometrial phase (secretory or proliferative). Patient-filled surveys using validated numeric rating scales documented the outcomes of "abdominal, pelvic and back pain", "dysmenorrhea (frequency)", "dysmenorrhea (intensity)", "score of dysmenorrhea", "dyspareunia (frequency)", "dyspareunia (intensity)" and "dysuria or dyschezia (frequency)" (36, 37). For EM cases, data for pain-related outcomes were available for 98.6% to 99.5% of participants, except for the "score of dysmenorrhea" (61.2%), "dysmenorrhea (intensity)" (35.4%), and "dyspareunia (intensity)" (59.3%) outcomes. In control cases, data was available for 86.7% to 97.4% of participants, except for "score of dysmenorrhea" (56.5%) and "dyspareunia (intensity)" (55.7%) outcomes. The patient's characteristics related to pain symptoms are presented in Supplementary Table 1. Pain related outcomes were dichotomized for the statistical analysis. The dichotomization for "abdominal, pelvic and back pain" was "yes or no". For "dysmenorrhea (frequency)", "dyspareunia (frequency)" and "dysuria or dyschezia (frequency)", the dichotomization was "infrequent (never, almost never or sometimes) or frequent (quite often or very often)". For "dyspareunia (intensity)" and "dysmenorrhea (intensity)", the dichotomization was "mild (no or slight pain) or moderate to severe (medium or strong pain)". For the "score of dysmenorrhea", the dichotomization was "scores of ≤5 or of >5". All participants provided an informed consent prior to their enrolment. This study was conducted in accordance with the declaration of Helsinki. This study was approved by the National Medical Ethics Committee in Slovenia (#0120-127/2016/6) and the ethics committee of the CHUQc – Université Laval (#2012-993).

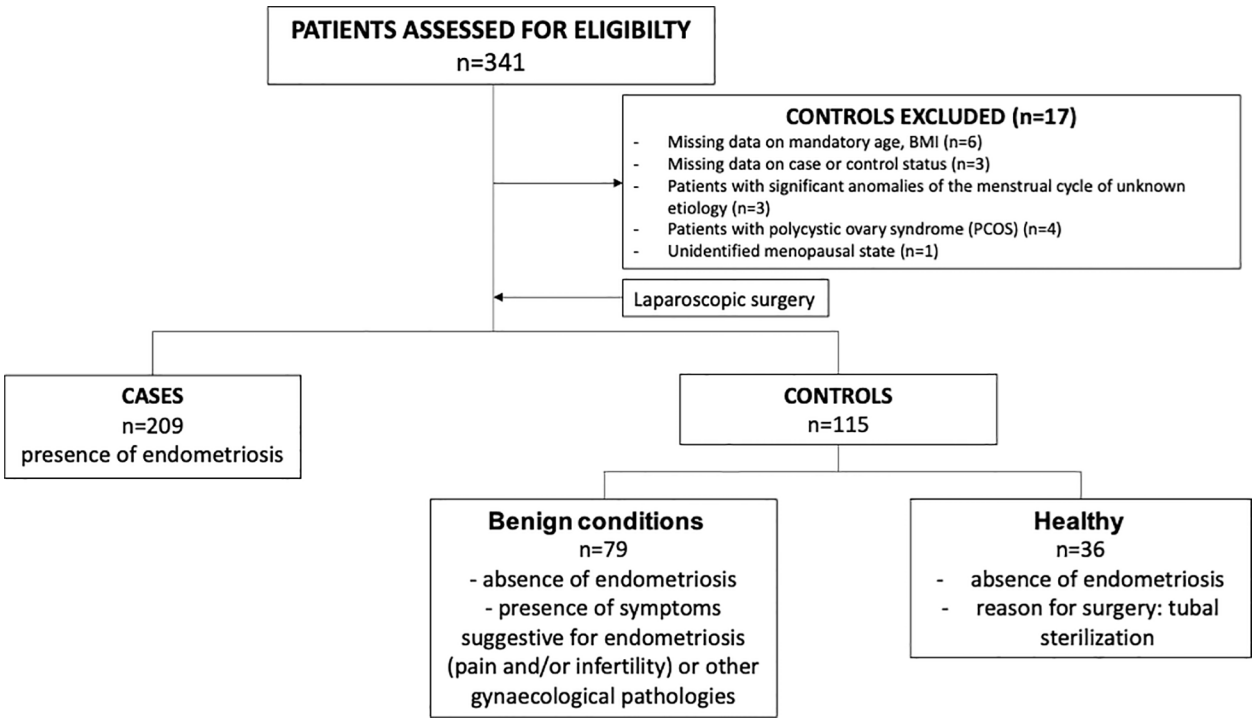


FIGURE 1
Flowchart of patient cohort.

10.3389/fendo.2022.1034614

TABLE 1 Characteristics and clinical data of endometriosis cases (n=209) and controls (n=115).

	Cases (n=209)	Controls (n=115)		
		All	Benign pathologies (n=79)	Healthy (n=36)
Age[1]				
Mean age ± SD (years)	31.57 ± 5.35	34.66 ± 6.79	32.03 ± 6.42	40.44 ± 2.87
Body mass index (BMI)[1]				
Mean ± SD	22.42 ± 3.66	24.88 ± 4.55	24.53 ± 4.67	25.66 ± 4.26
Type of disease				
Ovarian	35 (16.7%)	–	–	–
Ovarian and peritoneal	59 (28.2%)	–	–	–
Peritoneal	62 (29.7%)	–	–	–
Deep infiltrating	53 (25.4%)	–	–	–
Stage of disease				
1	49 (23.4%)	–	–	–
2	38 (18.2%)	–	–	–
3	67 (32.1%)	–	–	–
4	40 (19.1%)	–	–	–
Missing	15 (7.2%)	–	–	–
Smoking status				
Current	68 (32.5%)	37 (32.2%)	27 (34.2%)	10 (27.8%)
Former	11 (5.3%)	14 (12.2%)	9 (11.4%)	5 (13.9%)
Never	129 (61.7%)	62 (53.9%)	42 (53.2%)	20 (55.6%)
Missing	1 (0.5%)	2 (1.7%)	1 (1.3%)	1 (2.8%)
Hormonal therapy[2]				
Yes	38 (18.2%)	17 (14.8%)	15 (19.0%)	2 (5.6%)
No	171 (81.8%)	93 (80.9%)	62 (78.4%)	31 (86.1%)
Missing		5 (4.3%)	2 (2.5%)	3 (8.3%)
Per oral contraception[1,2]				
Yes	41 (19.6%)	27 (23.5%)	12 (15.2%)	15 (41.7%)
No	168 (80.4%)	85 (73.9%)	65 (82.3%)	20 (55.6%)
Missing	0 (0.0%)	3 (2.6%)	2 (2.5%)	1 (1.5%)
Endometrial phase[1]				
Secretory	83 (39.7%)	48 (41.7%)	28 (35.4%)	20 (55.6%)
Proliferative	112 (53.6%)	53 (46.1%)	38 (48.1%)	15 (41.7%)
Oral hormonal contraceptive	14 (6.7%)	11 (9.6%)	11 (13.9%)	0 (0.0%)
Missing	0 (0.0%)	3 (2.6%)	2 (2.5%)	1 (2.8%)

Surgical stages were classified using the revised American Society for Reproductive Medicine score (rASRM) classification of endometriosis. [1]*P*-values <0.0001 obtained using Pearson's chi square or Fisher's exact test when appropriate. [2]Last 3 months.

Quantification of estrogen derivatives

Blood samples were collected two days prior to surgery as described (32), and following strict standard operating procedures for collection, processing and storage at -80˚C to preserve stability of metabolites such as steroids. Briefly, 4 ml of blood sample was collected by venipuncture from the median cubital vein using BD Vacutainer tubes (#369032; Becton Dickinson and Company, NJ, USA). The collected samples were incubated for no more than 1 h at room temperature and then centrifuged at 1400 × g for 10 min at room temperature. The seperated serum was collected, aliquoted, and stored at -80°C until analysis. Only samples that were frozen/thawed once were used for analysis. A specific set of 16 estrogen derivatives were quantified in 250 µL of serum using a liquid-chromatography tandem mass spectrometry assay (LC-MS/MS) as described (29). The lower limit of quantification (LLOQ) was 5 pg/mL. Sums including all analytes and metabolic ratios were calculated for the different metabolic pathways. Catechol estrogens at levels below LLOQ (even if detected above the limit of detection) were considered undetected.

Statistical analysis

Differences in estrogen hormone levels between cases and controls, anatomic sites and surgical stages were determined by bivariate analyses on means of log transformed continuous hormone levels. The relationship between hormone levels was assessed using Spearman's rank-order correlation. Odd ratios (OR) were obtained using dichotomized hormone levels (independent variables) based on the median levels of controls as performed in previous studies (29) in a multivariate logistic regression model, adjusted for age, BMI, smoking status, oral contraception (last three months), hormonal therapy (last three months) and the endometrial phase (secretory or proliferative). Logistic models and Fisher's scoring were used to determine the ORs for pain related outcomes in cases. *P*-values were obtained using Pearson's chi square, Fisher's exact test, the one-way analysis of covariance F-test corrected with Tukey when appropriate or the Spearman Rho statistic test in the appropriate contexts. Results were considered statistically significant when $P < 0.05$. Statistical analyses were performed by the statistician (DS) using the software SAS 9.4 by SAS Institute Inc. (Cary, NC, USA). Due to the exploratory nature of the study, no adjustment for multiple comparison was done.

Results

Characteristics of EM and controls are despicted in Table 1. A total of 16 estrogen derivatives were quantified by MS in the serums of 341 women. Most estrogens and their oxidative metabolites (11 out of 16) were above LLOQ, except for 4OH-E_2, 17epi-E_3, 2MeO-E_2, 4MeO-E_1 and 4MeO-E_2 detected in less than 12% of the cohort. These five estrogens were thus excluded in subsequent statistical analyses (Supplementary Table 2). Levels of estrogens are displayed in Table 2 with E_1, E_2, 2OH-E_1, E_3, 16αOH-E_1 and 16keto-E_2 displaying the highest levels. In addition, 2OH-3MeOE_1 levels were higher in cases compared to controls (by 123%; $P = 0.02$) (Table 2). The analysis of hormone levels according to anatomic sites of EM (Table 3), showed higher levels of 2OH-E_1 (by 18%; $P = 0.009$), 2MeO-E_1 (38%; $P = 0.002$), sum of 2OH (7%; $P = 0.013$), sum of MeO (23%; $P = 0.036$), ratio of 2OH/sum E_1/E_2 (24%; $P = 0.023$), and ratio of 2OH-E_1/16αOH-E_1 (101%; $P = 0.033$) in cases diagnosed with ovarian EM. No evidence of an association was observed in relation to surgical stage (Supplementary Table 3).

The first objective aimed to establish the potential association of estrogens with the risk of developing EM. Women with higher circulating levels of specific catechol estrogens were shown to be more predisposed to EM risk in multivariable analysis adjusted for confounders including age, BMI, tobacco status, contraception, hormonal therapy and endometrial phase, which differ in EM cases compared to controls (Table 1). More specifically, women with higher levels of 2OH-3MeO-E_1 had an adjusted OR of 1.91 (95%CI 1.09-3.34; $P = 0.025$). This finding was also observed when restricted to healthy subjects and controls with benign pathologies (OR =2.61 (95%CI 0.84–8.09); $P = 0.097$ and 1.56 (95%CI 0.84–2.91); $P = 0.164$) but did not reached significance. A lower risk of EM was observed in association with elevated 16OH derivatives (with OR values of 0.22 for 16epi-E_3 (95%CI 0.06–0.83); $P = 0.025$) and 0.22 for 16keto-E_2 (95%CI 0.06–0.84); $P = 0.027$). Since 2OH-3MeO-E_1 was the main metabolite associated with the risk of EM, we evaluated whether the correlation of this metabolite with the other estrogens was different between controls and cases. We observed that 2OH-3MeO-E_1 was weakly but significantly correlated with 2-OH derivatives in EM cases at 0.20 ($P<0.05$) but not in controls (Table 4).

A secondary objective aimed to explore the relationship between hormone levels and symptoms of pain in EM cases (Table 5). Higher levels of 2OH-3MeO-E_1 were associated with the risk of pain in the abdominal, pelvic and back regions (OR =1.86 (95%CI 1.06-3.27) $P = 0.032$). Higher levels of 16αOH-E_1 were inversely associated with the risk of pain in the abdominal, pelvic and back regions (OR =0.55 (95%CI 0.31-0.97); $P = 0.038$). More frequent menstrual pain was associated with elevated E_1/E_2 (OR = 1.90 (95%CI 1.03-3.51); $P = 0.041$) whereas more severe menstrual pain was linked to a higher metabolic ratio of 4OH/sum of E_1/E_2 (OR =1.95; (95%CI 1.05-3.61); $P = 0.035$) (Table 5). Higher levels of 16keto-E_2 were associated with the risk of more severe dyspareunia experienced in the last three months (OR = 2.40 (95%CI 1.02-5.62); $P = 0.045$. Higher levels of 4OH-E_1 were associated with a reduced risk dysuria or dyschezia experienced

10.3389/fendo.2022.1034614

TABLE 2 Steroid levels for EM cases (n=209) and for controls (n=115) that included subjects with benign pathologies and healthy controls.

	Cases (n=209)		Controls (n=115)					
			All		Benign pathologies (n=79)		Healthy (n=36)	
Steroids	Median	10-90%	Median	10-90%	Median	10-90%	Median	10-90%
E_1	831.00	203.00-2830.00	882.00	262.00-2730.00	788.00	149.00-2830.00	1004.50	325.00-2130.00
E_2	138.00	8.86-396.00	118.00	17.20-320.00	100.00	13.20-320.00	163.50	87.70-345.00
2OH-E_1	96.40	9.70-247.00	93.10	11.70-227.00	73.90	11.40-212.00	123.50	12.60-262.00
2OH-E_2	10.60	2.50-37.90	12.40	2.50-41.10	10.40	2.50-27.90	17.55	2.50-48.80
4OH-E_1	8.77	2.50-25.90	7.59	2.50-22.50	7.65	2.50-25.00	7.42	2.50-21.50
16αOH-E_1	32.10	7.36-140.00	44.60	7.91-142.00	38.30	6.06-142.00	61.60	15.30-261.00
16epi-E_3	8.07	2.50-29.00	10.30	2.50-28.80	8.31	2.50-23.70	13.30	5.50-33.40
16keto-E_2	**31.70**	**8.05-130.00**	**40.80**	**7.07-142.00**	**31.10**	**6.55-92.00**	**58.00***	**22.50-269.00**
E_3	67.00	14.90-256.00	85.80	14.70-265.00	68.10	11.90-236.00	121.50	42.80-311.00
2MeO-E_1	24.50	2.50-80.90	18.40	2.50-60.60	15.30	2.50-61.60	25.35	2.50-60.60
2OH-3MeO-E_1	**5.58***	**2.50-16.00**	**2.50**	**2.50-13.60**	**2.50**	**2.50-12.10**	**2.50**	**2.50-20.60**
Sums								
E_1/E_2	1009.00	219.45-3102.00	1019.00	296.30-3040.00	885.40	182.33-3166.00	1138.50	460.00-2457.00
2OH	104.50	12.20-276.00	99.90	14.20-260.80	83.80	13.90-238.90	146.45	15.10-291.70
4OH	11.30	5.00-30.60	10.20	5.00-30.20	10.15	5.00-30.50	11.18	5.00-30.20
16OH	**152.10**	**33.70-551.30**	**204.11**	**37.36-591.80**	**140.21**	**31.88-428.20**	**264.90***	**69.24-758.90**
MeOs	41.19	16.33-99.39	33.00	15.83-79.51	30.00	15.83-87.60	39.07	15.63-79.30
OHs	302.20	54.62-837.80	358.63	69.23-801.10	288.25	57.52-648.80	474.50	211.26-941.60
CEs	352.40	81.72-928.61	390.22	98.36-876.50	312.61	82.52-734.78	539.77	242.16-1015.68
Ratios								
2OH/sum E_1/E_2	0.09	0.04-0.22	0.09	0.03-0.23	0.08	0.03-0.20	0.10	0.03-0.33
4OH/sum E_1/E_2	0.01	0.00-0.03	0.01	0.00-0.03	0.01	0.00-0.04	0.01	0.00-0.02
16OH/sum E_1/E_2	0.15	0.06-0.45	0.15	0.07-0.52	0.13	0.06-0.41	0.21	0.11-0.60
CEs/sum E_1/E_2	0.35	0.18-0.72	0.34	0.16-0.84	0.29	0.15-0.68	0.41	0.21-0.96
2OH/4OH	**8.14**	**2.06-20.2**	**9.09**	**2.13-22.8**	**8.38**	**1.74-22.32**	**11.46***	**3.02-29.70**
2OH/16OH	0.68	0.14-1.55	0.63	0.12-1.36	0.66	0.12-1.42	0.53	0.09-1.36
2OH/MeOs	2.46	0.80-4.08	2.79	0.71-5.00	2.71	0.71-4.84	3.13	1.21-5.35
4OH/16OH	0.08	0.02-0.27	0.06	0.01-0.24	0.08	0.02-0.34	0.05	0.01-0.13
4OH/MeOs	0.27	0.12-0.60	0.29	0.14-0.65	0.29	0.15-0.78	0.30	0.13-0.44
OHs/MeOs	**6.43**	**2.51-16.8**	**8.06**	**3.07-18.61**	**7.46**	**2.48-16.91**	**10.91***	**4.57-27.51**
2OH/16αOH-E_1	2.61	0.38-8.15	2.42	0.43-6.00	2.46	0.44-6.00	2.24	0.35-7.22

Steroids are reported in pg/mL. The sum of hydroxy derivatives (OHs) includes 2OH, 4OH and 16OH. The sum of all catechol estrogens (CEs) includes OHs and MeOs. *P*-values were obtained based on the one-way analysis of covariance F test with correction when necessary. Continuous means of hormone levels were log transformed and adjusted for age and BMI for statistical analysis. Significant *P* values <0.05 are highlighted with a star (*) and text in bold. The median values and the 10-90% intervals for E_2 were 123.00 (31.6 -417.0 pg/mL) for women in the proliferative phase and 169.00 (13.20-333.00 pg/mL) for women in the secretory phase.

10.3389/fendo.2022.1034614

TABLE 3 Steroid levels according to anatomic sites of disease in 209 endometriosis cases.

Steroids (pg/mL)	Median (10-90%)				*P*-value			
	Ovarian	Ovarian peritoneal	Peritoneal	Deep infiltrating	All	1 vs 3	1 vs 2	1 vs all
	n=35	n=59	n=62	n=53				
E_1	908.00 (258.00-3420.00)	723.00 (188.00-2990.00)	777.00 (188.00-3190.00)	873.00 (306.00-2160.00)				
E_2	143.00 (30.20-649.00)	138.00 (6.61-393.00)	130.50 (9.17-438.00)	139.00 (14.40-239.00)				
2OH-E_1	111.00 (53.70-455.00)	87.40 (7.06-211.00)	98.20 (7.76-234.00)	96.40 (20.40-196.00)	**0.033**	**0.022**	**0.011**	**0.009**
2OH-E_2	12.50 (2.50-67.40)	8.62 (2.50-36.60)	11.65 (2.50-33.50)	9.58 (2.50-35.20)				0.088
4OH-E_1	9.67 (2.50-35.10)	8.78 (2.50-24.40)	7.94 (2.50-22.10)	8.71 (2.50-24.20)				0.086
16αOH-E_1	28.70 (7.36-197.00)	27.20 (7.04-179.00)	39.00 (8.22-139.00)	34.70 (7.54-120.00)				
16epi-E_3	8.51 (2.50-29.20)	7.55 (2.50-31.00)	8.39 (2.50-28.10)	7.84 (2.50-25.20)				
16keto-E_2	40.50 (11.90-137.00)	34.10 (6.32-163.00)	26.80 (7.85-94.70)	30.00 (8.02-119.00)				
E_3	79.00 (23.70-233.00)	59.20 (14.90-245.00)	69.60 (9.06-329.00)	62.8 (17.00-225.00)				
2MeO-E_1	29.70 (10.80-234.00)	20.10 (2.50-75.50)	20.90 (2.50-63.20)	23.90 (5.48-80.90)	**0.011**	0.076	**0.029**	**0.002**
2OH-3MeO-E_1	5.65 (2.50-16.30)	2.50 (2.50-16.00)	2.50 (2.50-15.40)	6.05 (2.50-15.40)				
Sums								
E_1/E_2	1057.00 (295.20-3772.00)	857.00 (192.61-3474.00)	903.50 (196.86-3490.00)	1024.00 (351.80-2389.00)				0.058
2OH	117.70 (56.20-573.20)	98.77 (9.56-254.70)	126.55 (10.26-254.60)	104.50 (22.90-225.20)	0.083			**0.013**
4OH	13.80 (5.00-41.70)	11.37 (5.00-29.20)	10.44 (5.00-25.10)	11.21 (5.00-28.40)				
16OH	172.02 (45.00-540.10)	152.10 (33.70-682.20)	148.86 (32.51-542.00)	146.96 (32.26-444.90)				
MeOs	48.00 (26.20-269.56)	36.59 (16.33-99.39)	39.20 (12.50-80.10)	41.20 (15.48-103.70)				**0.036**
OHs	362.63 (114.39-1212.27)	267.50 (46.66-837.80)	299.54 (49.93-820.30)	343.91 (70.83-689.70)				
All CEs	404.57 (130.00-1283.07)	306.10 (79.50-885.49)	342.84 (72.00-850.37)	386.94 (102.43-738.61)				0.082
Ratios								
2OH/sum E_1/E_2	0.11 (0.04-0.27)	0.09 (0.03-0.18)	0.08 (0.03-0.20)	0.10 (0.04-0.22)	0.078			**0.023**
4OH/sum E_1/E_2	0.01 (0.00-0.03)	0.01 (0.00-0.04)	0.01 (0.00-0.03)	0.01 (0.01-0.03)				

(Continued)

TABLE 3 Continued

Steroids (pg/mL)	Median (10-90%)				*P*-value			
	Ovarian	Ovarian peritoneal	Peritoneal	Deep infiltrating	All	1 vs 3	1 vs 2	1 vs all
	n=35	n=59	n=62	n=53				
16OH/sum E_1/E_2	0.15 (0.07-0.30)	0.15 (0.06-0.38)	0.17 (0.08-0.48)	0.13 (0.06-0.58)				
CEs/sum E_1/E_2	0.32 (0.19-0.63)	0.36 (0.14-0.72)	0.34 (0.21-0.70)	0.34 (0.17-0.83)				
2OH/4OH	10.37 (2.87-23.33)	7.36 (1.00-17.20)	8.46 (1.77-26.80)	8.14 (3.02-18.93)				
2OH/16OH	0.89 (0.28-1.43)	0.58 (0.13-1.44)	0.55 (0.13-1.55)	0.76 (0.13-1.91)				0.090
2OH/MeOs	2.54 (1.22-4.50)	2.38 (0.49-3.90)	2.67 (0.49-4.46)	2.23 (0.88-3.90)				
4OH/16OH	0.09 (0.03-0.24)	0.09 (0.02-0.27)	0.07 (0.02-0.23)	0.09 (0.01-0.34)				
4OH/MeOs	0.24 (0.11-0.73)	0.28 (0.14-0.68)	0.27 (0.16-0.59)	0.29 (0.12-0.55)				
OHs/MeOs	6.32 (2.96-10.22)	5.96 (2.02-18.54)	7.42 (2.27-15.43)	5.79 (2.70-18.54)				0.075
2OH-E_1/16αOH-E_1	4.37 (1.29-8.69)	2.13 (0.22-7.64)	1.86 (0.35-5.88)	2.61 (0.51-8.79)				**0.033**

The sum of hydroxy derivatives (OHs) includes 2OH, 4OH and 16OH. The sum of all catechol estrogens (CEs) includes OHs and MeOs. Data represent bivariate analysis corrected with Tukey and adjusted for age and BMI. *P*-values were obtained with the F test using log transformed data. Only trends ($P<0.1$) and significant associations ($P<0.05$; in bold) are displayed.

TABLE 4 Spearman correlation coefficients among endogenous hormone levels in endometriosis cases (EM), benign conditions (BC) and healthy (H).

		E_1	E_2	2OH-E_1	2OH-E_2	4OH-E_1	16αOH-E_1	16epi-E_3	16keto-E_2	E_3	2MeO-E_1	3MeO-E_1
E_1	EM		**0.81***	**0.71***	**0.60***	**0.57***	**0.67***	**0.72***	**0.72***	**0.69***	**0.71***	0.09
	BC		**0.77***	**0.67***	**0.49***	**0.46***	**0.65***	**0.69***	**0.50***	**0.58***	**0.70***	0.00
	H		**0.68***	**0.53***	**0.33***	**0.68***	**0.41***	**0.64***	0.31	**0.37***	**0.59***	0.21
E_2	EM			**0.73***	**0.62***	**0.56***	**0.66***	**0.71***	**0.74***	**0.72***	**0.70***	0.07
	BC			**0.71***	**0.58***	**0.47***	**0.75***	**0.74***	**0.69***	**0.67***	**0.74***	-0.02
	H			**0.42***	0.26	**0.60***	**0.55***	**0.53***	**0.50***	0.31	**0.57***	-0.09
2OH-E_1	EM				**0.82***	**0.57***	**0.52***	**0.57***	**0.56***	**0.53***	**0.86***	**0.20***
	BC				**0.89***	**0.53***	**0.49***	**0.57***	**0.38***	**0.45***	**0.88***	-0.01
	H				**0.87***	**0.57***	0.22	**0.41***	0.04	0.17	**0.80***	-0.08
2OH-E_2	EM					**0.47***	**0.48***	**0.62***	**0.46***	**0.56***	**0.63***	**0.15***
	BC					**0.45***	**0.36***	**0.52***	**0.34***	**0.43***	**0.72***	0.01
	H					**0.50***	-0.03	0.27	-0.03	0.15	**0.64***	0.17
4OH-E_1	EM						**0.31***	**0.36***	**0.41***	**0.35***	**0.58***	0.08
	BC						**0.22***	**0.25***	0.17	0.17	**0.58***	-0.06

(Continued)

10.3389/fendo.2022.1034614

TABLE 4 Continued

		E_1	E_2	2OH-E_1	2OH-E_2	4OH-E_1	16αOH-E_1	16epi-E_3	16keto-E_2	E_3	2MeO-E_1	3MeO-E_1
	H						0.20	0.28	0.16	0.03	**0.67***	0.05
16αOH-E_1	EM							**0.80***	**0.78***	**0.82***	**0.49***	0.01
	BC							**0.85***	**0.81***	**0.82***	**0.49***	-0.11
	H							**0.69***	**0.73***	**0.60***	0.28	**-0.38***
16epi-E_3	EM								**0.81***	**0.92***	**0.47***	0.09
	BC								**0.79***	**0.92***	**0.52***	-0.08
	H								**0.56***	**0.84***	**0.34***	-0.26
16keto-E_2	EM									**0.85***	**0.51***	**0.14***
	BC									**0.79***	**0.34***	-0.01
	H									**0.51***	0.14	-0.10
E_3	EM										**0.45***	0.09
	BC										**0.38***	-0.06
	H										0.09	-0.18
2MeO-E_1	EM											**0.14***
	BC											0.02
	H											-0.09
2OH-3MeO-E_1	EM											
	BC											
	H											

Correlation values were similar with all controls (when BC and H were analyzed together). The *P*-values of <0.05 are identified with a (*) and highlighted in bold. Correlations were tested using the Spearman Rho statistic test.

in the last three months (OR =0.32 (95%CI 0.12-0.89); *P* =0.028) (Supplementary Table 4).

Discussion

EM is a complex estrogen-sensitive condition characterized by a chronic inflammation process for which the potential role of estrogen metabolites remains to be fully investigated. We report that higher levels of 2OH-3MeO-E_1 were associated with an increased risk of EM, with an approximately two-fold higher median level observed in circulation of EM cases compared to controls. An enrichment of the 2OH metabolic pathway, with significantly higher levels of 2OH-E_1, 2MeO-E_1, sum of MeO and ratio of 2OH-E_1/16αOH-E_1, was also observed in ovarian EM cases compared to cases affected with lesions at other anatomical sites. A perturbation of estrogen metabolism (2OH-3MeO-E_1 and 16αOH-E_1) was further associated with pain symptoms.

Estrogens and their receptors play a key role in the pathophysiology of EM. Studies reported higher levels of systemic and locally synthesized estrogens in EM cases promoting the growth of lesions (38). This increase in estrogens was attributed to the secretion of estrogens by the ovaries as well as their autocrine and paracrine action, and an increased aromatase activity in EM lesions that supports local E_2 synthesis (15, 17, 39–41). Additional changes in estrogen synthesis, as well as their metabolic and receptor pathways, have also been reported in support of an enhanced local production and action in EM lesions, creating a hyperestrogenic environment that affects hormone receptor function (38, 42–49). These changes may be reflected in circulation of EM cases with higher levels of E_2 and/or its metabolites. In our study, 2OH-3MeO-E_1 was associated with an increased risk of EM. This observation is consistent with elevated COMT expression in EM lesions (50) leading to the formation of 2OH-3MeO-E_1 from its precursor 2OH-E_1 (23), potentially contributing to higher systemic levels of this metabolite. In support, we showed that endometrial tissue can contribute to systemic estrogen levels in the context of endometrial cancer that significantly declined after surgery (29). In addition to significant higher circulating levels of

10.3389/fendo.2022.1034614

TABLE 5 Significant associations between pain and steroid levels in EM cases (n=209).

Steroids	Comparator Medians in pg/mL (n)	Outcome Medians in pg/mL (n)	OR (95%CI)	*P*-value
Abdominal, pelvic, and back pain (n=207)				
16αOH-E_1	No 42.60 (115)	Yes 26.15 (92)	0.55 (0.31-0.97)	**0.038**
2OH-3MeO-E_1	No 2.50 (115)	Yes 5.99 (92)	1.86 (1.06-3.27)	**0.032**
Dysmenorrhea (frequency) (n=208)				
sum E_1/E_2	Infrequent 857.00 (67)	Frequent 1064.90 (141)	1.90 (1.03-3.51)	**0.041**
Dysmenorrhea (intensity) (n=74)				
Ratio 4OH/sum E_1/E_2	Mild 0.01 (45)	Moderate to severe 0.01 (29)	1.95 (1.05-3.61)	**0.035**
Dyspareunia (intensity) (n=124)				
16keto-E_2	Mild 26.90 (95)	Moderate to severe 46.60 (29)	2.40 (1.02-5.62)	**0.045**
Dysuria or dyschezia (frequency) (n=208)				
4OH-E_1	Infrequent 8.87 (189)	Frequent 2.50 (19)	0.32 (0.12-0.89)	**0.028**

The sum of hydroxy derivatives (OHs) includes 2OH, 4OH and 16OH. The sum of all catechol estrogens (CEs) includes OHs and MeOs. Odds ratios (OR) and their P-values were obtained using a logistic regression model adjusted for age and BMI. The number of cases with data on clinical outcomes are identified next to each outcome. There was no evidence of an association for the "score of dysmenorrhea" and the "dyspareunia (frequency)" outcomes. Significant P-values (<0.05) are shown in bold.

2OH-3MeO-E_1metabolites in EM cases compared to controls, higher levels of 2MeO-E_1 and the sum of MeOs were observed but they did not reached significance. The biological properties of 2OH-3MeO-E_1 have been poorly studied. We further noted that this metabolite was less correlated in circulation with the other estrogen derivatives and particularly in control subjects, suggesting a dysregulation in the presence of EM lesions associated with its precursors such as 2OH-E_1, with a higher correlation coefficient for this metabolite in EM cases at 0.20 (P<0.05). In fact, 2OH-3MeO-E_1 was higher in cases compared to controls, supporting a potential EM origin. Consistent with our observation, a previous study evaluated a subset of estrogen metabolites in preoperative urine samples of 62 EM cases and 52 controls and found increased levels of the 2OH-3MeO-E_1 precursor 2OH-E_1 (51). Our findings that the 2OH pathway is significantly more elevated in ovarian EM cases is also consistent with a study that used proton nuclear magnetic resonance (H-NMR) spectroscopy to investigate potential non-invasive metabolomic markers in 31 infertile women with stage II and III EM cases and 15 healthy or control women (52). They found that levels of the antiangiogenic 2MeO-E_1/E_2 metabolites were higher in EM cases compared to controls. The enrichment of the 2OH metabolic pathway in ovarian EM cases is supported by higher tissular levels of CYP1A1, involved in the conversion of E_1 and E_2 to 2OH-E_1 and 2OH-E_2, reported to be 4-fold higher in the ovarian EM group (50, 53). Inversely, the 16OH pathway was inversely associated with EM, consistent with downregulation of the involved enzyme pathways (CYP3A) by inflammation (54, 55).

An estrogenic environment may be associated with more severe pain symptoms (13). Hence, the association between estrogen levels and pain outcomes may not be related only to the effect of estrogens, as E_2 metabolites have been documented to present receptor-independent biological activities and may contribute to the maintenance of the inflammatory milieu (16). Previous reports revealed that elevated ERβ is associated with proliferation, inflammation and pain transmission (46, 56, 57), coherent with the positive correlation observed in this study between 16keto-E_2 and dyspareunia in EM cases. However, the negative association between 16αOH-E_1 and pelvic, abdominal and back pain suggests more complex relationships. A component of pain in EM was shown to be related to inflammatory damage of nerve fibers with neuroprotective roles for ERβ (58–61). Also, a dysregulation of both the ERα and ERβ expression pathways was observed in the ectopic endometrium in EM compared to normal endometrium in favor of a superior ERβ to ERα ratio (46, 62, 63). 16-hydroxylated derivatives are amongst E_2 metabolites known to bind the ERβ receptor (24), which may explain the observed association with pain. Other studies showed that the ERα was correlated with symptoms in deep infiltrating EM (64) and that it could favor hyperalgesia by altering calcium release (61). Since the 2OH metabolites are known to bind ERα (24), this could explain the association between pain outcomes and 2OH metabolites, such as 2OH-3MeO-E_1. Additional studies are required to

uncover the precise biological function of the 2OH-3MeO-E_1 metabolite.

This pilot study provides a comprehensive quantification of estrogens in the circulation of EM cases and controls based on a sensitive mass-spectrometry assay. It is comprised of a significant sample size, surgical and histologic confirmation of case and control status, adjustment for confounding factors and examination of pain symptoms. A limitation is the fact that the control group also included patients with gynecological conditions other than EM, which may influence the hormonal milieu (40, 65). Although cases and controls differed in confounding factors such as menstrual phase, these variables were included in the multivariate model for EM risk. Exploratory analyses in relation to pain symptoms were adjusted for age and BMI. Additional studies could provide levels of progesterone and its metabolites, shown to be dysregulated in EM and recognized to counteract the effect of E_2 (48, 66), whereas the endometriotic intratissue estrogen levels may not reflect the corresponding systemic levels. Due to the exploratory nature of the study, no correction for multiple testing was applied, but our initial findings warrant replication in other cohorts.

Conclusions

We conclude that the 2OH-3MeO-E_1 metabolite represents a potential adverse feature of EM and that the 2OH pathway is associated with the risk of ovarian endometriotic lesions. Data also suggest an association between the 2OH metabolic pathway and the risk of unfavorable pain outcomes.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

Ethics statement

The studies involving human participants were reviewed and approved by National Medical Ethics Committee in Slovenia (#0120-127/2016/6) and the Ethics committee of the CHUQc – Université Laval (#2012-993). The patients/participants provided their written informed consent to participate in this study.

Author contributions

Study concept and design: TR, CG. Patient recruitment and clinical data: MP, AV, JO, TR. Conducted experiments and mass spectrometry: PC, VT. Statistical analyses: DS. Drafting of the manuscript: J-PE, CG. Critical revision of the manuscript for important intellectual content: All authors. Obtaining funding: TR, CG.

Funding

This work was supported by research grants from the Canadian Institutes of Health Research (CIHR) (FRN-167269 to CG), the Canada Research Chair Program (CG) and grant from Slovenian Research Agency J3-1755 and EU H2020-MSCA-RISE grant TRENDO (101008193) to TR, J-PE received a studentship award from the Fonds de recherche du Québec – Santé. C.G. holds the Canada Research Chair in Pharmacogenomics (Tier I).

Acknowledgments

The authors thank all study participants who donated their samples, the personnel from the University Medical Centre Ljubljana, Slovenia, especially, Dr. Martina Ribič Pucelj, Marika Andonova, Vera Troha Poljančič and Tatjana Lončar and the researchers from the Institute of Biochemistry Medical Faculty University of Ljubljana Dr. Katja Vouk, Dr. Vida Kocbek, Dr. Tamara Knific and Dr. Teja Klančič, who contributed to sample and data collection.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.1034614/full#supplementary-material

References

1. Rowlands IJ, Abbott JA, Montgomery GW, Hockey R, Rogers P, Mishra. GD. Prevalence and incidence of endometriosis in Australian women: A data linkage cohort study. *Bjog* (2021) 128:657–65. doi: 10.1111/1471-0528.16447

2. Johnson NP, Hummelshoj L, Adamson GD, Keckstein J, Taylor HS, Abrao MS, et al. World endometriosis society consensus on the classification of endometriosis. *Hum Reprod* (2017) 32:315–24. doi: 10.1093/humrep/dew293

3. Marinho MCP, Magalhaes TF, Fernandes LFC, Augusto KL, Brilhante AVM, Bezerra L. Quality of life in women with endometriosis: An integrative review. *J Womens Health (Larchmt)* (2018) 27:399–408. doi: 10.1089/jwh.2017.6397

4. Tomassetti C, D'Hooghe T. Endometriosis and infertility: Insights into the causal link and management strategies. *Best Pract Res Clin Obstet Gynaecol* (2018) 51:25–33. doi: 10.1016/j.bpobgyn.2018.06.002

5. Koninckx PR, Ussia A, Adamyan L, Wattiez A, Donnez J. Deep endometriosis: definition, diagnosis, and treatment. *Fertil Steril* (2012) 98:564–71. doi: 10.1016/j.fertnstert.2012.07.1061

6. Nisolle M, Donnez J. Peritoneal endometriosis, ovarian endometriosis, and adenomyotic nodules of the rectovaginal septum are three different entities. *Fertil Steril* (1997) 68:585–96. doi: 10.1016/S0015-0282(97)00191-X

7. Zondervan KT, Becker CM, Koga K, Missmer SA, Taylor RN, Viganò. P. Endometriosis. *Nat Rev Dis Primers* (2018) 4:9. doi: 10.1038/s41572-018-0008-5

8. Lagana AS, Garzon S, Gotte M, Vigano P, Franchi M, Ghezzi F, et al. The pathogenesis of endometriosis: Molecular and cell biology insights. *Int J Mol Sci* (2019) 20:5615. doi: 10.3390/ijms20225615

9. Saunders PTK, Horne AW. Endometriosis: Etiology, pathobiology, and therapeutic prospects. *Cell* (2021) 184:2807–24. doi: 10.1016/j.cell.2021.04.041

10. Bulun SE, Cheng YH, Yin P, Imir G, Utsunomiya H, Attar E, et al. Progesterone resistance in endometriosis: link to failure to metabolize estradiol. *Mol Cell Endocrinol* (2006) 248:94–103. doi: 10.1016/j.mce.2005.11.041

11. Burney RO, Talbi S, Hamilton AE, Vo KC, Nyegaard M, Nezhat CR, et al. Gene expression analysis of endometrium reveals progesterone resistance and candidate susceptibility genes in women with endometriosis. *Endocrinology* (2007) 148:3814–26. doi: 10.1210/en.2006-1692

12. Mercorio A, Gianpaolino P, Romano A, Dällenbach P, Pluchino N. Is intracrinology of endometriosis relevant in clinical practice? a systematic review on estrogen metabolism. *Front Endocrinol* (2022) 13:950866. doi: 10.3389/fendo.2022.950866

13. Greaves E, Temp J, Esnal-Zufiurre A, Mechsner S, Horne AW, Saunders PT. Estradiol is a critical mediator of macrophage-nerve cross talk in peritoneal endometriosis. *Am J Pathol* (2015) 185:2286–97. doi: 10.1016/j.ajpath.2015.04.012

14. Bulun SE. Endometriosis. *N Engl J Med* (2009) 360:268–79. doi: 10.1056/NEJMra0804690

15. Chantalat E, Valera MC, Vaysse C, Noirrit E, Rusidze M, Weyl A, et al. Estrogen receptors and endometriosis. *Int J Mol Sci* (2020) 21. doi: 10.3390/ijms21082815

16. Garcia-Gomez E, Vazquez-Martinez ER, Reyes-Mayoral C, Cruz-Orozco OP, Camacho-Arroyo I, Cerbon M. Regulation of inflammation pathways and inflammasome by sex steroid hormones in endometriosis. *Front Endocrinol* (2019) 10:935. doi: 10.3389/fendo.2019.00935

17. Mori T, Ito F, Koshiba A, Kataoka H, Tanaka Y, Okimura H, et al. Aromatase as a target for treating endometriosis. *J Obstet Gynaecol Res* (2018) 44:1673–81. doi: 10.1111/jog.13743

18. Noble LS, Simpson ER, Johns A, Bulun SE. Aromatase expression in endometriosis. *J Clin Endocrinol Metab* (1996) 81:174–9. doi: 10.1210/jcem.81.1.8550748

19. Zeitoun K, Takayama K, Sasano H, Suzuki T, Moghrabi N, Andersson S, et al. Deficient 17beta-hydroxysteroid dehydrogenase type 2 expression in endometriosis: failure to metabolize 17beta-estradiol. *J Clin Endocrinol Metab* (1998) 83:4474–80. doi: 10.1210/jcem.83.12.5301

20. Gupta D, Hull ML, Fraser I, Miller L, Bossuyt PM, Johnson N, et al. Endometrial biomarkers for the non-invasive diagnosis of endometriosis. *Cochrane Database Syst Rev* (2016) 4:Cd012165. doi: 10.1002/14651858.CD012165

21. Rizner TL. Noninvasive biomarkers of endometriosis: myth or reality? *Expert Rev Mol Diagn* (2014) 14:365–85. doi: 10.1586/14737159.2014.899905

22. Cavalieri EL, Rogan EG. Depurinating estrogen-DNA adducts, generators of cancer initiation: Their minimization leads to cancer prevention. *Clin Transl Med* (2016) 5:12. doi: 10.1186/s40169-016-0088-3

23. Dawling S, Roodi N, Mernaugh RL, Wang X, Parl FF. Catechol-o-methyltransferase (COMT)-mediated metabolism of catechol estrogens: comparison of wild-type and variant COMT isoforms. *Cancer Res* (2001) 61:6716–22.

24. Zhu BT, Han GZ, Shim JY, Wen Y, Jiang XR. Quantitative structure-activity relationship of various endogenous estrogen metabolites for human estrogen receptor alpha and beta subtypes: Insights into the structural determinants favoring a differential subtype binding. *Endocrinology* (2006) 147:4132–50. doi: 10.1210/en.2006-0113

25. Fotsis T, Zhang Y, Pepper MS, Adlercreutz H, Montesano R, Nawroth PP, et al. The endogenous oestrogen metabolite 2-methoxyoestradiol inhibits angiogenesis and suppresses tumour growth. *Nature* (1994) 368:237–9. doi: 10.1038/368237a0

26. Samartzis EP, Imesch P, Twiehaus A, Dubey RK, Leeners B. The estrogen metabolites 2-methoxyestradiol and 2-hydroxyestradiol inhibit endometriotic cell proliferation in estrogen-receptor-independent manner. *Gynecol Endocrinol* (2016) 32:529–33. doi: 10.3109/09513590.2015.1137094

27. Schumacher G, Neuhaus P. The physiological estrogen metabolite 2-methoxyestradiol reduces tumor growth and induces apoptosis in human solid tumors. *J Cancer Res Clin Oncol* (2001) 127:405–10. doi: 10.1007/s004320000233

28. Merriam GR, MacLusky NJ, Picard MK, Naftolin F. Comparative properties of the catechol estrogens, I: Methylation by catechol-o-methyltransferase and binding to cytosol estrogen receptors. *Steroids* (1980) 36:1–11. doi: 10.1016/0039-128X(80)90062-8

29. Audet-Delage Y, Gregoire J, Caron P, Turcotte V, Plante M, Ayotte P, et al. Estradiol metabolites as biomarkers of endometrial cancer prognosis after surgery. *J Steroid Biochem Mol Biol* (2018) 178:45–54. doi: 10.1016/j.jsbmb.2017.10.021

30. Emond JP, Lacombe L, Caron P, Turcotte V, Simonyan D, Aprikian A, et al. Urinary oestrogen steroidome as an indicator of the risk of localised prostate cancer progression. *Br J Cancer* (2021) 125:78–84. doi: 10.1038/s41416-021-01376-z

31. Zondervan KT, Cardon LR, Kennedy SH. What makes a good case-control study? design issues for complex traits such as endometriosis. *Hum Reprod* (2002) 17:1415–23. doi: 10.1093/humrep/17.6.1415

32. Knific T, Vouk K, Vogler A, Osredkar J, Gstöttner M, Wenzl R, et al. Models including serum CA-125, BMI, cyst pathology, dysmenorrhea or dyspareunia for diagnosis of endometriosis. *Biomark Med* (2018) 12:737–47. doi: 10.2217/bmm-2017-0426

33. Kocbek V, Vouk K, Bersinger NA, Mueller MD, Rižner TL. Panels of cytokines and other secretory proteins as potential biomarkers of ovarian endometriosis. *J Mol Diagn* (2015) 17:325–34. doi: 10.1016/j.jmoldx.2015.01.006

34. Vouk K, Hevir N, Ribić-Pucelj M, Haarpaintner G, Scherb H, Osredkar J, et al. Discovery of phosphatidylcholines and sphingomyelins as biomarkers for ovarian endometriosis. *Hum Reprod* (2012) 27:2955–65. doi: 10.1093/humrep/des152

35. Haas D, Shebl O, Shamiyeh A, Oppelt P. The rASRM score and the enzian classification for endometriosis: Their strengths and weaknesses. *Acta Obstet Gynecol Scand* (2013) 92:3–7. doi: 10.1111/aogs.12026

36. Vincent K, Kennedy S, Stratton P. Pain scoring in endometriosis: entry criteria and outcome measures for clinical trials. report from the art and science of endometriosis meeting. *Fertil Steril* (2010) 93:62–7. doi: 10.1016/j.fertnstert.2008.09.056

37. Bourdel N, Alves J, Pickering G, Ramilo I, Roman H, Canis M. Systematic review of endometriosis pain assessment: How to choose a scale? *Hum Reprod Update* (2015) 21:136–52. doi: 10.1093/humupd/dmu046

38. Huhtinen K, Desai R, Stahle M, Salminen A, Handelsman DJ, Perheentupa A, et al. Endometrial and endometriotic concentrations of estrone and estradiol are determined by local metabolism rather than circulating levels. *J Clin Endocrinol Metab* (2012) 97:4228–35. doi: 10.1210/jc.2012-1154

39. Blakemore J, Naftolin F. Aromatase: Contributions to physiology and disease in women and men. *Physiol (Bethesda)* (2016) 31:258–69. doi: 10.1152/physiol.00054.2015

40. Rižner TL. The important roles of steroid sulfatase and sulfotransferases in gynecological diseases. *Front Pharmacol* (2016) 7:30. doi: 10.3389/fphar.2016.00030

41. Utsunomiya H, Cheng YH, Lin Z, Reierstad S, Yin P, Attar E, et al. Upstream stimulatory factor-2 regulates steroidogenic factor-1 expression in endometriosis. *Mol Endocrinol* (2008) 22:904–14. doi: 10.1210/me.2006-0302

42. Bulun SE, Monsavais D, Pavone ME, Dyson M, Xue Q, Attar E, et al. Role of estrogen receptor-beta in endometriosis. *Sem Rep Med* (2012) 30:39–45. doi: 10.1055/s-0031-1299596

43. Delvoux B, Groothuis P, D'Hooghe T, Kyama C, Dunselman G, Romano A. Increased production of 17beta-estradiol in endometriosis lesions is the result of impaired metabolism. *J Clin Endocrinol Metab* (2009) 94:876–83. doi: 10.1210/jc.2008-2218

44. Mori T, Ito F, Koshiba A, Kataoka H, Takaoka O, Okimura H, et al. Local estrogen formation and its regulation in endometriosis. *Reprod Med Biol* (2019) 18:305–11. doi: 10.1002/rmb2.12285

45. Tang ZR, Zhang R, Lian ZX, Deng SL, Yu K. Estrogen-receptor expression and function in female reproductive disease. *Cells* (2019) 8:1123. doi: 10.3390/cells8101123

46. Yilmaz BD, Bulun SE. Endometriosis and nuclear receptors. *Hum Reprod Update* (2019) 25:473–85. doi: 10.1093/humupd/dmz005

47. Smuc T, Pucelj MR, Sinkovec J, Husen B, Thole H, Rizner TL. Expression analysis of the genes involved in estradiol and progesterone action in human ovarian endometriosis. *Gynecol Endocrinol* (2007) 23:105–11. doi: 10.1080/09513590601152219

48. Smuc T, Rizner TL. Aberrant pre-receptor regulation of estrogen and progesterone action in endometrial cancer. *Mol Cell Endocrinol* (2009) 301:74–82. doi: 10.1016/j.mce.2008.09.019

49. Rizner TL. Estrogen metabolism and action in endometriosis. *Mol Cell Endocrinol* (2009) 307:8–18. doi: 10.1016/j.mce.2009.03.022

50. Hevir N, Ribič-Pucelj M, Rižner TL. Disturbed balance between phase I and II metabolizing enzymes in ovarian endometriosis: a source of excessive hydroxy-estrogens and ROS? *Mol Cell Endocrinol* (2013) 367:74–84. doi: 10.1016/j.mce.2012.12.019

51. Othman ER, Markeb AA, Khashbah MY, Abdelaal II, ElMelegy TT, Fetih AN, et al. Markers of local and systemic estrogen metabolism in endometriosis. *Reprod Sci* (2021) 28:1001–11. doi: 10.1007/s43032-020-00383-4

52. Ghazi N, Arjmand M, Akbari Z, Mellati AO, Saheb-Kashaf H, Zamani. Z. (1)H NMR- based metabolomics approaches as non- invasive tools for diagnosis of endometriosis. *Int J Reprod BioMed* (2016) 14:1–8. doi: 10.29252/ijrm.14.1.1

53. Piccinato CA, Neme RM, Torres N, Sanches LR, Cruz Derogis PB, Brudniewski HF, et al. Increased expression of CYP1A1 and CYP1B1 in ovarian/peritoneal endometriotic lesions. *Reproduction* (2016) 151:683–92. doi: 10.1530/REP-15-0581

54. Harvey RD, Morgan ET. Cancer, inflammation, and therapy: effects on cytochrome p450-mediated drug metabolism and implications for novel immunotherapeutic agents. *Clin Pharmacol Ther* (2014) 96:449–57. doi: 10.1038/clpt.2014.143

55. Rižner TL. Estrogen biosynthesis, phase I and phase II metabolism, and action in endometrial cancer. *Mol Cell Endocrinol* (2013) 381:124–39. doi: 10.1016/j.mce.2013.07.026

56. Han SJ, Jung SY, Wu SP, Hawkins SM, Park MJ, Kyo S, et al. Estrogen receptor β modulates apoptosis complexes and the inflammasome to drive the pathogenesis of endometriosis. *Cell* (2015) 163:960–74. doi: 10.1016/j.cell.2015.10.034

57. Monsivais D, Dyson MT, Yin P, Coon JS, Navarro A, Feng G, et al. ERβ- and prostaglandin E2-regulated pathways integrate cell proliferation *via* ras-like and estrogen-regulated growth inhibitor in endometriosis. *Mol Endocrinol* (2014) 28:1304–15. doi: 10.1210/me.2013-1421

58. Sommer C, Leinders M, Üçeyler N. Inflammation in the pathophysiology of neuropathic pain. *Pain* (2018) 159:595–602. doi: 10.1097/j.pain.0000000000001122

59. Howard FM. Endometriosis and mechanisms of pelvic pain. *J Minim Invasive Gynecol* (2009) 16:540–50. doi: 10.1016/j.jmig.2009.06.017

60. Kiguchi N, Kobayashi D, Saika F, Matsuzaki S, Kishioka S. Pharmacological regulation of neuropathic pain driven by inflammatory macrophages. *Int J Mol Sci* (2017) 18:2296. doi: 10.3390/ijms18112296

61. Chen Q, Zhang W, Sadana N, Chen X. Estrogen receptors in pain modulation: Cellular signaling. *Biol Sex Differ* (2021) 12:22. doi: 10.1186/s13293-021-00364-5

62. Xue Q, Lin Z, Cheng YH, Huang CC, Marsh E, Yin P, et al. Promoter methylation regulates estrogen receptor 2 in human endometrium and endometriosis. *Biol Reprod* (2007) 77:681–7. doi: 10.1095/biolreprod.107.061804

63. Vouk K, Smuc T, Guggenberger C, Ribič-Pucelj M, Sinkovec J, Husen B, et al. Novel estrogen-related genes and potential biomarkers of ovarian endometriosis identified by differential expression analysis. *J Steroid Biochem Mol Biol* (2011) 125:231–42. doi: 10.1016/j.jsbmb.2011.03.010

64. Pluchino N, Mamillapalli R, Wenger JM, Ramyead L, Drakopoulos P, Tille JC, et al. Estrogen receptor-α immunoreactivity predicts symptom severity and pain recurrence in deep endometriosis. *Fertil Steril* (2020) 113:1224–31.e1. doi: 10.1016/j.fertnstert.2020.01.036

65. Vander Borght M, Wyns C. Fertility and infertility: Definition and epidemiology. *Clin Biochem* (2018) 62:2–10. doi: 10.1016/j.clinbiochem.2018.03.012

66. Marquardt RM, Kim TH, Shin JH, Jeong JW. Progesterone and estrogen signaling in the endometrium: What goes wrong in endometriosis? *Int J Mol Sci* (2019) 20:3822. doi: 10.3390/ijms20153822

frontiers | Frontiers in Endocrinology

TYPE Review
PUBLISHED 07 March 2023
DOI 10.3389/fendo.2023.1110824

OPEN ACCESS

EDITED BY
Mainak Dutta,
Birla Institute of Technology and Science, United Arab Emirates

REVIEWED BY
Julio Cesar Rosa-e-Silva,
University of São Paulo, Brazil
Xiaona Lin,
Sir Run Run Shaw Hospital, China

*CORRESPONDENCE
Cansu Uzuner
cansu.uzuner@gmail.com

SPECIALTY SECTION
This article was submitted to Reproduction, a section of the journal Frontiers in Endocrinology

RECEIVED 29 November 2022
ACCEPTED 21 February 2023
PUBLISHED 07 March 2023

CITATION
Uzuner C, Mak J, El-Assaad F and Condous G (2023) The bidirectional relationship between endometriosis and microbiome.
Front. Endocrinol. 14:1110824.
doi: 10.3389/fendo.2023.1110824

The bidirectional relationship between endometriosis and microbiome

Cansu Uzuner[1]*, Jason Mak[1], Fatima El-Assaad[2] and George Condous[1]

[1]Endometriosis Ultrasound and Advanced Endosurgery Unit, Sydney Medical School Nepean, University of Sydney, Nepean Hospital, Sydney, NSW, Australia, [2]University of New South Wales Microbiome Research Centre, St. George and Sutherland Clinical Campuses, School of Clinical Medicine, Faculty of Medicine and Health, University of New South Wales, Sydney, NSW, Australia

Endometriosis has been described by many different theories of pathogenesis over the years. It is now also appreciated to be a state of chronic inflammation, and the role of immune dysfunction in its development has been proven. There is increasing evidence to support the role of the microbiome in the formation and progression of endometriosis *via* inflammatory pathways. The dysbiosis seen in endometriosis is thought to be both causative and a consequence of the pathogenesis. Gut, peritoneal fluid and female reproductive tract microbiota has been studied to understand if there are any microbiome signatures specific to endometriosis. New research on how to manipulate the microbiome for better detection and treatment of endometriosis is emerging.

KEYWORDS

endometriosis, microbiome, microbiota, dysbiosis, inflammation, immune response

Introduction

Endometriosis is an inflammatory disease characterized by the presence of endometrium-like tissue outside of the endometrium and myometrium (1, 2). It has a variety of subtypes and clinical presentations, ranging from being asymptomatic to causing chronic pain and infertility (2, 3). Endometriosis affects a significant proportion of the world's population – estimated to be present in up to 10% of females, and up to 50% of women with infertility (1). It also has significant healthcare costs, with the most recent study in 2022 appraising the direct cost of endometriosis to be US$1459 to US$20,239 per patient per year, and indirect cost to be between US$4,572 and US$14,079 (4). An Australian study in 2019 projected the total economic burden per year in the reproductive aged population (at 10% prevalence) to be Int$6.50 billion (5).

Recently, an increased understanding of the role of microbiota and immune dysbiosis in many diseases has also brought to light the possibility of their role in development of endometriosis. The knowledge on the human microbiome and its definition has been rapidly expanding due to new developments in sequencing methods and analytical techniques (6). With this growing field, terminology can lead to confusion. Microbiota is

defined as the community of microorganisms living in or on the human body site – it includes bacteria, archaea (single celled organisms without nuclei), fungi, eukaryotes and viruses. Microbiome is defined as the collective genomes of these microbes (7, 8). Microbiota has an associated theatre of activity – structural elements, metabolites, signal molecules, and the surrounding environmental conditions – that supports local immune, metabolic and epithelial function (9). When there is dysbiosis – defined as an imbalance or impairment of the microbiota – this support breaks down (3, 9, 10). Microbes and their metabolites can translocate to different body sites and can trigger an immune response and inflammation that is involved in multitude of diseases such as metabolic disorders, many neurological disorders, arthritis, psoriasis, inflammatory bowel disease and cancer (10–12).

Pathogenesis of endometriosis

There have been many postulations of pathogenesis of endometriosis including retrograde menstruation, immune dysfunction, inflammation, hormone dysregulation, coelomic metaplasia, lymphatic or hematological metastasis, stem cell dysfunction and genetic and epigenetic factors (13–16). A combination of these theories is likely in play together to lead to this chronic disease.

Immunopathology of endometriosis

Peritoneal endometriosis has been described as a chronic inflammatory state (17, 18). It is thought that the inflammatory state and the immune dysfunction in the peritoneum are both the cause and result of endometriosis. Immune dysregulation also leads to poor immunosurveillance as an appropriate response cannot be mounted to the refluxed endometrial cells and debris. This allows ectopic endometrial cells to persist in the peritoneal cavity (10, 19).

Firstly, the peritoneal inflammation plays a role in the development of the disease as well as the symptomology of the disease – pain and subfertility (17). The theory of retrograde menstruation is the most widely accepted pathogenesis of endometriosis (15). However, this theory alone does not explain disease prevalence, as women without endometriosis also display retrograde menstruation. Once endometrial cells are in the peritoneal cavity, they are required to adhere and proliferate to lead to endometriosis (10, 19). The inflammation and altered immunity create the right environment for cellular adhesion and endometriosis development and disease progression (10, 17, 18).

Oxidative stress is thought to be a key contributor to this inflammatory process (18, 20, 21). Reactive oxygen species (ROS) are intermediaries produced by the normal oxygen metabolism and have been implicated in his process (17, 18, 21). As a protective mechanism, cells cultivate antioxidant systems to counteract the ROS. When there is an imbalance between the ROS and antioxidants, with an abundance of ROS and deficiency in antioxidants, oxidative stress occurs (18, 20). In endometriosis, this imbalance is postulated to arise from erythrocytes in the peritoneal cavity and their toxic by-products of heme and iron. Free heme and iron lead to formation of ROS (17, 18, 21). This oxidative stress not only leads to cellular damage but also can alter cellular function *via* affecting protein activity and gene expression. The transcription factor called nuclear factor kappa-B (NF-κB) induces expression of multiple genes encoding proinflammatory cytokines, growth factors, adhesion molecules and enzymes, and has been implicated in peritoneal endometriosis by aiding in endometrial cell adhesion, proliferation and neovascularization (10, 17, 21–23).

Once the endometriotic implants adhere to the peritoneum, they require the help of cytokines and growth factors such as vascular endothelial growth factor (VEGF), tumour necrosis factor-α (TNF-α) and interleukin-8 (IL-8) for angiogenesis and lesion proliferation (10, 19, 24). These are primarily expressed by macrophages in the peritoneal cavity *via* increased activation of NF-κB pathways. There have been studies that support this theory by demonstrating increased number of macrophages, monocytes, and inflammatory mediators such as complements and cytokines in the peritoneal fluid of women with endometriosis (25, 26).

The dysregulation of both the innate and adaptive immunity are involved in the immunopathology of endometriosis. It has been shown that ectopic endometrial deposits, compared to matched eutopic endometrium of the same patients as well as to the endometrial tissues of the control group, have elevated expression of molecular genes associated with immune system process activation (27). Genes encoding for proinflammatory cytokines and receptors, cell adhesion molecules, complement proteins and angiogenesis are increased, and genes involved in regulation of inflammation, NK and cytotoxic T-cell activity, and cellular apoptosis are aberrantly expressed in ectopic endometrial tissues (27). These findings support the immune dysregulation in endometriosis.

Role of genetics and epigenetics in pathogenesis of endometriosis

Higher rates of endometriosis are seen in the relatives of women affected with endometriosis (28, 29). Twin studies have also supported the genetic influences on endometriosis by demonstrating a concordance ratio of 2:1 between monozygotic and dizygotic twins and a genetic risk ratio of 2.34 for endometriosis for a sibling, as well as 47-51% of endometriosis variation to be attributable to additive genetic effects (30, 31). As our understanding of genes and their role in disease has exponentially grown, there have been many studies conducted to determine the genes involved in specific condition such as endometriosis. Genome-wide association studies (GWAS) have discovered up to 27 significant loci associated with endometriosis but the challenge of understanding the functional consequences of these loci remain (32–35). There are genes associated with steroidogenesis and sex hormone receptorial activity, leading to dysregulation of estrogen and progesterone receptor ligand signaling, genes involved in inflammation and immune response, neoangiogenesis and DNA

reparation, and genes coding for metabolism regulation and cell growth postulated to be instrumental in establishment of endometriosis (33). Furthermore, genes have been shown to regulate their neighboring genes by epigenetic mechanisms. Epigenetics is defined as heritable changes in gene function that are not associated with DNA sequence changes but involves processes such as DNA methylation and histone modification (34, 36). Epigenetic mechanisms have been demonstrated to be involved in regulating immune processes such as cytokine expression, T-cell differentiation, antigen presentation and regulation of transcription factors, such as NF-κB, which has been implicated in immunopathogenesis of endometriosis (10, 36, 37). The genetic and epigenetic theory is also supported by the finding that there are gene expression and molecular differences found in the endometrium of women with endometriosis is compared to the endometrium of healthy controls, as well as between the eutopic and ectopic endometrium of women with endometriosis (36, 38–40).

Microbiome of endometriosis

Alterations in the microbiota of gut, peritoneal fluid and female reproductive tract in subjects with endometriosis compared to healthy controls have been demonstrated in increasing number of both human and animal studies (10, 41–51). It is not too clear whether these alterations are a result of endometriosis or whether they are the cause of endometriosis. However experimental animal models support a bidirectional relationship between endometriosis and microbiota changes (42, 50). In a particular study in mice who had surgically induced endometriosis, a reduction in the size of the endometriotic lesions was seen after treatment with antibiotics. After fecal microbiota transfer from endometriotic mice, regrowth of the lesions and associated inflammation was seen (42).

Gut microbiota

Gut microbiota has been the most studied body site in endometriosis microbiome research. The gut microbiome is dominated by bacteria, especially the members of the phyla *Bacteroidetes* and *Firmicutes*. In most healthy humans, percentage of each of these two dominant phyla can vary but the combined percentage tends to be approximately 95%. In a disease state, the gut microbiome can shift to represent large percentages of other bacterial phyla, such as *Proteobacteria*, *Verrucomicrobia*, *Actinobacteria*, or *Fusobacteria* (7, 52).

Interestingly, a systematic review conducted in 2019 on the microbial signatures of endometriosis found the following results (3). At the phylum level, *Actinobacteria*, *Firmicutes*, *Proteobacteria* and *Verrucomicrobia* were identified as being significantly higher in the gut of the endometriosis cohort, compared with controls. In contrast, *Lactobacillaceae* was found to be significantly decreased. They concluded that the levels of *Proteobacteria*, *Enterobacteriaceae*, *Streptococcus* and *E. coli* were elevated across various microbiome sites in endometriosis cohorts.

Since this systematic review, a number of studies exploring the role of gut microbiome in endometriosis have been published (41, 48, 53). Svensson et al's 2021 study did not find any significant differences in the abundance of bacterial classes between patient with or without isolated ovarian endometriosis, involvement of the gastrointestinal tract, gastrointestinal symptoms, or hormonal treatment (48). However, other studies have demonstrated that in the gut microbiota, more women in the endometriosis group had *Shigella and Escherichia* dominance (41).

Female reproductive tract microbiota

The female reproductive tract microbiota can be divided into the vagina, cervix, endometrium, fallopian tubes and ovaries. Majority of the studies in the microbiota of the female reproductive tract in endometriosis have focused on the cervix and the vagina. It has been found that the distribution of microbiota is similar in the cervical mucus of women with and without endometriosis regardless of the phases of the menstrual cycle, however the abundance of each changes (54). *Lactobacilli* is the predominant species in the vagina and the cervix. In addition to this, the abundance of *Corynebacterium*, *Enterobacteriaceae*, *Flavobacterium*, *Pseudomonas*, and *Streptococcus* are increased in the endometriosis group compared to the control group, with *Enterobacteriaceae* and *Streptococcus* being the more noteworthy candidates (54). A recent review has summarized that bacterial vaginosis-associated bacteria and Lactobacillus depletion in the cervicovaginal microbiome were associated with endometriosis and infertility in the majority of studies they analyzed (53). Another noteworthy finding is that reduced richness and diversity of cervical microbiome were detected in patients with more severe endometriosis symptoms including higher CA125 levels, more severe pain and infertility (55). This study suggested that cervical microbiome has an important role in regulating the pathogenesis of the associated complications of endometriosis and concluded that a more diverse cervical microbiome is associated with better clinical outcomes.

A small study of 14 participants with Stage III-IV endometriosis and 14 healthy controls revealed that the vaginal, cervical and gut microbiota composition among the endometriosis group were similar; but it showed that some potentially pathogenic species were increased in the cervical and stool microbiome in women with endometriosis compared to the control group (41). In the cervical microbiota, *Gardnerella*, *Streptococcus*, *Escherichia*, *Shigella*, and *Ureoplasma* were increased. Interestingly they observed a total absence of a particular genus, *Atopobium* in vaginal and cervical microbiota. *Atopobium* has been recently implicated as a gynecological pathogen potentially associated with endometrial cancer, and lower incidence of it was seen in women with benign gynecological pathologies (56). It is unclear that this association is causal or coincidental. It could be proposed that the absence of *Atopobium* can be related to occurrence benign gynecological pathologies, in which endometriosis a part of.

Less studies have been performed exploring the relationship with viruses, particularly human papilloma virus (HPV)

with endometriosis (3). Majority of these studies have found HPV detection to be higher and therefore associated with endometriosis (57–59).

Peritoneal microbiota

Microbiota diversity of the peritoneal fluid was shown to be similar in women with an without endometriosis (51). However, the abundance of these microbiota differed (47, 51). *Acidovorax*, *Devosia*, *Methylobacterium*, *Phascolarctobacterium*, and *Streptococcus* were more abundant in the peritoneal fluid of endometriosis patients than the controls, while *Brevundimonas* and *Stenotrophomonas* were less abundant (51). Another study reported the abundance of *Acinetobacter*, *Pseudomonas*, *Streptococcus*, and *Enhydrobacter* to be significantly increased while the abundance of *Propionibacterium*, *Actinomyces*, and *Rothia* to be significantly decreased in the endometriosis group compared with those in the control group (47). A third study concluded *Sphingobium*, *Pseudomonadaceae*, *Sphingomonas*, *Acinetobacter*, *Erysipelothrix*, *Clostridiales*, *Micrococcaceae*, *Vagococcus*, *Dysgonomonas*, *Pseudomonas viridiflava*, *Shewanella*, *Tissierellaceae* were enriched in the peritoneal fluid of endometriosis patients compared to the control group (49). When the microbiota of deep endometriosis lesions were examined by Hernandes et al. in 2020, *Alishewanella, Enterococcus* and *Pseudomonas* were demonstrated to be more abundant (43). *Acinetobacter* and *Pseudomonas* prove to be persistently present in several of these studies (43, 47, 49). These findings not only support that microbiome composition is altered in the peritoneal environment in women with endometriosis but also point to the possibility of finding a peritoneal fluid microbial signature specific to endometriosis.

Microbiome's role in pathogenesis of endometriosis

The effects of dysbiosis could be contributing to the pathogenesis of endometriosis *via* inflammation and immune modulation and there is new evidence suggesting a role of the microbiome in development of endometriosis (10, 41, 43, 49, 53, 60–62).

Bacterial contamination theory

A "bacterial contamination" theory for endometriosis progression of endometriosis has been postulated. Khan et al. (63) examined *Escherichia coli* (*E. coli*) concentrations in the menstrual blood of women with endometriosis in comparison to control groups. They found that there was increased number of *E. coli* colony formation in women with endometriosis, especially those with peritoneal endometriosis in addition to ovarian endometriomas. They suggested that *E. coli* contamination of the menstrual blood would be a constant source of bacterial endotoxin or a lipopolysaccharide in the peritoneal cavity. The primary inflammation caused by the lipopolysaccharides would lead to the secretion of secondary inflammatory mediators such as NF-κB in the peritoneal cavity *via* promoting Toll-like receptor 4 (TLR4) which are present on macrophages and other immune cells (60). This would start the cascade of endometriosis development as explained with the immunopathology of endometriosis.

The bacterial contamination theory has since been discussed (10, 60) and is supported by other research demonstrating increased levels of *Proteobacteria*, which is a phylum of bacteria that produces lipopolysaccharides, in endometriosis cohorts (41, 45, 50, 54, 63, 64). Furthermore, a large cohort study of over 140,000 women demonstrated that there is a three-fold increased risk of developing endometriosis in women with a history of pelvic inflammatory disease (PID) compared to the control cohort (62). A similar result has been exhibited by another study in which double the incidence of endometriosis was seen in women with a lower genital tract infection (61).

Estrobolomes in development of endometriosis

Another possible mechanism of how microbiome can influence endometriosis development and progression can be explained by the altered estrogen metabolism that is seen with dysbiosis (65). It is known that endometriosis is an estrogen driven condition (66, 67). Certain dysbiotic gut microbiota are known as 'estrobolomes' whose products can metabolize estrogen, increasing the circulating levels of estrogen in the body (3, 53, 68). These estrobolomes are known to secrete β-glucuronidase and β-glucosidases which deconjugate estrogen, which in turn increases the re-absorption of free estrogens in the gut (68, 69). It is theorized that this can lead to a hyperestrogenic state and contribute to progressing of endometriosis (53, 65). Multiple genera in the gut microbiome encode for β-glucuronidase, including *Bacteroides*, *Bifidobacterium*, *Escherichia* and *Lactobacillus* (3, 69). Interestingly, some studies have found higher levels of *Bifidobacterium* and *Escherichia* in endometriosis groups over control groups (50, 63). It is also known that there is dysbiosis in the Bacteroidetes/Firmicutes ratios, which are the two dominant phyla in the gut, in women with endometriosis (10, 50).

Genetic and epigenetic factors

Bacterial induced epigenetic deregulation of host cells has been well studied (70–72). Dysbiosis and female genital tract infections may induce genetic and epigenetic incidents, leading to increased oxidative stress and changes in the immune responses, which in turn could play a role in the formation of endometriosis (72). A study on *Mycoplasma genitalium* revealed that the gene expression of peritoneal fluid cells of women with endometriosis who are colonized with *Mycoplasma genitalium* were significantly downregulated which in turn would inhibit immune cells recruited to the site (73). Viruses are also known to be mutagenic

and this has been proven in many malignancies caused by carcinogenic viruses such as HPV, human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV) and Ebstein-Barr virus (EBV) (74–76). There have only been a few studies assessing the relationship between viruses, particularly HPV, and endometriosis, and future studies are needed in this area (57–59). Furthermore, it has been reported that inflammation itself can cause aberrant DNA methylation patterns leading to hypermethylation which in turn effects the production of certain transcription factors and receptors (such as HOXA10 and progesterone receptor B) that is seen in endometriosis (39, 77, 78).

Clinical implications

Performance of different body sites

Only a few studies have compared microbiota of different body sites to assess which site is the best performer in predicting endometriosis. One of the studies examined the gut, peritoneal fluid and cervical mucus. It demonstrated that the gut and peritoneal fluid have higher richness and diversity in microbiota compared to cervical mucus and concluded that the gut microbiota is the top performing predictor of endometriosis out of the three (44). Another study compared samples from lower third of vagina, posterior vaginal fornix, cervical mucus, endometrium and peritoneal fluid (49). Each site had a different microbiota distribution. Significant difference of the community diversity began showing in the cervical mucus of endometriosis patients and gradually increased upward the reproductive tract, suggesting the upper female reproductive tract is better indicator for the risk of endometriosis if used as a screening tool (49).

Microbiome in predicting endometriosis stage

There is limited evidence available in the differences between microbiotas of different endometriosis stages and all the available evidence is based on studies with small numbers (44, 79). The studies that investigated this question used revised-ASRM (rASRM) stages (80). A study of 21 participants with endometriosis found no difference between gut microbiota of early (Stage I and II) and advanced stages (Stage III and IV) of endometriosis (44). A larger study with 59 participants (34 with endometriosis, 24 control) assessed gut and vaginal samples collected at two different time periods within the menstrual cycle (79). They reported that 9/35 (25.7%) had Stage I, 12/35 (34.2%) had Stage II, 3/35 (11.4%) had Stage III, and 10/35 (28.5%) had Stage IV endometriosis. They grouped Stage I and II together and Stage II and IV together for comparative analysis. The analysis did not show any significant differences in the microbiota between either two groups of stages of endometriosis or control groups. However, they concluded that vaginal microbiome was predictive of the stage of the disease based on an operational taxonomical unit (OTU) from the genus *Anaerococcus*. Both of these studies used 16S rRNA gene sequencing for analysis. Other studies that performed a sub-analysis on rASRM stages any briefly mentioned that there was no significant difference between the different stages (45, 73).

Evidence so far in altering microbiome to treat endometriosis

Evidence on the role of microbiome and dysbiosis in the development of endometriosis is rapidly mounting. Therapeutic manipulation of the microbiome in treatment and prevention of endometriosis is a very real possibility. Animal studies have already established this possibility (42). Figure 1 summaries the current interventions that have a potential in treatment of endometriosis. Chadchan et al's study on mice (42) with surgically induced endometriosis found higher levels of *Bacteroidetes* and lower levels of *Firmicutes* in the gut microbiota composition compared to the control mice. Metronidazole was used on the mice with endometriosis to target *Bacteroidetes* and this demonstrated reduction in the endometriosis lesion size and cell proliferation. Furthermore, they proved a reduced inflammatory response in the treated mice by measuring lower levels of inflammatory cytokines and mediators in the peritoneal fluid and endometriotic lesions.

Probiotics is another promising treatment option that has proven itself with other benign gynecological conditions such as candida vulvovaginitis and bacterial vaginosis (81). There have been two mice studies that show some potential in its benefit in the treatment of endometriosis (82, 83). Both studies investigated the role of oral *Lactobaccilus gassseri* and displayed both the suppression of development endometriosis as well as and suppression of growth of already present endometriosis. The mechanism postulated was immunostimulatory activity *via* activation of NK cells and reduction in development of ectopic endometriotic lesions.

Although there are limited animal studies on antibiotics and probiotics on treatment of endometriosis (42, 82, 83), there have not been any studies to date to investigate the specific role of probiotics or prebiotics in helping resolve the dysbiosis associated with endometriosis in hopes to assist in its treatment. Urgent future research is needed to study the role of probiotic and antibiotic therapy further in human subjects. Conversely it is important to remember that excessive use of antibiotics can have the adverse effect of altering healthy commensal microbiota and contributing to antimicrobial resistance.

Lastly, the therapeutic anti-inflammatory effect of polyunsaturated fatty acids (PUFAs) such as omega-3 and omega-6 on multitude of diseases is well established (84, 85) and increasing evidence on beneficial effects of PUFAs on endometriosis is becoming available. Women with endogenously higher serum PUFAs levels have been shown to be 82% less likely to have endometriosis compared to women with low PUFAs levels (86) and a high dietary consumption of omega-3 demonstrates a lower incidence of laparoscopically confirmed endometriosis compared to individuals with a low dietary intake of omega-3 (87). A study on

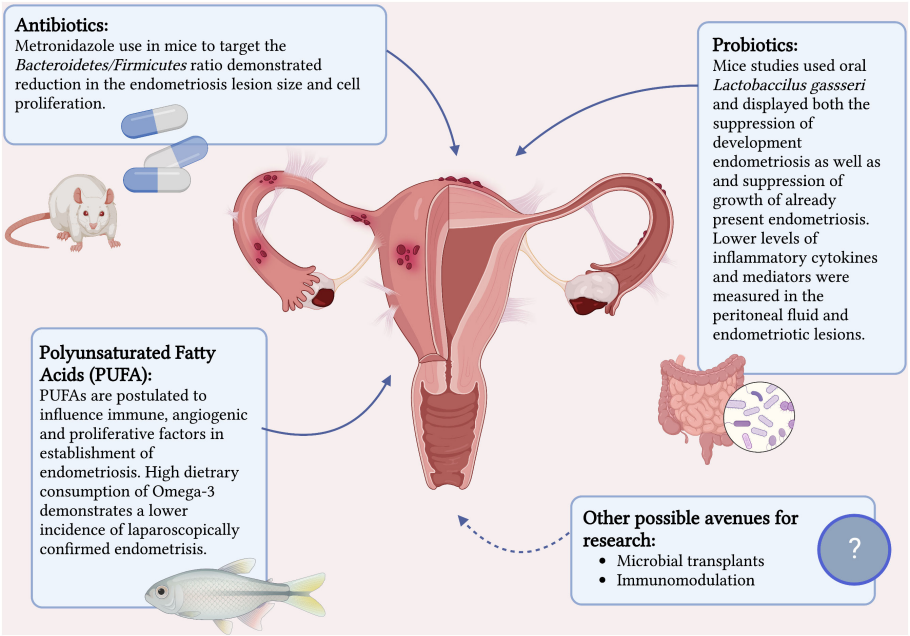


FIGURE 1
Current interventions that have a potential in treatment of endometriosis. Created with BioRender.com.

mice has confirmed this by exhibiting a 99-fold lower level of inflammatory cytokine IL-6 in mice with endogenously high levels of omega-3 PUFAs as well as a reduction of proliferation in endometriosis-like lesions when donor tissue was transferred to a PUFA rich host environment (88). They demonstrated that omega-3 PUFA levels influence immune, angiogenic, and proliferative factors implicated in the early establishment of endometriosis.

Future directions

The majority of research in microbiota and endometriosis has been focused on bacteria. The most common analytic method used in these studies has been the 16sRNA sequencing. 16sRNA sequencing uses a single gene in bacteria and is used to differentiate bacterial taxa and their relative abundance. However, it does not distinguish between the different strains of each genus of bacteria. Different strains of the genus of bacteria are genomically distinct and one strain can cause significant illness whilst another strain could be considered a probiotic (7). Shotgun metagenomics and metabolomics are newer analytical methods that have become more accessible in the recent years. They examine a wider range of microbiota and microbiome, although both come with analytical limitations due to the ongoing developments in the field. Shotgun metagenomics fragments all the DNA from a sample and sequences these fragments. It can infer a complete list of microbial strains including viruses and fungi. Metabolomics is the study of the nonprotein small molecules including products of metabolism (7, 89). Further research with these methods may yield different results, especially on different types of microbiota, such as fungi or viruses and new associations with endometriosis may be discovered.

Discussion

Microbiome testing has potential be used a non-invasive test to detect endometriosis. There is a significant delay in diagnosis of endometriosis, with the average time between onset of symptoms to diagnosis being 8 years and the range reported as 4-12 years in different studies (90–92). Previously, laparoscopic diagnosis was considered the gold standard but has the disadvantage of being invasive. As imaging techniques have improved over the years, transvaginal ultrasound and MRI for diagnosis of deep endometriosis have proven to have great accuracy and now accepted as first line diagnostic tools (1, 93). However, they are limited by the availability of skilled sonographers, sonologists and radiologists. In addition, the techniques to improve diagnosis of superficial endometriosis on ultrasound are still relatively new (94, 95). If available in the future, a simple microbiota test could complement the imaging modalities well in non-invasive diagnosis of endometriosis. Currently there is no evidence for microbiome signatures of different stages of endometriosis or predicting infertility. Further research is needed to be able to make this a possibility. Discovering the microbial signature of endometriosis would also create avenues for future research into developing methods to alter the microbiome *via* probiotics, microbial transplants, or immunomodulation to alter the disease.

Author contributions

CU wrote the manuscript. JM, FE-A and GC outlined the content of the review, reviewed, edited and approved the final

draft. GC supervised the project. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Becker CM, Bokor A, Heikinheimo O, Horne A, Jansen F, Kiesel L, et al. ESHRE guideline: endometriosis. *Hum Reprod Open* (2022) 2022(2):hoac009. doi: 10.1093/hropen/hoac009

2. International working group of Aagl EE, Wes TC, Johnson NP, Petrozza J, Abrao MS, Einarsson JI, et al. An international terminology for endometriosis, 2021. *J Minim Invasive Gynecol* (2021) 28(11):1849–59. doi: 10.1093/hropen/hoab029

3. Leonardi M, Hicks C, El-Assaad F, El-Omar E, Condous G. Endometriosis and the microbiome: A systematic review. *BJOG* (2020) 127(2):239–49. doi: 10.1111/1471-0528.15916

4. Darba J, Marsa A. Economic implications of endometriosis: A review. *Pharmacoeconomics* (2022) 40(12):1143–58. doi: 10.1007/s40273-022-01211-0

5. Armour M, Lawson K, Wood A, Smith CA, Abbott J. The cost of illness and economic burden of endometriosis and chronic pelvic pain in Australia: A national online survey. *PloS One* (2019) 14(10):e0223316. doi: 10.1371/journal.pone.0223316

6. Ursell LK, Metcalf JL, Parfrey LW, Knight R. Defining the human microbiome. *Nutr Rev* (2012) 70 Suppl 1:S38–44. doi: 10.1111/j.1753-4887.2012.00493.x

7. Allaband C, McDonald D, Vazquez-Baeza Y, Minich JJ, Tripathi A, Brenner DA, et al. Microbiome 101: Studying, analyzing, and interpreting gut microbiome data for clinicians. *Clin Gastroenterol Hepatol* (2019) 17(2):218–30. doi: 10.1016/j.cgh.2018.09.017

8. Turnbaugh PJ, Ley RE, Hamady M, Fraser-Liggett CM, Knight R, Gordon JI. The human microbiome project. *Nature* (2007) 449(7164):804–10. doi: 10.1038/nature06244

9. Berg G, Rybakova D, Fischer D, Cernava T, Verges MC, Charles T, et al. Microbiome definition re-visited: Old concepts and new challenges. *Microbiome* (2020) 8(1):103. doi: 10.1186/s40168-020-00875-0

10. Jiang I, Yong PJ, Allaire C, Bedaiwy MA. Intricate connections between the microbiota and endometriosis. *Int J Mol Sci* (2021) 22(11):5644. doi: 10.3390/ijms22115644

11. Cho I, Blaser MJ. The human microbiome: at the interface of health and disease. *Nat Rev Genet* (2012) 13(4):260–70. doi: 10.1038/nrg3182

12. Rinninella E, Raoul P, Cintoni M, Franceschi F, Miggiano GAD, Gasbarrini A, et al. What is the healthy gut microbiota composition? A changing ecosystem across age, environment, diet, and diseases. *Microorganisms* (2019) 7(1):14. doi: 10.3390/microorganisms7010014

13. Vercellini P, Vigano P, Somigliana E, Fedele L. Endometriosis: Pathogenesis and treatment. *Nat Rev Endocrinol* (2014) 10(5):261–75. doi: 10.1038/nrendo.2013.255

14. Riccio L, Santulli P, Marcellin L, Abrao MS, Batteux F, Chapron C. Immunology of endometriosis. *Best Pract Res Clin Obstet Gynaecol* (2018) 50:39–49. doi: 10.1016/j.bpobgyn.2018.01.010

15. Sampson JA. Metastatic or embolic endometriosis, due to the menstrual dissemination of endometrial tissue into the venous circulation. *Am J Pathol* (1927) 3(2):93–110 43.

16. Burney RO, Giudice LC. Pathogenesis and pathophysiology of endometriosis. *Fertil Steril* (2012) 98(3):511–9. doi: 10.1016/j.fertnstert.2012.06.029

17. Lousse JC, Van Langendonckt A, Defrere S, Ramos RG, Colette S, Donnez J. Peritoneal endometriosis is an inflammatory disease. *Front Biosci (Elite Ed)* (2012) 4 (1):23–40. doi: 10.2741/e358

18. Van Langendonckt A, Casanas-Roux F, Donnez J. Oxidative stress and peritoneal endometriosis. *Fertil Steril* (2002) 77(5):861–70. doi: 10.1016/S0015-0282(02)02959-X

19. Symons LK, Miller JE, Kay VR, Marks RM, Liblik K, Koti M, et al. The immunopathophysiology of endometriosis. *Trends Mol Med* (2018) 24(9):748–62. doi: 10.1016/j.molmed.2018.07.004

20. Clower L, Fleshman T, Geldenhuys WJ, Santanam N. Targeting oxidative stress involved in endometriosis and its pain. *Biomolecules* (2022) 12(8):1055. doi: 10.3390/biom12081055

21. Donnez J, Binda MM, Donnez O, Dolmans MM. Oxidative stress in the pelvic cavity and its role in the pathogenesis of endometriosis. *Fertil Steril* (2016) 106 (5):1011–7. doi: 10.1016/j.fertnstert.2016.07.1075

22. Gonzalez-Ramos R, Donnez J, Defrere S, Leclercq I, Squifflet J, Lousse JC, et al. Nuclear factor-kappa b is constitutively activated in peritoneal endometriosis. *Mol Hum Reprod* (2007) 13(7):503–9. doi: 10.1093/molehr/gam033

23. Gonzalez-Ramos R, Van Langendonckt A, Defrere S, Lousse JC, Mettlen M, Guillet A, et al. Agents blocking the nuclear factor-kappaB pathway are effective inhibitors of endometriosis in an *in vivo* experimental model. *Gynecol Obstet Invest* (2008) 65(3):174–86. doi: 10.1159/000111148

24. Iwabe T, Harada T, Tsudo T, Nagano Y, Yoshida S, Tanikawa M, et al. Tumor necrosis factor-alpha promotes proliferation of endometriotic stromal cells by inducing interleukin-8 gene and protein expression. *J Clin Endocrinol Metab* (2000) 85(2):824–9. doi: 10.1210/jcem.85.2.6335

25. Deaton JL, Honore GM, Huffman CS, Bauguess P. Early transvaginal ultrasound following an accurately dated pregnancy: The importance of finding a yolk sac or fetal heart motion. *Hum Reprod* (1997) 12(12):2820–3. doi: 10.1093/humrep/12.12.2820

26. Gazvani R, Templeton A. Peritoneal environment, cytokines and angiogenesis in the pathophysiology of endometriosis. *Reproduction* (2002) 123(2):217–26. doi: 10.1530/rep.0.1230217

27. Ahn SH, Khalaj K, Young SL, Lessey BA, Koti M, Tayade C. Immune-inflammation gene signatures in endometriosis patients. *Fertil Steril* (2016) 106 (6):1420–31.e7. doi: 10.1016/j.fertnstert.2016.07.005

28. Simpson JL, Elias S, Malinak LR, Buttram VCJr. Heritable aspects of endometriosis. *I. Genet Stud Am J Obstet Gynecol* (1980) 137(3):327–31. doi: 10.1016/0002-9378(80)90917-5

29. Moen MH, Magnus P. The familial risk of endometriosis. *Acta Obstet Gynecol Scand* (1993) 72(7):560–4. doi: 10.3109/00016349309058164

30. Treloar SA, O'Connor DT, O'Connor VM, Martin NG. Genetic influences on endometriosis in an Australian twin sample. *Fertil Steril* (1999) 71(4):701–10. doi: 10.1016/S0015-0282(98)00540-8

31. Saha R, Pettersson HJ, Svedberg P, Olovsson M, Bergqvist A, Marions L, et al. Heritability of endometriosis. *Fertil Steril* (2015) 104(4):947–52. doi: 10.1016/j.fertnstert.2015.06.035

32. Rahmioglu N, Nyholt DR, Morris AP, Missmer SA, Montgomery GW, Zondervan KT. Genetic variants underlying risk of endometriosis: Insights from meta-analysis of eight genome-wide association and replication datasets. *Hum Reprod Update* (2014) 20(5):702–16. doi: 10.1093/humupd/dmu015

33. Deiana D, Gessa S, Anardu M, Daniilidis A, Nappi L, D'Alterio MN, et al. Genetics of endometriosis: A comprehensive review. *Gynecol Endocrinol* (2019) 35 (7):553–8. doi: 10.1080/09513590.2019.1588244

34. Borghese B, Zondervan KT, Abrao MS, Chapron C, Vaiman D. Recent insights on the genetics and epigenetics of endometriosis. *Clin Genet* (2017) 91(2):254–64. doi: 10.1111/cge.12897

35. Giacomini E, Minetto S, Li Piani L, Pagliardini L, Somigliana E, Vigano P. Genetics and inflammation in endometriosis: Improving knowledge for development of new pharmacological strategies. *Int J Mol Sci* (2021) 22(16):9033. doi: 10.3390/ijms22169033

36. Guo SW. Epigenetics of endometriosis. *Mol Hum Reprod* (2009) 15(10):587–607. doi: 10.1093/molehr/gap064

37. Fitzpatrick DR, Wilson CB. Methylation and demethylation in the regulation of genes, cells, and responses in the immune system. *Clin Immunol* (2003) 109(1):37–45. doi: 10.1016/S1521-6616(03)00205-5

38. Koninckx PR, Ussia A, Adamyan L, Wattiez A, Gomel V, Martin DC. Pathogenesis of endometriosis: The genetic/epigenetic theory. *Fertil Steril* (2019) 111 (2):327–40. doi: 10.1016/j.fertnstert.2018.10.013

39. Wu Y, Halverson G, Basir Z, Strawn E, Yan P, Guo SW. Aberrant methylation at HOXA10 may be responsible for its aberrant expression in the endometrium of patients with endometriosis. *Am J Obstet Gynecol* (2005) 193(2):371–80. doi: 10.1016/j.ajog.2005.01.034

40. Meola J, Rosa e Silva JC, Dentillo DB, da Silva WAJr., Veiga-Castelli LC, Bernardes LA, et al. Differentially expressed genes in eutopic and ectopic endometrium of women with endometriosis. *Fertil Steril* (2010) 93(6):1750–73. doi: 10.1016/j.fertnstert.2008.12.058

41. Ata B, Yildiz S, Turkgeldi E, Brocal VP, Dinleyici EC, Moya A, et al. The endobiota study: Comparison of vaginal, cervical and gut microbiota between women with stage 3/4 endometriosis and healthy controls. *Sci Rep* (2019) 9(1):2204. doi: 10.1038/s41598-019-39700-6

42. Chadchan SB, Cheng M, Parnell LA, Yin Y, Schriefer A, Mysorekar IU, et al. Antibiotic therapy with metronidazole reduces endometriosis disease progression in mice: A potential role for gut microbiota. *Hum Reprod* (2019) 34(6):1106–16. doi: 10.1093/humrep/dez041

43. Hernandes C, Silveira P, Rodrigues Sereia AF, Christoff AP, Mendes H, Valter de Oliveira LF, et al. Microbiome profile of deep endometriosis patients: Comparison of vaginal fluid, endometrium and lesion. *Diagnostics (Basel)* (2020) 10(3):163. doi: 10.3390/diagnostics10030163

44. Huang L, Liu B, Liu Z, Feng W, Liu M, Wang Y, et al. Gut microbiota exceeds cervical microbiota for early diagnosis of endometriosis. *Front Cell Infect Microbiol* (2021) 11:788836. doi: 10.3389/fcimb.2021.788836

45. Khan KN, Fujishita A, Kitajima M, Hiraki K, Nakashima M, Masuzaki H. Intra-uterine microbial colonization and occurrence of endometritis in women with endometriosisdagger. *Hum Reprod* (2014) 29(11):2446–56. doi: 10.1093/humrep/deu222

46. Le N, Cregger M, Fazleabas A, Braundmeier-Fleming A. Effects of endometriosis on immunity and mucosal microbial community dynamics in female olive baboons. *Sci Rep* (2022) 12(1):1590. doi: 10.1038/s41598-022-05499-y

47. Lee SR, Lee JC, Kim SH, Oh YS, Chae HD, Seo H, et al. Altered composition of microbiota in women with ovarian endometrioma: Microbiome analyses of extracellular vesicles in the peritoneal fluid. *Int J Mol Sci* (2021) 22(9):4608. doi: 10.3390/ijms22094608

48. Svensson A, Brunkwall L, Roth B, Orho-Melander M, Ohlsson B. Associations between endometriosis and gut microbiota. *Reprod Sci* (2021) 28(8):2367–77. doi: 10.1007/s43032-021-00506-5

49. Wei W, Zhang X, Tang H, Zeng L, Wu R. Microbiota composition and distribution along the female reproductive tract of women with endometriosis. *Ann Clin Microbiol Antimicrob* (2020) 19(1):15. doi: 10.1186/s12941-020-00356-0

50. Yuan M, Li D, Zhang Z, Sun H, An M, Wang G. Endometriosis induces gut microbiota alterations in mice. *Hum Reprod* (2018) 33(4):607–16. doi: 10.1093/humrep/dex372

51. Yuan W, Wu Y, Chai X, Wu X. The colonized microbiota composition in the peritoneal fluid in women with endometriosis. *Arch Gynecol Obstet* (2022) 305(6):1573–80. doi: 10.1007/s00404-021-06338-7

52. Human Microbiome Project C. Structure, function and diversity of the healthy human microbiome. *Nature* (2012) 486(7402):207–14. doi: 10.1038/nature11234

53. Salliss ME, Farland LV, Mahnert ND, Herbst-Kralovetz MM. The role of gut and genital microbiota and the estrobolome in endometriosis, infertility and chronic pelvic pain. *Hum Reprod Update* (2021) 28(1):92–131. doi: 10.1093/humupd/dmab035

54. Akiyama K, Nishioka K, Khan KN, Tanaka Y, Mori T, Nakaya T, et al. Molecular detection of microbial colonization in cervical mucus of women with and without endometriosis. *Am J Reprod Immunol* (2019) 82(2):e13147. doi: 10.1111/aji.13147

55. Chang CY, Chiang AJ, Lai MT, Yan MJ, Tseng CC, Lo LC, et al. A more diverse cervical microbiome associates with better clinical outcomes in patients with endometriosis: A pilot study. *Biomedicines* (2022) 10(1):174. doi: 10.3390/biomedicines10010174

56. Walther-Antonio MR, Chen J, Multinu F, Hokenstad A, Distad TJ, Cheek EH, et al. Potential contribution of the uterine microbiome in the development of endometrial cancer. *Genome Med* (2016) 8(1):122. doi: 10.1186/s13073-016-0368-y

57. Heidarpour M, Derakhshan M, Derakhshan-Horeh M, Kheirollahi M, Dashti S. Prevalence of high-risk human papillomavirus infection in women with ovarian endometriosis. *J Obstet Gynaecol Res* (2017) 43(1):135–9. doi: 10.1111/jog.13188

58. Oppelt P, Renner SP, Strick R, Valletta D, Mehlhorn G, Fasching PA, et al. Correlation of high-risk human papilloma viruses but not of herpes viruses or chlamydia trachomatis with endometriosis lesions. *Fertil Steril* (2010) 93(6):1778–86. doi: 10.1016/j.fertnstert.2008.12.061

59. Rocha RM, Souza RP, Gimenes F, Consolaro MEL. The high-risk human papillomavirus continuum along the female reproductive tract and its relationship to infertility and endometriosis. *Reprod BioMed Online* (2019) 38(6):926–37. doi: 10.1016/j.rbmo.2018.11.032

60. Khan KN, Fujishita A, Hiraki K, Kitajima M, Nakashima M, Fushiki S, et al. Bacterial contamination hypothesis: A new concept in endometriosis. *Reprod Med Biol* (2018) 17(2):125–33. doi: 10.1002/rmb2.12083

61. Lin WC, Chang CY, Hsu YA, Chiang JH, Wan L. Increased risk of endometriosis in patients with lower genital tract infection: A nationwide cohort study. *Med (Baltimore)* (2016) 95(10):e2773. doi: 10.1097/MD.0000000000002773

62. Tai FW, Chang CY, Chiang JH, Lin WC, Wan L. Association of pelvic inflammatory disease with risk of endometriosis: A nationwide cohort study involving 141,460 individuals. *J Clin Med* (2018) 7(11):379. doi: 10.3390/jcm7110379

63. Khan KN, Kitajima M, Hiraki K, Yamaguchi N, Katamine S, Matsuyama T, et al. Escherichia coli contamination of menstrual blood and effect of bacterial endotoxin on endometriosis. *Fertil Steril* (2010) 94(7):2860–3.e1-3. doi: 10.1016/j.fertnstert.2010.04.053

64. Khan KN, Fujishita A, Masumoto H, Muto H, Kitajima M, Masuzaki H, et al. Molecular detection of intrauterine microbial colonization in women with endometriosis. *Eur J Obstet Gynecol Reprod Biol* (2016) 199:69–75. doi: 10.1016/j.ejogrb.2016.01.040

65. Baker JM, Al-Nakkash L, Herbst-Kralovetz MM. Estrogen-gut microbiome axis: Physiological and clinical implications. *Maturitas* (2017) 103:45–53. doi: 10.1016/j.maturitas.2017.06.025

66. Huhtinen K, Stahle M, Perheentupa A, Poutanen M. Estrogen biosynthesis and signaling in endometriosis. *Mol Cell Endocrinol* (2012) 358(2):146–54. doi: 10.1016/j.mce.2011.08.022

67. Kitawaki J, Kado N, Ishihara H, Koshiba H, Kitaoka Y, Honjo H. Endometriosis: the pathophysiology as an estrogen-dependent disease. *J Steroid Biochem Mol Biol* (2002) 83(1-5):149–55. doi: 10.1016/S0960-0760(02)00260-1

68. Plottel CS, Blaser MJ. Microbiome and malignancy. *Cell Host Microbe* (2011) 10(4):324–35. doi: 10.1016/j.chom.2011.10.003

69. Kwa M, Plottel CS, Blaser MJ, Adams S. The intestinal microbiome and estrogen receptor-positive female breast cancer. *J Natl Cancer Inst* (2016) 108(8):djw029. doi: 10.1093/jnci/djw029

70. Bierne H, Hamon M, Cossart P. Epigenetics and bacterial infections. *Cold Spring Harb Perspect Med* (2012) 2(12):a010272. doi: 10.1101/cshperspect.a010272

71. Jenner RG, Young RA. Insights into host responses against pathogens from transcriptional profiling. *Nat Rev Microbiol* (2005) 3(4):281–94. doi: 10.1038/nrmicro1126

72. Koninckx PR, Ussia A, Tahlak M, Adamyan L, Wattiez A, Martin DC, et al. Infection as a potential cofactor in the genetic-epigenetic pathophysiology of endometriosis: A systematic review. *Facts Views Vis Obgyn* (2019) 11(3):209–16.

73. Campos GB, Marques LM, Rezende IS, Barbosa MS, Abrao MS, Timenetsky J. Mycoplasma genitalium can modulate the local immune response in patients with endometriosis. *Fertil Steril* (2018) 109(3):549–60.e4. doi: 10.1016/j.fertnstert.2017.11.009

74. Serraino D, Piselli P, Scognamiglio P. Viral infections and cancer: epidemiological aspects. *J Biol Regul Homeost Agents* (2001) 15(3):224–8.

75. Butel JS. Viral carcinogenesis: revelation of molecular mechanisms and etiology of human disease. *Carcinogenesis* (2000) 21(3):405–26. doi: 10.1093/carcin/21.3.405

76. Rapp F. Current knowledge of mechanisms of viral carcinogenesis. *Crit Rev Toxicol* (1984) 13(2):197–204. doi: 10.3109/10408448409034082

77. Hsiao KY, Wu MH, Tsai SJ. Epigenetic regulation of the pathological process in endometriosis. *Reprod Med Biol* (2017) 16(4):314–9. doi: 10.1002/rmb2.12047

78. Lagana AS, Garzon S, Gotte M, Vigano P, Franchi M, Ghezzi F, et al. The pathogenesis of endometriosis: Molecular and cell biology insights. *Int J Mol Sci* (2019) 20(22):5615. doi: 10.3390/ijms20225615

79. Perrotta AR, Borrelli GM, Martins CO, Kallas EG, Sanabani SS, Griffith LG, et al. The vaginal microbiome as a tool to predict rASRM stage of disease in endometriosis: A pilot study. *Reprod Sci* (2020) 27(4):1064–73. doi: 10.1007/s43032-019-00113-5

80. Revised American fertility society classification of endometriosis: 1985. *Fertil Steril* (1985) 43(3):351–2. doi: 10.1016/s0015-0282(16)48430-x

81. Gholiof M, Adamson-De Luca E, Wessels JM. The female reproductive tract microbiotas, inflammation, and gynecological conditions. *Front Reprod Health* (2022) 4:963752. doi: 10.3389/frph.2022.963752

82. Itoh H, Sashihara T, Hosono A, Kaminogawa S, Uchida M. Lactobacillus gasseri OLL2809 inhibits development of ectopic endometrial cell in peritoneal cavity *via* activation of NK cells in a murine endometriosis model. *Cytotechnology* (2011) 63(2):205–10. doi: 10.1007/s10616-011-9343-z

83. Uchida M, Kobayashi O. Effects of lactobacillus gasseri OLL2809 on the induced endometriosis in rats. *Biosci Biotechnol Biochem* (2013) 77(9):1879–81. doi: 10.1271/bbb.130319

84. Simopoulos AP. Omega-3 fatty acids in inflammation and autoimmune diseases. *J Am Coll Nutr* (2002) 21(6):495–505. doi: 10.1080/07315724.2002.10719248

85. Tortosa-Caparros E, Navas-Carrillo D, Marin F, Orenes-Pinero E. Anti-inflammatory effects of omega 3 and omega 6 polyunsaturated fatty acids in cardiovascular disease and metabolic syndrome. *Crit Rev Food Sci Nutr* (2017) 57(16):3421–9. doi: 10.1080/10408398.2015.1126549

86. Hopeman MM, Riley JK, Frolova AI, Jiang H, Jungheim ES. Serum polyunsaturated fatty acids and endometriosis. *Reprod Sci* (2015) 22(9):1083–7. doi: 10.1177/1933719114565030

87. Missmer SA, Chavarro JE, Malspeis S, Bertone-Johnson ER, Hornstein MD, Spiegelman D, et al. A prospective study of dietary fat consumption and endometriosis risk. *Hum Reprod* (2010) 25(6):1528–35. doi: 10.1093/humrep/deq044

88. Attaman JA, Stanic AK, Kim M, Lynch MP, Rueda BR, Styer AK. The anti-inflammatory impact of omega-3 polyunsaturated fatty acids during the establishment of endometriosis-like lesions. *Am J Reprod Immunol* (2014) 72(4):392–402. doi: 10.1111/aji.12276

89. Riesenfeld CS, Schloss PD, Handelsman J. Metagenomics: Genomic analysis of microbial communities. *Annu Rev Genet* (2004) 38:525–52. doi: 10.1146/annurev.genet.38.072902.091216

90. Agarwal SK, Chapron C, Giudice LC, Laufer MR, Leyland N, Missmer SA, et al. Clinical diagnosis of endometriosis: A call to action. *Am J Obstet Gynecol* (2019) 220 (4):354.e1–e12. doi: 10.1016/j.ajog.2018.12.039

91. Ghai V, Jan H, Shakir F, Haines P, Kent A. Diagnostic delay for superficial and deep endometriosis in the united kingdom. *J Obstet Gynaecol* (2020) 40(1):83–9. doi: 10.1080/01443615.2019.1603217

92. Kiesel L, Sourouni M. Diagnosis of endometriosis in the 21st century. *Climacteric* (2019) 22(3):296–302. doi: 10.1080/13697137.2019.1578743

93. Leonardi M, Uzuner C, Mestdagh W, Lu C, Guerriero S, Zajicek M, et al. Diagnostic accuracy of transvaginal ultrasound for detection of endometriosis using international deep endometriosis analysis (IDEA) approach: Prospective international pilot study. *Ultrasound Obstet Gynecol* (2022) 60(3):404–13. doi: 10.1002/uog.24936

94. Chowdary P, Stone K, Ma T, Readman E, McIlwaine K, Druitt M, et al. Multicentre retrospective study to assess diagnostic accuracy of ultrasound for superficial endometriosis-are we any closer? *Aust N Z J Obstet Gynaecol* (2019) 59 (2):279–84. doi: 10.1111/ajo.12911

95. Leonardi M, Robledo KP, Espada M, Vanza K, Condous G. SonoPODography: A new diagnostic technique for visualizing superficial endometriosis. *Eur J Obstet Gynecol Reprod Biol* (2020) 254:124–31. doi: 10.1016/j.ejogrb.2020.08.051

frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 23 March 2023
DOI 10.3389/fendo.2023.1093683

OPEN ACCESS

EDITED BY
Mainak Dutta,
Birla Institute of Technology and Science, United Arab Emirates

REVIEWED BY
Takashi Kajitani,
Sakura no Seibo Junior College, Japan
Martin Stimpfel,
University Medical Centre Ljubljana, Slovenia

*CORRESPONDENCE
Fang Li
fang_li@tongji.edu.cn
Bowen Lin
bowenlin0512@163.com

SPECIALTY SECTION
This article was submitted to Reproduction, a section of the journal Frontiers in Endocrinology

RECEIVED 09 November 2022
ACCEPTED 10 March 2023
PUBLISHED 23 March 2023

CITATION
Lei L, Xu X, Gong C, Lin B and Li F (2023) Integrated analysis of genome-wide gene expression and DNA methylation profiles reveals candidate genes in ovary endometriosis.
Front. Endocrinol. 14:1093683.
doi: 10.3389/fendo.2023.1093683

Integrated analysis of genome-wide gene expression and DNA methylation profiles reveals candidate genes in ovary endometriosis

Lei Lei[1], Xinxin Xu[1], Chengchen Gong[2], Bowen Lin[3,4]* and Fang Li[1]*

[1]Department of Gynecology, Shanghai East Hospital, Tongji University School of Medicine, Shanghai, China, [2]Department of Dermatology, Shanghai East Hospital, Tongji University School of Medicine, Shanghai, China, [3]Department of Cardiology, Shanghai East Hospital, Tongji University School of Medicine, Shanghai, China, [4]Research Center for Translational Medicine, East Hospital, Tongji University School of Medicine, Shanghai, China

Background: The incidence of endometriosis (EMs), a common disease in gynecology, has increased over the years. Women suffer from the symptoms caused by EMs, such as chronic pelvic pain, dysmenorrhea, and infertility. However, the etiology and pathophysiology of EMs remain unclear. This study aimed to identify candidate genes of endometriosis through integrated analysis of genome-wide gene expression and DNA methylation profiles.

Results: Eutopic and ectopic endometrial tissues were collected from patients who were diagnosed as ovarian EMs. Genome-wide methylation profiling identified 17551 differentially methylated loci, with 9777 hypermethylated and 7774 hypomethylated loci. Differentially methylated loci were mainly concentrated in the gene body and intergenic regions. Genome-wide gene expression profiling identified 1837 differentially expressed genes (DEGs), with 1079 genes upregulated and 758 downregulated in ectopic groups. Integrated analysis revealed that DNA methylation was negatively correlated to gene expression in most genomic regions, such as exon, 3'UTR, 5'UTR, and promoter. We also identified promoter-related (53 downregulated and 113 upregulated) and enhancer-related DMGs (212 downregulated and 232 upregulated), which were significantly correlated to the gene expression. Further validation of the top-ranked genes belonging to differentially methylated genes (DMGs) and DEGs revealed that *TMEM184A*, *GREM2*, *SFN*, *KIR3DX1*, *HPGD*, *ESR1*, *BST2*, *PIK3CG* and *RNASE1* were significant candidate genes in ovarian endometriosis.

Conclusion: Our study revealed the significance of DNA methylation in the gene expression in ovary endometriosis, which provides new insights and a molecular foundation for understanding the underlying mechanisms of endometriosis.

KEYWORDS

DNA methylation, gene expression, epigenetics, endometriosis, multi-omic analysis

Introduction

Endometrial tissue that exhibits growing activity outside the uterine cavity and grows abnormally is defined as endometriosis (EMs) (1). Lesions typically occur in the peritoneal cavity, including the ovaries, uterorectal depressions, and uterosacral ligaments (2). EMs affected 5% to 15% of women of reproductive age (3). Patients with EMs suffer a series of symptoms, such as pelvic pain, dysmenorrhea, and infertility (4). EMs exhibit biological behaviors of malignant tumors as a benign disease, such as cell proliferation, implant growth, infiltration, distant metastasis, and recurrence (2). The current mainstream etiological theories cannot fully explain the pathogenesis of EMs, including 'menstrual blood reflux theory', 'lymphatic and venous dissemination theory' (5), and 'body cavity epithelial metaplasia theory' (6), immunological mechanism, angiogenesis mechanism, cell proliferation, and apoptosis imbalance mechanism, and in-place endometrial determinism (7). In recent years, the incidence of EMs has significantly increased, and the patient's onset age tends toward younger, calling for a deeper understanding of the pathogenesis of EMs.

DNA methylation is important in the regulation of gene expression (8). Several studies reported that DNA methylation might play an essential role in the pathogenesis of EMs (9). With the development of sequencing techniques, genome-wide methylation differences between eutopic and ectopic endometrial tissues have been investigated in recent years (10–14). However, few studies linked genome-wide DNA methylation with the changes in gene expression in EMs (10, 15), suggesting DNA methylation changes were associated with altered gene expression in endometrial function or dysfunction. Herein, we integrated the genome-wide DNA methylation with the gene expression of EMs to find candidate genes in EMs.

Materials and methods

Patient enrollment and sample collection

Thirteen female patients who were diagnosed as ovarian EMs were enrolled. DNA methylation profiles were performed on 6 patient specimens, RNA sequencing was performed on 3 patient specimens, and quantitative reverse transcription PCR (qRT-PCR) verifications were performed on 7 patient specimens. Patients (Hans, 27-42 years old) in Shanghai East Hospital undergoing laparoscopic and postoperative pathological diagnosis of ovarian EMs were selected. The typical ectopic endometrial tissue and the matched eutopic endometrium from each patient were collected in the proliferation phase. All the participants had regular menstrual cycles and had not received hormonal medication 3 months before surgery. The participants had no history of other organic diseases, such as hypertension, diabetes, tumors, chronic infections, etc. Patients with benign gynecologic diseases (leiomyoma, ovarian serous cyst, PCOS) were excluded. All patients provided written informed consent in this study. This study was approved by the Ethics Committee of the Shanghai East Hospital of Tongji University.

DNA and RNA extraction

All the samples were kept in RNAlater (Ambion, AM7020) when obtained during laparoscopic surgery. Ectopic and eutopic tissues were extracted for genomic DNA detection according to the manufacturer's instructions (QIAamp DNA Mini Kit, 51304). Total RNA was extracted from ectopic and eutopic tissues using RNAiso plus reagent (Takara, 9109) following the manufacturer's instructions. Briefly, tissues were grinded and crushed in liquid nitrogen, and then added 300 μL chloroform/isoamyl alcohol (24:1) and mixed thoroughly. After centrifuging, the supernatant was moved to a 1.5 mL centrifuge tube, mixed with isopropyl alcohol, and then purified to RNA for the subsequent experiments.

DNA methylation array

At least 500 ng genomic DNA was extracted and bisulfate converted (Zymo EZ-96 DNA Methylation-Direct Kit, D5023) before being processed to the methylation array. Illumina Human Methylation 450 K BeadChips were applied to get the whole genome methylation states of the samples. The experimental data were sorted out and encoded, and the data was double-converted and double-calibrated by EpidData. Background adjustment and quantile normalization pretreatment were performed according to the raw data of each probe methylation site of the Illumina methylated HM450K BeadChip chip. Then the degree of methylation at the probe level was analyzed. β value was calculated and normalized to quantify the degree of methylation. In this study, the average β value ≥ 0.2 and P-value < 0.05 was considered as significant.

Differential methylation analysis

After standardizing the original methylation data, the Pearson Correlation Coefficiency (PCC) was used to evaluate the methylation profile correlation between the ectopic and eutopic groups. The correlation between ectopic and eutopic samples was high, indicating the overall reproducibility of the samples. Since not all the samples were in accordance with normal distribution, the Wilcox test was applied to do differential methylation analysis. The criteria for screening the difference site need to meet both (1) the original $P < 0.05$ and (2) the difference of ectopic and eutopic group beta value (delta beta value) greater than or equal to 0.2. The results were also calculated with FDR (false discovery rate) corrected by multiple hypothesis testing to assess differential methylation levels. The differential DMR biological function analysis was performed using the hypergeometric distribution algorithm or Fisher test. Biological pathway analysis was enriched in KEGG (Kyoto Encyclopedia of Genes and Genomes, www.genome.jp/kegg/) database.

RNA-sequencing and analysis

RNA libraries were constructed with a VAHTS Universal V6 RNA-seq Library Prep Kit for MGI (Vazyme, NRM604). DNBSEQ-T7 high-throughput sequencing platform of BGI was used for sequencing. The raw data was filtered to clean data after trimming low quality reads and adapters. Clean data were mapped to mm10 mouse reference genome using Hisat2 software (version 2.2.1). The reads were quantified by featureCounts (version 2.0.1). The differential gene analysis was performed by Deseq2 (version 1.32.0). Genes with adjusted P value < 0.05 and the abs (Log2(Fold Change)) ≥ 1 was considered as differentially expressed genes (DEGs). The enrichment of Gene Ontology (GO) was conducted by clusterProfiler package (v4.0.5).

Quantitative reverse transcription PCR

Total RNA was extracted from ectopic and eutopic samples. cDNA was obtained after reverse transcription reversal by Evo M-MLV RT Kit (Accurate Biology Co. Ltd, AG11601). The qRT-PCR was performed by Evo M-MLV One Step RT-PCR Kit (Accurate Biology Co. Ltd, AG11607). Each target gene was compared to β-actin. The expression of target mRNA was calculated based on $2^{-\Delta\Delta Ct}$ method. The primers used in this study were listed in the Supplementary Table S2.

Statistical analysis

All the statistical analyses were performed using R software (version 4.0.1). P-values less than 0.05 were considered statistically significant. The Kolmogorov-Smirnov test was applied to evaluate the normality of the distribution of the variables. For qRT-PCR, statistical analyses were conducted using a student t-test (data with normal distribution) or Mann-Whitney test (data with skewed distribution) as appropriate by GraphPad Prism software 8.0 (GraphPad Software Inc).

Results

Genome-wide DNA methylation changes between eutopic and ectopic endometrial tissues

We first obtained the genome-wide DNA methylation changes of eutopic and ectopic endometrial tissues (Figure 1A). The clinical characteristics of the patients were summarized in Supplementary Table S1. A total of 17551 differentially methylated loci were identified, of which 9777 were hypermethylated and 7774 were hypomethylated (Figure 1B). To get view of the distribution of differentially methylation loci, we annotated the locations to different functional elements in the genome. Hypermethylated and hypomethylated loci in ectopic groups were mainly located in gene body (39.13% of hypermethylated loci, and 37.97% of hypomethylated loci) and intergenic regions (36.22% of hypermethylated loci, and 30.89% of hypomethylated loci) (Figure 1C). Furthermore, the CpG island probe distribution of both hypermethylated and hypomethylated loci occurred in the OpenSea region (Figure 1D). Next, we performed KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway analysis of differentially methylated loci. Both hypermethylated and hypomethylated genes in ectopic were enriched in ECM-receptor interaction, human papillomavirus (HPV) infection, focal adhesion, PI3K-Akt signaling pathway and Wnt signaling pathway. Moreover, axon guidance, estrogen signaling pathway, glutamatergic synapse, protein digestion and absorption, and relaxin signaling pathway were only enriched in hypermethylated genes. Besides, apelin signaling pathway, cholinergic synapse and Hippo signaling pathway were only enriched in hypomethylated genes in the ectopic group (Figure 1E). The above results indicated that DNA methylation was associated with various pathways in the eutopic and ectopic endometrial tissues.

Transcriptome analysis of ectopic and eutopic samples

To view the transcriptional difference between ectopic and eutopic endometrial tissues. We performed RNA sequencing of ectopic and eutopic endometrial tissues (3 ectopic *vs* 3 eutopic, Supplementary Table S1). As indicated in Principal Component Analysis (PCA), ectopic and eutopic endometrial tissues were significantly separated and the samples in each group were closely related (Figure 2A), suggesting that ectopic and eutopic endometrial tissues were transcriptionally different. Differential gene expression analysis showed that there were 1837 differentially expressed genes (DEGs) (Figure 2B) in the ectopic compared to eutopic samples, with 1079 genes upregulated and 758 downregulated. Next, we compared our RNA-seq data with another RNA-seq dataset comparing the ectopic with eutopic endometria in eight patients with ovarian endometriosis (GSE105764) (16). Venn plot showed that 70.4% upregulated and 60.95% downregulated DEGs in our RNA-seq data were overlapped with DEGs in GSE105764 dataset (Figure 2C), validating the reliability of our RNA-seq data. The top 10 upregulated and downregulated genes in ectopic endometrial tissues were shown in Figure 2D. The upregulated DEGs in ectopic samples were enriched in leukocyte migration, muscle system process, humoral immune in response (Figure 2E), and downregulated DEGs are enriched in functions such as nuclear division, chromosome segregation, and mitotic nuclear division (Figure 2F). In summary, ectopic and eutopic endometrial tissues were transcriptionally different from each other.

Integrated analysis of DNA methylation and gene expression reveals candidate genes in endometriosis

DNA methylation was important in the gene expression regulation (8). To connect DNA methylation with gene expression in endometriosis, we further integrated our DNA

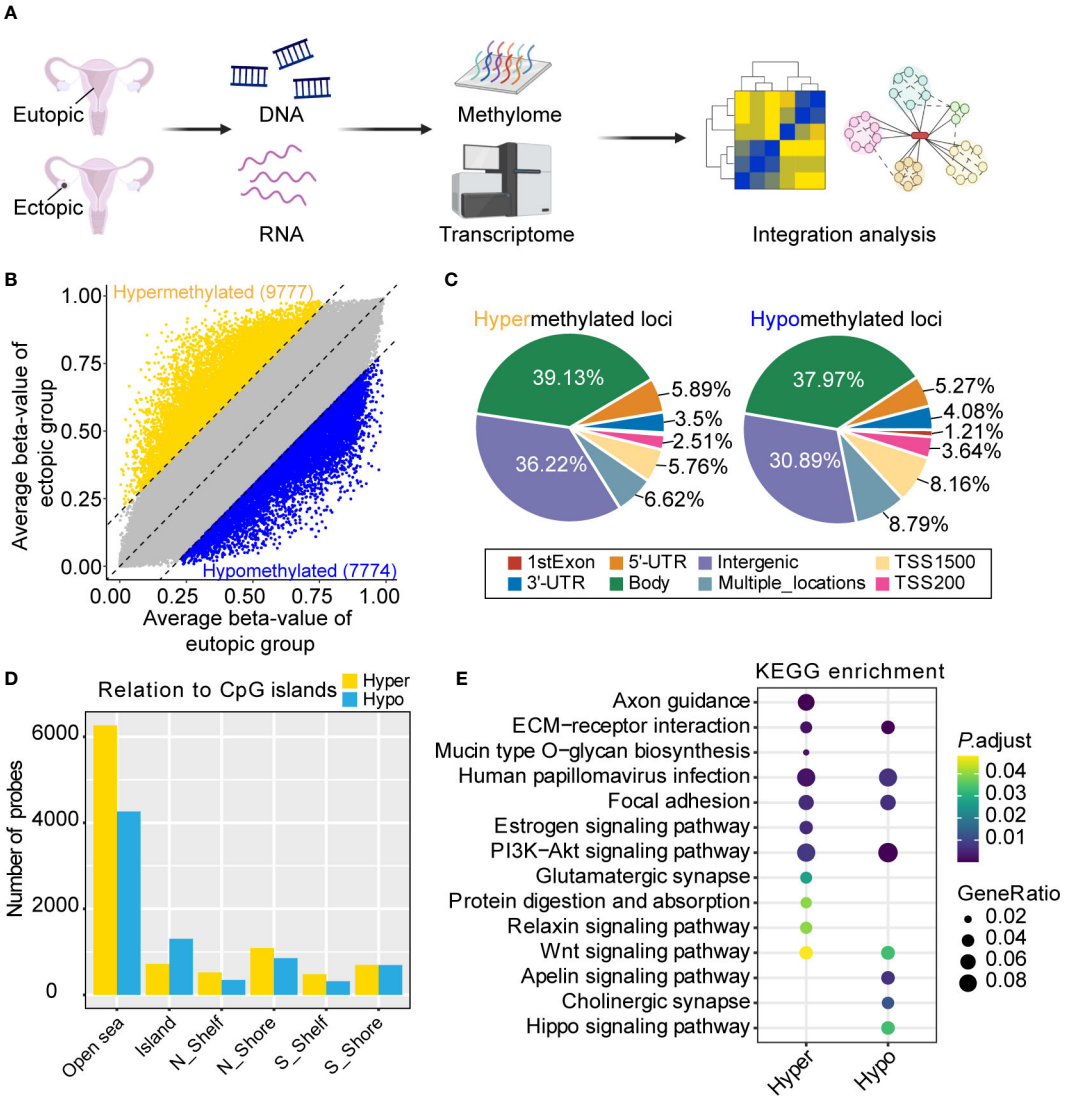


FIGURE 1
Genome-wide DNA methylation analysis of eutopic and ectopic endometrial tissues. **(A)** Schematic diagram of the study. Created with BioRender.com. **(B)** Differential methylated loci in eutopic and ectopic endometrial tissues. **(C)** The distribution of hypermethylated (left) and hypomethylated (right) loci in ectopic endometrial tissues. **(D)** CpG island probe distribution of hypermethylated and hypomethylated loci in ectopic endometrial tissues. **(E)** Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis of the hypermethylated and hypomethylated loci in the ectopic group.

methylation and RNA-seq data. First, we divided the DNA methylation states to six groups (1st exon, 3'UTR, 5'UTR, gene body, multiple locations, and promoter) according to the position of methylated loci. As expected, the level of DNA methylation (delta β-value) was negatively correlated with gene expression in most of the groups (1st exon, 5'UTR, multiple locations, and promoter) (Figure 3A). We further defined genes with differentially methylated loci in promoters as 'promoter-related DMGs (differentially methylated genes)' and genes with differentially methylated loci not in promoters as 'enhancer-related DMGs'. According to the relation of level of methylation (delta β-value) and gene expression, genes were separated to four quadrants (part 1 to part 4, Figure 3A). Almost half of promoter-related DMGs (48.0%, 113/235) were in part 4 (Figure 3B). Promoter-related DMGs in part 4 were enriched in sialic acid binding and lipase activity (Figure 3C). Furthermore, promoter-related DMGs in part 2 were specifically enriched in channel activity. For enhancer-related DMGs, 70.25% were in part 1 and 4 (Figure 3D). DMGs in part 1 were enriched in transcriptional activity (nuclear receptor activity, ligand-activated transcription factor activity, and DNA-binding transcription activator), steroid hormone receptor activity and channel activity (Figure 3E). These results indicated that most DMGs in promoter and enhancer were negatively correlated with gene expression.

Validation of candidate genes in endometriosis

To validate the candidate genes in endometriosis, we listed the top 15 DMGs (ranked by delta β-value) whose expression was

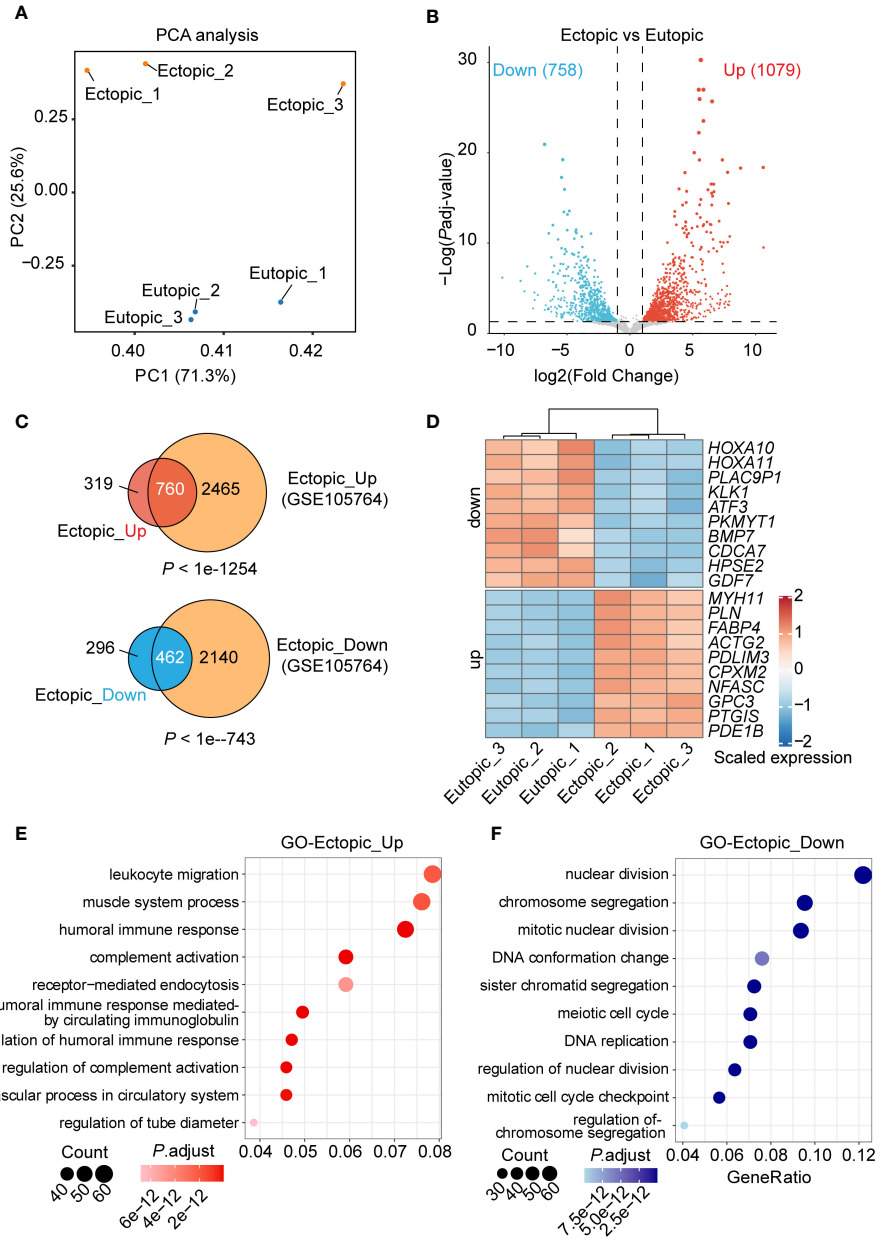


FIGURE 2
Genome-wide gene expression analysis of eutopic and ectopic endometrial tissues. **(A)** Principal components analysis (PCA) analysis of the eutopic and ectopic samples. **(B)** Differentially expressed genes (DEGs) analysis of eutopic and ectopic samples. **(C)** Venn plots of the DEGs and DEGs in the GSE105764 dataset. **(D)** Heatmap of the top 10 upregulated and downregulated DEGs in RNA-seq. **(E)** GO analysis of the upregulated genes in ectopic samples. **(F)** GO analysis of the downregulated genes in ectopic samples.

upregulated (Table 1) and downregulated (Table 2). After filtering the past reported genes, 10 unreported DMGs (*TMEM184A*, *GREM2*, *SFN*, *KIR3DX1*, *HPGD*, *ESR1*, *CASS4*, *BST2*, *PIK3CG* and *RNASE1*) were kept. We further detected the expression of these 10 DMGs in ectopic and eutopic endometrial tissues (7 ectopic *vs* 7 eutopic, Supplementary Table S1). *TMEM184A*, *GREM2*, *SFN*, *KIR3DX1*, *HPGD*, and *ESR1* were significantly downregulated in ectopic tissues, and *BST2*, *PIK3CG* and *RNASE1* were significantly upregulated in ectopic tissues (Figure 4). In summary, we validated the top candidate genes in endometriosis which might be regulated by DNA methylation.

Discussions

EMs, vividly known as 'a kind of benign cancer' and women's pelvic 'sandstorm', has established itself as one of the most common diseases in gynecology by its high incidence and its early onset age,

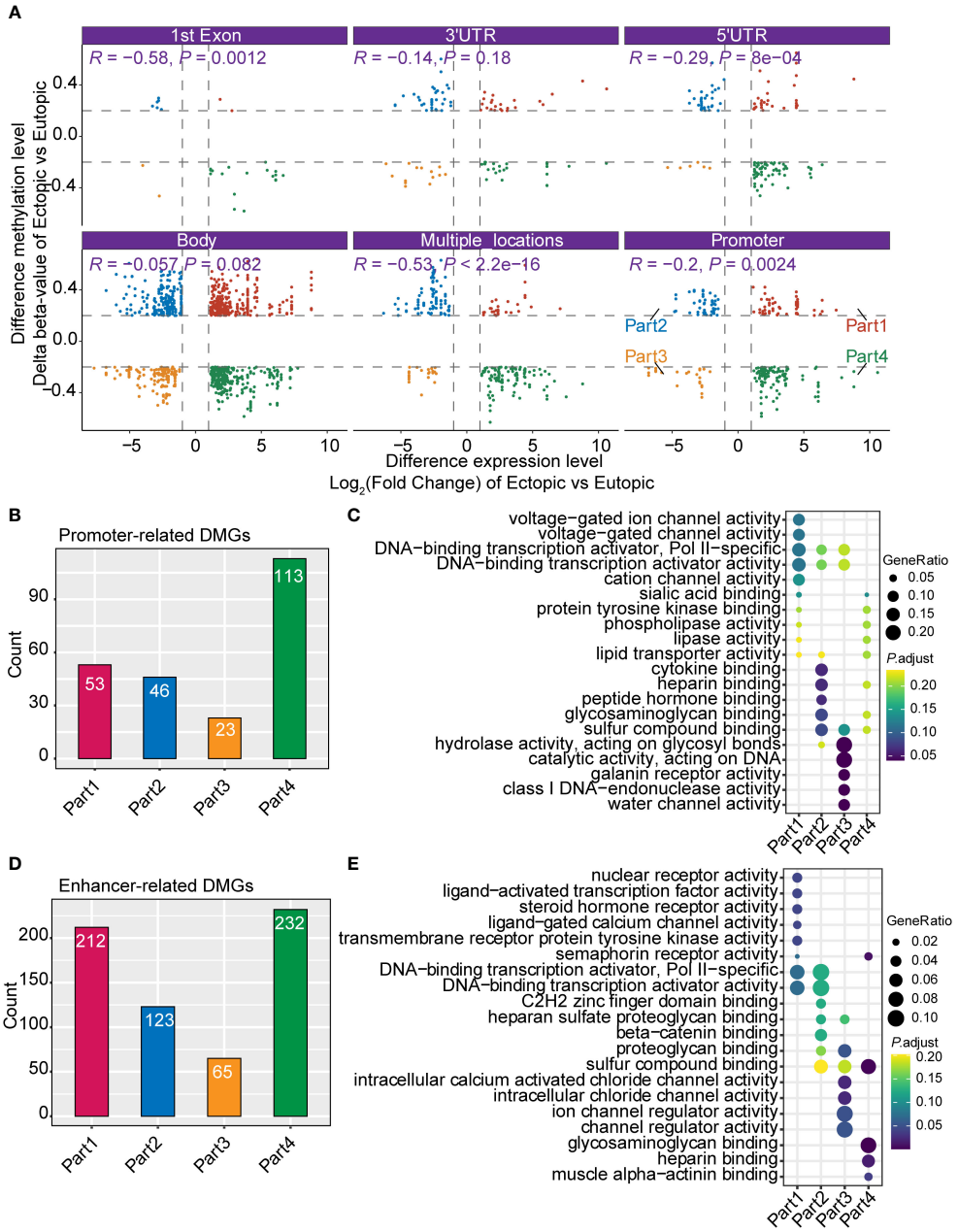


FIGURE 3
Integrated analysis of DNA methylation and gene expression profiles. **(A)** Correlation of the DNA methylation and gene expression grouped by different genomic positions (1st Exon, 3′UTR, 5′UTR, gene body, multiple locations, and promoter). **(B)** Bar plot shows the count of promoter-related differentially methylated genes (DMGs). **(C)** Gene Ontology (GO) of different groups of promoter-related DMGs (part1-part4 in Figure 3A). **(D)** Bar plot shows the count of enhancer-related DMGs. **(E)** GO analysis of different groups of enhancer-related DMGs.

Symptoms like chronic pelvic pain, dysmenorrhea, and infertility caused by EMs have seriously threatened women's health and reduced their life quality. EMs is one of the focuses of global reproductive health issues. According to the available literature reports, it is estimated that about 15% to 30% of women in the childbearing period would be affected by the disease, involving about 270 million people worldwide (17).

Although the theory of menstrual blood reflux proposed by Sampson J.J., who was the father of endometriosis in the world, has been accepted for a century and a half (5), the related factors and pathogenesis of EMs are still incomplete. Due to the wide range of lesions and diverse morphologies, malignant biological behaviors such as metastasis, invasion, and recurrence, and the possibility of malignant transformation, EMs is vividly called 'benign cancer' (1). More and more studies believe that its similar biological behavior to malignant tumors may be one of the important pathogenesis of EMs (18–21). The occurrence and development of malignant lesions are marked by the accumulation of tumor suppressor genes and oncogene genetic variations, especially by the abnormal methylation of tumor suppressor genes. Studies have confirmed

TABLE 1 Top candidate genes which were hypermethylated and downregulated in ectopic endometrial tissues.

Probe_ID	Gene	beta_case avg	beta_control avg	beta_change	RELATION_TO_CPG_ISLAND	log2FoldChange	padj
cg06738242	*TWIST2*	0.554053551	0.15610387	0.397949684	Island	-2.6571473	0.00015233
cg23210268	*GREM2*	0.699915101	0.30227628	0.397638816		-3.3286895	0.00018973
cg15188268	*ADAMTS19*	0.817201563	0.43219189	0.385009671	N_Shore	-3.6976664	6.26E-05
cg09386458	*QPCT*	0.886765946	0.51097633	0.375789613	N_Shore	-2.1138241	0.00406449
cg23037932	*KIR3DX1*	0.815017046	0.45292026	0.362096786		-4.7201206	0.00906221
cg22920700	*OSR2*	0.579597099	0.22700124	0.352595857	N_Shore	-1.7278745	0.02790383
cg01352551	*SLC24A3*	0.793752782	0.46820021	0.325552568	N_Shore	-1.5072888	0.03022538
cg23719157	*STRA6*	0.78738477	0.46561667	0.321768098		-2.4999297	0.01348782
cg13213527	*ZNF516*	0.775537145	0.45945426	0.316082885		-1.9614117	0.00040565
cg16549596	*SFN*	0.649653471	0.33389726	0.315756214	N_Shore	-2.9059607	0.00244178
cg04555941	*HPGD*	0.417078247	0.11941307	0.297665176	S_Shore	-2.5526777	0.00058597
cg00335591	*TMEM184A*	0.857047129	0.56488322	0.292163912		-1.8289676	0.04205992
cg25338972	*ESR1*	0.515218373	0.22405393	0.291164441		-2.5746547	0.00330811
cg13468624	*PSORS1C3*	0.694573016	0.41112169	0.283451322	N_Shore	-2.0015838	0.03245501
cg02992645	*OVGP1*	0.796760034	0.53732872	0.259431316		-2.9074884	0.00245663

that abnormal methylation of CpG islands regulated by non-methylated tumor suppressor genes is closely related to the occurrence and metastasis of human malignant tumors (22). A large number of studies have confirmed that EMs is a genetic disease caused by the interaction of multiple locus genes and environmental factors (23–26). HOXA10, hMLH1, PTEN promoter region 5' CpG island methylation pattern is currently known to be related to the occurrence and development of EMs. HOXA10 gene can inhibit tumor cell proliferation, reduce invasion ability, and inhibit tumor cell metastasis. Recent studies abroad have found that the reduced expression of the HOXA10 gene in EMs is similar to malignant lesions. EMs animal model studies have further supported that methylation of the promoter region of the HOXA10 gene may be involved in the pathogenesis of EMs (27). PTEN is currently recognized as a tumor suppressor gene, and PTEN methylation is closely related to the occurrence and development of various tumors. Abnormalities in this gene have been demonstrated in many human cancers, especially in ovarian endometrioidal carcinomas, endometrial cancers, and gliomas (28). Salvesen et al. confirmed that PTEN gene methylation was prevalent in endometrial cancer, and tumor suppressor gene methylation was associated with advanced tumor metastasis in endometrial cancer and plays an important role in tumor progression (28). KF. Tam et al. (29) explored the relationship between DNA methylation and ovarian tumors, including PTEN genes. They concluded that the methylation rate of borderline ovarian tumors and ovarian cancer was significantly higher than that of benign ovarian tumors and normal ovarian tissue.

Our study identified a series of candidate genes and pathways in EMs. Of note, in KEGG pathway analysis, both hypermethylated and hypomethylated genes in ectopic were enriched in human papillomavirus (HPV) infection, suggesting a potential link between endometriosis and HPV infection. The prevalence of high-risk HPV was significantly higher in patients with EMs than in those without EMs (30). Besides, HPV-infected endometrial cells were reported to be spread through retrograde menstruation (31). The persistent HPV infection of endometriosis lesions was proposed to contribute to malignant progression. Since EMs is considered a common gynecologic problem with multifactorial origins, HPV infection is a highly possible pathophysiologic causes of EMs. We also validated the differential expression of top-ranked genes (*TMEM184A*, *GREM2*, *SFN*, *KIR3DX1*, *HPGD*, *ESR1*, *BST2*, *PIK3CG* and *RNASE1*) in EMs and normal tissues. *TMEM184A*, *GREM2*, *SFN*, *KIR3DX1*, *HPGD*, and *ESR1* were hypermethylated and significantly downregulated in ectopic endometrial tissues. Conversely, *BST2*, *PIK3CG* and *RNASE1* were hypomethylated and upregulated in ectopic endometrial tissues.

SFN is highly expressed in the esophagus-mucous membrane, skin, small salivary glands, and vagina (32–34), indicating its essential role in maintaining the homeostasis of a series of tissues and organs. The higher expression of SFN was significantly associated with a better prognosis of endometrial cancer (35). Accordingly, SFN was downregulated in ectopic endometrial tissues in our study, suggesting the loss of function of SFN might be involved in the pathophysiology of EMs. HPGD was reported to be involved in the pathophysiology of endometriosis (36). Decreased expression of HPGD is associated with abnormal prostaglandin metabolism in endometriosis (35, 37). Besides, the decreased expression of HPGD might be regulated by the miR-218-5p in endometrial adenocarcinoma tissue (35). ESR1 encodes estrogen receptors and ligand-activated transcription factors that can form homodimers or heterodimers with estrogen receptors. ERβ is widely expressed during endometrial hyperplasia in infertile

TABLE 2 Top candidate genes which were hypomethylated and upregulated in ectopic endometrial tissues.

Probe_ID	Gene	beta_case_avg	beta_control_avg	beta_change	RELATION_TO_CPG_ISLAND	log2FoldChange	padj
cg24587601	*CASS4*	0.137575599	0.72258402	0.58500842		1.77916735	0.01566374
cg01329005	*BST2*	0.108425528	0.63957445	0.531148922		2.95333954	1.53E-05
cg13214190	*PIK3CG*	0.329104785	0.81439809	0.485293304	N_Shelf	1.75014039	0.01724619
cg13718960	*RNASE1*	0.327354776	0.79572139	0.468366613		2.68147978	1.05E-05
cg20986996	*TCF21*	0.211925743	0.67962279	0.467697044	N_Shore	6.09318374	5.74E-12
cg00123072	*THBS4*	0.453287548	0.88810567	0.434818118	N_Shore	2.15240829	0.00020269
cg26928972	*CSTA*	0.348577804	0.7816603	0.433082493		5.87099798	8.69E-13
cg15338159	*FHL2*	0.412303334	0.82354861	0.411245276		3.14284341	0.00012948
cg19876649	*MYOM1*	0.399121642	0.75975417	0.360632532		2.51276745	3.61E-05
cg13661129	*NR5A1*	0.469142795	0.82427195	0.355129158	S_Shelf	8.7981021	5.00E-19
cg24315815	*PLSCR4*	0.311694902	0.66008554	0.348390633	S_Shore	1.857896	0.01098051
cg04413226	*FMO1*	0.436500059	0.78104022	0.344540159		3.61678571	4.07E-09
cg01089639	*JAK3*	0.348527172	0.66969015	0.32116298	S_Shore	2.04877678	0.01319003
cg18525352	*NUAK1*	0.055519124	0.37199013	0.316471009	S_Shore	1.83605399	0.00401288
cg26683398	*LTC4S*	0.302200139	0.61640078	0.314200641	N_Shelf	3.06959132	4.57E-05

10.3389/fendo.2023.1093683

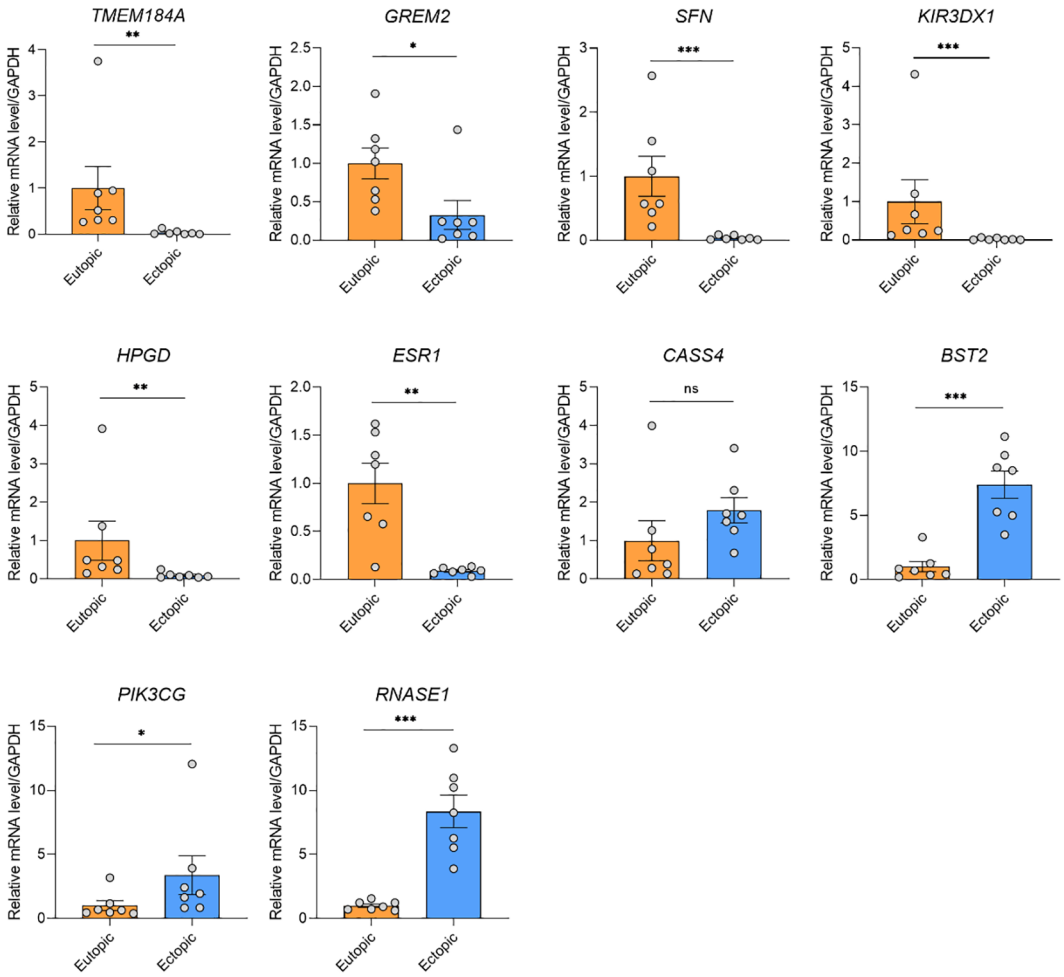


FIGURE 4
qRT-PCR validation of the gene expression of the candidate genes. *TMEM184A*, *GREM2*, *SFN*, *KIR3DX1*, *HPGD*, and *ESR1* were significantly downregulated in ectopic tissues, and *BST2*, *PIK3CG* and *RNASE1* were significantly upregulated in ectopic tissues. ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$, ns, not significant.

women while decreasing or absent in women of normal childbearing age, suggesting that overexpression of ERβ may be related to infertility. Mutated ER in exon 8 with high activity *in vivo* may cause precocious puberty in girls (38). As reported, genetic variants in ESR1 were reported to change the susceptibility to endometriosis and might influence the fertility status in endometriosis patients (39). Moreover, ESR1 amplification might be one mechanism for ER over-expression in endometrial carcinoma (40). BST2 was one of the proteins that were found to be related to tumor metastasis (41). The anti-BST2 antibody had a potent antitumor effect against endometrial cancer both *in vitro* and *in vivo*, indicating that anti-BST2 antibody might be a potential therapeutic strategy for endometrial cancer. Interestingly, There are few studies of *TMEM184A*, *GREM2*, *KIR3DX1*, *BST2* and *RNASE1* in EMs, which needs more investigations to figure out their function in EMs in the future.

There are some limitations in this study. The low number of included patients might limit the statistical power of the findings. Further large-scale studies and investigations are needed to validate the findings. Besides, all the patients enrolled were Asian, which might introduce confounding when interpreting the results. With the increasing sequencing data of EMs being reported, meta-analyses of these sequencing data might give a more comprehensive view of the pathophysiology of EMs.

Conclusion

In conclusion, we screened the differentially methylated and expressed genes through genome-wide DNA methylation and transcriptome sequencing of ectopic and eutopic endometrial tissues. Further integrated analysis identified a series of potential candidate genes in EMs. We also verified *TMEM184A*, *GREM2*, *SFN*, *KIR3DX1*, *HPGD*, *ESR1*, *BST2*, *PIK3CG* and *RNASE1* as key candidate genes in EMs.

Data availability statement

The data presented in the study are deposited in the CNGB Sequence Archive (CNSA) of China National GeneBank DataBase (CNGBdb) repository, accession number CNP0003813.

Ethics statement

The studies involving human participants were reviewed and approved by Ethics Committee of the Shanghai East Hospital of Tongji University. The patients/participants provided their written informed consent to participate in this study.

Author contributions

FL and BL conceived and designed the project. LL and FL performed all operations. BL analyzed the data and drew the figures. LL and BL wrote the manuscript. XX, CG, BL and FL revised the manuscript. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by the National Key R&D Program of China (2021YFC2701200) to FL, and fund program of Shanghai Municipal Commission of Health and Family Planning (No. 202040132) to LL.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1093683/full#supplementary-material

References

1. Olive DL, Pritts EA. Treatment of endometriosis. *N Engl J Med* (2001) 345 (4):266–75. doi: 10.1056/NEJM200107263450407

2. Macer ML, Taylor HS. Endometriosis and infertility: a review of the pathogenesis and treatment of endometriosis-associated infertility. *Obstet Gynecol Clin North Am* (2012) 39(4):535–49. doi: 10.1016/j.ogc.2012.10.002

3. Giudice LC, Kao LC. Endometriosis. *Lancet* (2004) 364(9447):1789–99. doi: 10.1016/S0140-6736(04)17403-5

4. Giudice LC. Clinical practice. *Endometriosis. N Engl J Med* (2010) 362(25):2389–98. doi: 10.1056/NEJMcp1000274

5. Nisolle M, Donnez J. Peritoneal endometriosis, ovarian endometriosis, and adenomyotic nodules of the rectovaginal septum are three different entities. *Fertil Steril* (1997) 68(4):585–96. doi: 10.1016/s0015-0282(97)00191-x

6. Eskenazi B, Warner ML. Epidemiology of endometriosis. *Obstet Gynecol Clin North Am* (1997) 24(2):235–58. doi: 10.1016/s0889-8545(05)70302-8

7. Santamaria X, Massasa EE, Taylor HS. Migration of cells from experimental endometriosis to the uterine endometrium. *Endocrinology* (2012) 153(11):5566–74. doi: 10.1210/en.2012-1202

8. Greenberg MVC, Bourc'his D. The diverse roles of DNA methylation in mammalian development and disease. *Nat Rev Mol Cell Biol* (2019) 20(10):590–607. doi: 10.1038/s41580-019-0159-6

9. Koukoura O, Sifakis S, Spandidos DA. DNA Methylation in endometriosis (Review). *Mol Med Rep* (2016) 13(4):2939–48. doi: 10.3892/mmr.2016.4925

10. Houshdaran S, Nezhat CR, Vo KC, Zelenko Z, Irwin JC, Giudice LC. Aberrant endometrial DNA methylome and associated gene expression in women with endometriosis. *Biol Reprod* (2016) 95(5):93. doi: 10.1095/biolreprod.116.140434

11. Naqvi H, Ilagan Y, Krikun G, Taylor HS. Altered genome-wide methylation in endometriosis. *Reprod Sci* (2014) 21(10):1237–43. doi: 10.1177/1933719114532841

12. Nasu K, Kawano Y, Tsukamoto Y, Takano M, Takai N, Li H, et al. Aberrant DNA methylation status of endometriosis: epigenetics as the pathogenesis, biomarker and therapeutic target. *J Obstet Gynaecol Res* (2011) 37(7):683–95. doi: 10.1111/j.1447-0756.2011.01663.x

13. Saare M, Modhukur V, Suhorutshenko M, Rajashekar B, Rekker K, Soritsa D, et al. The influence of menstrual cycle and endometriosis on endometrial methylome. *Clin Epigenet* (2016) 8:2. doi: 10.1186/s13148-015-0168-z

14. Wang L, Zhao J, Li Y, Wang Z, Kang S. Genome-wide analysis of DNA methylation in endometriosis using illumina human methylation 450 K BeadChips. *Mol Reprod Dev* (2019) 86(5):491–501. doi: 10.1002/mrd.23127

15. Houshdaran S, Zelenko Z, Irwin JC, Giudice LC. Human endometrial DNA methylome is cycle-dependent and is associated with gene expression regulation. *Mol Endocrinol* (2014) 28(7):1118–35. doi: 10.1210/me.2013-1340

16. Zhao L, Gu C, Ye M, Zhang Z, Li L, Fan W, et al. Integration analysis of microRNA and mRNA paired expression profiling identifies deregulated microRNA-transcription factor-gene regulatory networks in ovarian endometriosis. *Reprod Biol Endocrinol* (2018) 16(1):4. doi: 10.1186/s12958-017-0319-5

17. Nezhat C, Lewis M, Kotikela S, Veeraswamy A, Saadat L, Hajhosseini B, et al. Robotic versus standard laparoscopy for the treatment of endometriosis. *Fertil Steril* (2010) 94(7):2758–60. doi: 10.1016/j.fertnstert.2010.04.031

18. Choudhuri S. From waddington's epigenetic landscape to small noncoding RNA: some important milestones in the history of epigenetics research. *Toxicol Mech Methods* (2011) 21(4):252–74. doi: 10.3109/15376516.2011.559695

19. Wu Y, Strawn E, Basir Z, Halverson G, Guo SW. Promoter hypermethylation of progesterone receptor isoform b (PR-b) in endometriosis. *Epigenetics* (2006) 1(2):106–11. doi: 10.4161/epi.1.2.2766

20. Xue Q, Lin Z, Cheng YH, Huang CC, Marsh E, Yin P, et al. Promoter methylation regulates estrogen receptor 2 in human endometrium and endometriosis. *Biol Reprod* (2007) 77(4):681–7. doi: 10.1095/biolreprod.107.061804

21. Xue Q, Lin Z, Yin P, Milad MP, Cheng YH, Confino E, et al. Transcriptional activation of steroidogenic factor-1 by hypomethylation of the 5' CpG island in endometriosis. *J Clin Endocrinol Metab* (2007) 92(8):3261–7. doi: 10.1210/jc.2007-0494

22. van Kaam KJ, Delvoux B, Romano A, D'Hooghe T, Dunselman GA, Groothuis PG. Deoxyribonucleic acid methyltransferases and methyl-CpG-binding domain proteins in human endometrium and endometriosis. *Fertil Steril* (2011) 95(4):1421–7. doi: 10.1016/j.fertnstert.2011.01.031

23. Matsuzaki S, Canis M, Pouly JL, Wattiez A, Okamura K, Mage G. Cyclooxygenase-2 expression in deep endometriosis and matched eutopic endometrium. *Fertil Steril* (2004) 82(5):1309–15. doi: 10.1016/j.fertnstert.2004.03.059

24. Dyson MT, Roqueiro D, Monsivais D, Ercan CM, Pavone ME, Brooks DC, et al. Genome-wide DNA methylation analysis predicts an epigenetic switch for GATA factor expression in endometriosis. *PloS Genet* (2014) 10(3):e1004158. doi: 10.1371/journal.pgen.1004158

25. Ren F, Wang DB, Li T, Chen YH, Li Y. Identification of differentially methylated genes in the malignant transformation of ovarian endometriosis. *J Ovarian Res* (2014) 7:73. doi: 10.1186/1757-2215-7-73

26. Senthong A, Kitkumthorn N, Rattanatanyong P, Khemapech N, Triratanachart S, Mutirangura A. Differences in LINE-1 methylation between endometriotic ovarian cyst and endometriosis-associated ovarian cancer. *Int J Gynecol Cancer* (2014) 24 (1):36–42. doi: 10.1097/IGC.0000000000000021

27. Penna I, Du H, Ferriani R, Taylor HS. Calpain5 expression is decreased in endometriosis and regulated by HOXA10 in human endometrial cells. *Mol Hum Reprod* (2008) 14(10):613–8. doi: 10.1093/molehr/gan055

28. Salvesen HB, MacDonald N, Ryan A, Jacobs IJ, Lynch ED, Akslen LA, et al. PTEN methylation is associated with advanced stage and microsatellite instability in endometrial carcinoma. *Int J Cancer* (2001) 91(1):22–6. doi: 10.1002/1097-0215(20010101)91:1<22::aid-ijc1002>3.0.co;2-s

29. Tam KF, Liu VW, Liu SS, Tsang PC, Cheung AN, Yip AM, et al. Methylation profile in benign, borderline and malignant ovarian tumors. *J Cancer Res Clin Oncol* (2007) 133(5):331–41. doi: 10.1007/s00432-006-0178-5

30. Heidarpour M, Derakhshan M, Derakhshan-Horeh M, Kheirollahi M, Dashti S. Prevalence of high-risk human papillomavirus infection in women with ovarian endometriosis. *J Obstet Gynaecol Res* (2017) 43(1):135–9. doi: 10.1111/jog.13188

31. Oppelt P, Renner SP, Strick R, Valletta D, Mehlhorn G, Fasching PA, et al. Correlation of high-risk human papilloma viruses but not of herpes viruses or chlamydia trachomatis with endometriosis lesions. *Fertil Steril* (2010) 93(6):1778–86. doi: 10.1016/j.fertnstert.2008.12.061

32. Esfandyari S, Aleyasin A, Noroozi Z, Taheri M, Khodarahmian M, Eslami M, et al. The protective effect of sulforaphane against oxidative stress through activation of NRF2/ARE pathway in human granulosa cells. *Cell J* (2021) 23(6):692–700. doi: 10.22074/cellj.2021.7393

33. Zhang H, Wu J, Li Y, Jin G, Tian Y, Kang S. Identification of key differentially Methylated/Expressed genes and pathways for ovarian endometriosis by bioinformatics analysis. *Reprod Sci* (2022) 29(5):1630–43. doi: 10.1007/s43032-021-00751-8

34. Coudyzer P, Lemoine P, Po C, Jordan BF, van der Smissen P, Courtoy PJ, et al. Induction of post-menstrual regeneration by ovarian steroid withdrawal in the functionalis of xenografted human endometrium. *Hum Reprod* (2015) 30(5):1156–68. doi: 10.1093/humrep/dev043

35. Tang FH, Chang WA, Tsai EM, Tsai MJ, Kuo PL. Investigating novel genes potentially involved in endometrial adenocarcinoma using next-generation sequencing and bioinformatic approaches. *Int J Med Sci* (2019) 16(10):1338–48. doi: 10.7150/ijms.38219

36. Sinreih M, Anko M, Kene NH, Kocbek V, Rizner TL. Expression of AKR1B1, AKR1C3 and other genes of prostaglandin F2alpha biosynthesis and action in ovarian endometriosis tissue and in model cell lines. *Chemico-biological Interact* (2015) 234:320–31. doi: 10.1016/j.cbi.2014.11.009

37. Cobellis L, Razzi S, De Simone S, Sartini A, Fava A, Danero S, et al. The treatment with a COX-2 specific inhibitor is effective in the management of pain related to endometriosis. *Eur J Obstet Gynecol Reprod Biol* (2004) 116(1):100–2. doi: 10.1016/j.ejogrb.2004.02.007

38. Li B, Liu L, Fu X, Zhou WQ, Zou DT, Zhao XY, et al. A novel mutation of estrogen receptor gene detected in girls with precocious puberty. *Yi Chuan Xue Bao* (2005) 32(10):1011–7.

39. Lamp M, Peters M, Reinmaa E, Haller-Kikkatalo K, Kaart T, Kadastik U, et al. Polymorphisms in ESR1, ESR2 and HSD17B1 genes are associated with fertility status in endometriosis. *Gynecol Endocrinol* (2011) 27(6):425–33. doi: 10.3109/09513590.2010.495434

40. Lebeau A, Grob T, Holst F, Seyedi-Fazlollahi N, Moch H, Terracciano L, et al. Oestrogen receptor gene (ESR1) amplification is frequent in endometrial carcinoma and its precursor lesions. *J Pathol* (2008) 216(2):151–7. doi: 10.1002/path.2405

41. Fang KH, Kao HK, Chi LM, Liang Y, Liu SC, Hseuh C, et al. Overexpression of BST2 is associated with nodal metastasis and poorer prognosis in oral cavity cancer. *Laryngoscope* (2014) 124(9):E354–60. doi: 10.1002/lary.24700

frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 12 May 2023
DOI 10.3389/fendo.2023.1084927

OPEN ACCESS

EDITED BY
Mainak Dutta,
Birla Institute of Technology and Science, United Arab Emirates

REVIEWED BY
Shalem Leemaqz,
Flinders University, Australia
Alice Albu,
Carol Davila University of Medicine and Pharmacy, Romania

*CORRESPONDENCE
Lei Jin
leijintongjih@qq.com
Fei Li
1151877287@qq.com

RECEIVED 31 October 2022
ACCEPTED 01 May 2023
PUBLISHED 12 May 2023

CITATION
Liu S, Guo Y, Li F and Jin L (2023) Influence of ovarian reserves on assisted reproductive and perinatal outcomes in patients with endometriosis: a retrospective study.
Front. Endocrinol. 14:1084927.
doi: 10.3389/fendo.2023.1084927

Influence of ovarian reserves on assisted reproductive and perinatal outcomes in patients with endometriosis: a retrospective study

Shuai Liu, Yaxin Guo, Fei Li* and Lei Jin*

Reproductive Medicine Center, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China

Objective: To investigate the association between different ovarian reserves and reproductive and adverse perinatal outcomes in patients with endometriosis.

Design: Retrospective study.

Setting: Reproductive Medicine Center in a hospital.

Patients: Patients surgically diagnosed with endometriosis were divided into three groups according to their ovarian reserve: diminished ovarian reserve (DOR) group (n=66), normal ovarian reserve (NOR) group (n=160), and high ovarian reserve (HOR) group (n=141).

Intervention(s): None.

Main Outcome Measures: Live birth rate (LBR), cumulative live birth rate (CLBR), and adverse perinatal outcome for singleton live births.

Results: There were significantly higher live birth and cumulative live birth rates in endometriosis patients with NOR or HOR than in those with DOR. For adverse perinatal outcomes, patients with NOR or HOR had no significant association with preterm birth, gestational hypertension, placenta previa, fetal malformation, abruptio placentae, macrosomia, or low birth weight, except for a decreased risk of gestational diabetes mellitus.

Conclusion: Our study revealed that although patients with endometriosis with NOR and HOR had increased reproductive outcomes, patients with endometriosis with DOR had still an acceptable live birth rate and a similar cumulative live birth rate with available oocytes. Moreover, patients with NOR and HOR might not exhibit a decreased risk of abnormal perinatal outcomes, except for gestational diabetes mellitus. Multicenter prospective studies are needed to further clarify the relationship.

KEYWORDS

endometriosis, assisted reproduction, diminished ovarian reserve, live birth rate, cumulative live birth rate, abnormal perinatal outcome

1 Introduction

Ovarian reserve is defined as the quantity and quality of follicles within the ovary. Numerous markers, such as age, baseline antral follicle count (AFC), follicle stimulating hormone (FSH) level, and anti-Müllerian hormone (AMH) level, have been evaluated to assess ovarian reserve and predict ovarian response and reproductive potential (1).

Endometriosis is an estrogen-dependent disease in women of reproductive age that is characterized by the presence of endometrial-like tissue outside the uterine cavity (2). Endometriosis affects approximately 10% of women of reproductive age, and its prevalence among infertile women is 5–50% (3). On the one hand, patients with endometriosis often have a high risk of obstetric complications (4). A recent study found that endometriosis may adversely affect perinatal outcomes, especially due to increased risk of placenta abruption and operative delivery (5). On the other hand, the cause of infertility in women with endometriosis is multifactorial, and diminished ovarian reserve (DOR) is of major concern in women with endometriosis-associated infertility (6). Cystectomy and surgery for endometriosis, as well as the endometriomas themselves, may cause DOR. Usually, DOR results in a decreased fecundability, along with a reduction in oocyte quantity and a decrease in oocyte quality (7). Patients with DOR also have a high risk of obstetric complications. DOR, specifically defined as an AFC of six or less, is associated with a higher incidence of preeclampsia and multiple placental fetal vascular lesions (8). Study also found that younger women with low prognosis and normal ovarian reserve have a higher probability for live births and better perinatal outcomes compared with older women with poor or normal ovarian reserve (9). Nevertheless, Sunkara et al. (10) found an increased risk of adverse obstetric outcomes among women with excessive ovarian response while no increased risk among women with poor ovarian response. Whether the ovarian reserve influences perinatal outcomes in patients with endometriosis remains unclear. Therefore, it is important to determine whether there are differences in perinatal outcomes in patients with endometriosis with different ovarian reserves who become pregnant using assisted reproductive technology (ART).

In this study, we evaluated the risk of abnormal perinatal outcomes in patients with endometriosis with normal ovarian reserve (NOR) or high ovarian reserve (HOR) compared with DOR.

2 Materials and methods

2.1 Patients

We retrospectively reviewed patients who underwent their first fresh cycle of *in vitro* fertilization (IVF) or intracytoplasmic sperm injection (ICSI) between January 1, 2016 and December 12, 2019 at the Reproductive Medicine Center of Tongji Hospital, Tongji Medical College of Huazhong University of Science and Technology (Wuhan, China). This study was approved by the ethical committee of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology (TJ-IRB20220119). Patients were surgically diagnosed with endometriosis (laparotomy or laparoscopy) and histologically confirmed from biopsies. Patients with endometrial cysts in the ovaries and adenomyosis were included in the study.

Patients were excluded from the study if they met any of the following exclusion criteria: 1) polycystic ovary syndrome; 2) endocrine diseases; 3) donated oocyte; 4) hypertension; 5) autoimmune disease.

Patients were divided into three groups by their ovarian reserve according to a clinical guideline (11): DOR group: age > 35 years or AMH < 1.1 or AFC < 5 or FSH > 12; NOR group: age ≤ 35 years and 1.1 ≤ AMH ≤ 4.5, and 7 ≤ AFC ≤14 and FSH ≤ 10; HOR group: age ≤ 35 years and AMH > 4.5 or AFC > 20.

2.2 Controlled ovarian stimulation protocol

The controlled ovarian stimulation(COS) protocol was individually selected according to ovarian reserve testing and other characteristics of GnRH agonist (GnRH-AGO) or antagonist (GnRH-ANTA) treatment. Human chorionic gonadotropin (hCG) (10,000 IU, EMD Serono) was used to trigger ovulation when one or two leading follicles attained a mean diameter of 18 mm. Transvaginal ultrasound-guided oocyte retrieval was conducted 34–36 h after hCG administration. The oocyte maturation rate was defined as the number of MII oocytes divided by the number of retrieved oocytes. Normal fertilization was defined as 2PN. The normal fertilization rate was defined as the number of 2PN divided by the number of retrieved oocytes in *in vitro* fertilization (IVF) or 2PN divided by the number of MII in ICSI. All embryos were checked on the morning of Day 3 after oocyte retrieval. Fewer than two embryos of the best quality were selected for transfer on Day 3.

2.3 Main outcome measures

We collected data on maternal age, follicle-stimulating hormone (FSH) level, body mass index (BMI), AFC, AMH level, duration of infertility, and type of endometriosis.

The COS outcomes included endometrial thickness, no. of retrieved oocytes, MII oocytes, 2PN zygotes, oocyte maturation rate, and normal fertilization rate.

The ART outcomes included biochemical pregnancy, clinical pregnancy, pregnancy loss, live birth, cumulative pregnancy, and cumulative live birth. Biochemical pregnancy was defined as a pregnancy when a woman has a positive pregnancy test, but no gestational sac can be visualized by ultrasound. Clinical pregnancy was defined as viable intrauterine pregnancy (gestational sac with fetal heart activity) confirmed by ultrasound. Clinical pregnancies and live births were calculated according to the first fresh transfer cycle. Cumulative pregnancy and live birth were calculated by the first fresh transfer cycle and subsequent frozen cycles until live birth, or all embryos were used. Women without a live birth by December 31, 2019 were considered as non-live births as a conservative estimate.

The adverse singleton perinatal outcomes included preterm birth, placenta previa, fetal malformation, gestational hypertension, gestational diabetes mellitus, low birth weight (< 2,500 g), and macrosomia (> 4,000 g).

2.4 Statistical analysis

Continuous data are presented as means ± standard deviations (SDs), and categorical data are presented as percentages (%). Continuous variables, with normal distribution and homogeneity of variance, were compared using the one-way analysis of variance test while continuous variables with non-normal or heterogeneity, were compared using the Kruskal–Wallis test. Categorical variables were compared using the chi-square test, Bonferroni correction was applied to all multiple comparisons, and subgroup analysis was performed with Cochran-Mantel-Haenszel test with common odds ratios calculated. The relationships among variables, subsequent clinical pregnancy, and live birth were assessed using binomial logistic regression analysis with enter method, and the odds ratio (OR) with 95% confidence intervals (CI) was calculated. A two-tailed P<0.05 was considered statistically significant. All analyses were performed using the Statistical Package for Social Sciences (SPSS version 26.0, IBM Corp, Armonk, NY, USA).

3 Results

3.1 Baseline characteristics of patients, and COS and ART outcomes

A total of 367 participants were included in the study during their first fresh cycle. When subdividing cycles according to ovarian reserve DOR (n=66), NOR (n=160), and HOR (n=141), ovarian reserve increased with decreasing age and FSH and increasing AMH levels. AFC significantly increased from DOR to NOR to HOR. The duration of infertility and BMI were comparable among the three groups. GnRH protocols differ significantly among DOR, NOR, and HOR. The detailed baseline characteristics and COS outcomes can be seen in the Table 1.

Regarding the COS and ART outcomes, there were no significant differences regarding endometrial thickness, normal fertilization rate, biochemical pregnancy, and pregnancy loss among the three groups. The number of retrieved oocytes; no. of MII and no. of 2PN were significantly increased in the HOR group compared with those in the DOR and NOR groups. The oocyte maturation rate was significantly lower in the HOR group than in the DOR group (P= 0.044). Clinical pregnancy and cumulative pregnancy increased with increasing ovarian reserve from DOR to NOR to HOR and exhibited a significant difference between DOR and HOR (P=0.015), whereas live birth and cumulative live birth increased with increasing ovarian reserve from DOR to NOR to HOR with significant differences between DOR and NOR (P=0.027), DOR and HOR (P<0.001) in live birth and DOR and NOR (P=0.048), DOR and HOR (P<0.001) in cumulative live birth. The single live birth was significantly lower in DOR group than in HOR group (P=0.012). The multiple live birth, single and multiple cumulative live birth were comparable among the three groups.

Binary logistic regression analysis was performed to evaluate the effects of ovarian reserve, COS protocols, and type of endometriosis on clinical pregnancy, live births, cumulative pregnancy, and cumulative live births in women with endometriosis. The results in Table 2 indicate that ovarian reserve was significantly associated with clinical pregnancy between HOR and DOR group (P=0.013, OR=2.276, 95%CI: 1.190~4.352) and comparable between NOR and DOR group (P>0.05). For live birth, ovarian reserve was significantly associated between NOR and DOR (P=0.031, OR=2.004 95%CI 1.068~3.763), HOR and DOR group (P=0.002, OR=2.874 95%CI 1.489~5.547). For cumulative pregnancy, ovarian reserve was significantly associated with cumulative pregnancy between HOR and DOR group (P=0.037, OR=2.225, 95%CI: 1.051~4.709) and comparable between NOR and DOR group (P>0.05), while patients with adenomyosis was significantly associated with cumulative pregnancy compared with those with endometriosis cysts (P=0.047, OR=0.463 95%CI 0.217~0.989). For cumulative live birth, ovarian reserve was significantly associated between HOR and DOR group (P=0.020, OR=2.266, 95%CI: 1.140~4.507) and comparable between NOR and DOR group (P>0.05). Other characteristics did not suggest any significant variation in the model.

Subgroup analysis with Cochran-Mantel-Haenszel test was performed by stratifying the women into GnRH-AGO and GnRH-ANTA groups. Figure 1 shows that in the GnRH-AGO group, clinical pregnancy, live birth, cumulative pregnancy, and cumulative live birth were comparable among the ovarian reserve groups. In the GnRH-ANTA group, live births were significantly lower in the DOR group (21.4%) than in the NOR group (60%), with a common odds ratio= 2.024(P=0.025) and homogeneity of the Odds Ratio(P>0.05), whereas clinical pregnancy, cumulative pregnancy, and cumulative live births were similar among the three groups.

3.2 Singleton perinatal outcomes

We then investigated the effect of ovarian reserve on singleton perinatal outcomes. 37 patients achieved cumulative live births with 29 singleton live births in the DOR group, among 116 patients in the NOR group, 84 had singleton live births, and among 112 patients in the HOR group, 86 had singleton live births. Singleton perinatal outcomes included abnormal outcomes are reported in Table 3. There were significant differences regarding gestational diabetes mellitus between DOR and NOR group (P=0.039), DOR and HOR group(P=0.015), but no significant differences were observed in the remaining outcomes.

For abnormal perinatal outcome, binary logistic regression analysis was conducted for ovarian reserve, type of endometriosis, and COS protocol. The correlation was that ovarian reserve had a significant association with the risk of gestational diabetes mellitus, and the NOR (7.1%) (OR: 0.199, 95% CI: 0.057–0.697, P=0.012) and HOR (5.8%) (OR: 0.151, 95% CI: 0.040–0.568, P=0.005) groups had a decreased risk of gestational diabetes mellitus when compared with the DOR group (24.1%) (OR: 1.000). No association was observed with the remaining abnormal perinatal outcomes. The risks of low birth weight, macrosomia, preterm birth, gestational hypertension, placenta previa, fetal malformation, and abruptio placentae were similar among the three groups.

10.3389/fendo.2023.1084927

TABLE 1 Baseline characteristics and COS and ART outcomes.

Variable		GROUP			P value	
	DOR N=66	NOR N=160	HOR N=141	D VS N	D VS H	N VS H
Age, y	33.24 ± 4.22	29.48 ± 2.88	29.33 ± 2.77			
FSH, IU/L	8.48 ± 2.65	7.45 ± 1.51	7.01 ± 1.82			
AFC	7.77 ± 4.76	9.52 ± 2.00	15.53 ± 4.67			
AMH, ng/ml	2.49 ± 2.33	2.77 ± 0.92	7.38 ± 2.91			
BMI, kg/m^2	20.94 ± 2.49	21.46 ± 2.50	20.95 ± 2.22	ns	ns	ns
GnRH protocol						
antagonist	28(42.4%)	20(12.5%)	9(6.4%)	<0.001	<0.001	ns
agonist	38(57.6%)	140(87.5%)	132(93.6%)	<0.001	<0.001	ns
Duration of infertility, y	3.29 ± 3.11	3.21 ± 2.10	3.02 ± 1.93	ns	ns	ns
Type of endometriosis						
endometriosis cysts	48(72.7%)	138(86.3%)	126(89.4%)	ns	ns	ns
adenomyosis	13(19.7%)	12(7.5%)	11(9.8%)	0.024	0.039	ns
cysts co-occurrence with adenomyosis	5(7.6%)	10(6.3%)	4(2.8%)	ns	ns	ns
Endometrial thickness, mm	11.76 ± 2.62	12.54 ± 2.71	12.24 ± 2.38	ns	ns	ns
No. of oocytes retrieved	8.39 ± 4.50	9.73 ± 4.48	13.94 ± 4.20	ns	<0.001	<0.001
No. of MII	7.76 ± 4.32	8.69 ± 4.24	12.20 ± 4.04	ns	<0.001	<0.001
Oocyte maturation rate	0.92 ± 0.10	0.89 ± 0.12	0.88 ± 0.13	ns	0.044	ns
No. of 2PN	5.38 ± 3.32	6.13 ± 3.42	8.40 ± 3.43	ns	<0.001	<0.001
Normal fertilization rate	0.66 ± 0.22	0.66 ± 0.21	0.64 ± 0.18	ns	ns	ns
Biochemical pregnancy	5(7.6%)	8(5.0%)	5(3.5%)	ns	ns	ns
Pregnancy loss	10(15.2%)	12(7.5)	10(7.1%)	ns	ns	ns
Clinical pregnancy	32(48.5%)	96(60%)	97(68.8%)	ns	0.015	ns
Live birth	22(33.3%)	84(52.5%)	87(61.7%)	0.027	<0.001	ns
single	17(25.8%)	59(36.9%)	66(46.8%)	ns	0.012	ns
multiple	5(7.6%)	25(15.6%)	21(14.9%)	ns	ns	ns
Cumulative pregnancy	44(66.7%)	129(80.6%)	120(85.1%)	ns	0.006	ns
Cumulative live birth	37(56.1%)	116(72.5%)	112(79.4%)	0.048	<0.001	ns
single	29(43.9%)	84(52.5%)	86(61.0%)	ns	ns	ns
multiple	8(12.1%)	32(20.0%)	26(18.4%)	ns	ns	ns

Continuous data are reported as mean ± standard deviation. Categorical data are reported as n (%). One-way analysis of variance test was used for the continuous data, and chi-square test was used for categorical data. P<0.05 was considered statistically significant. ns, not statistically significant; DOR, diminished ovarian reserve; NOR, normal ovarian reserve; HOR, high ovarian reserve; AFC, antral follicle count, FSH, follicle stimulating hormone; AMH, anti-Müllerian hormone.

4 Discussion

This study aimed to explore the relationship between reproductive and perinatal outcomes and ovarian reserve in patients with endometriosis. The results showed that patients with endometriosis with NOR and HOR had increased reproductive outcomes compared to patients with DOR, but the reproductive outcomes in patients with DOR were still acceptable. Regarding adverse perinatal outcomes, patients with NOR and HOR had a decreased risk of gestational diabetes mellitus compared to patients with DOR. Patients with DOR and HOR did not exhibit a higher risk of abnormal perinatal outcomes in the remaining outcomes.

Ovarian reserve testing, which is closely associated with reproductive outcomes, is a useful option for physicians to assess ovarian reserve. Diminished ovarian reserve is defined as decreased oocyte quality, quantity, or reproductive potential, resulting in infertility (12). In our study, we found that the reproductive

10.3389/fendo.2023.1084927

TABLE 2 Binary logistic regression analysis on reproductive outcomes.

Reproductive outcome	variable	P	ORs	95% C.I.	
				Lower	Upper
Clinical pregnancy	GnRH protocol				
	antagonist		1.000		
	agonist	0.845	1.064	0.573	1.976
	ovarian reserve	0.039			
	DOR		1.000		
	NOR	0.164	1.544	0.837	2.849
	HOR	0.013	2.276	1.190	4.352
	Type of endometriosis	0.875			
	endometriosis cysts		1.000		
	adenomyosis	0.724	0.879	0.430	1.797
	co-occurrence	0.728	1.189	0.447	3.162
	Constant	0.791	0.919		
Live birth	GnRH protocol				
	antagonist		1.000		
	agonist	0.296	1.396	0.746	2.610
	ovarian reserve	0.007			
	DOR		1.000		
	NOR	0.031	2.004	1.068	3.763
	HOR	0.002	2.874	1.489	5.547
	Type of endometriosis	0.977			
	endometriosis cysts		1.000		
	adenomyosis	0.925	0.966	0.471	1.983
	co-occurrence	0.851	1.096	0.422	2.849
	Constant	0.008	0.411		
Cumulative pregnancy	GnRH protocol				
	antagonist		1.000		
	agonist	0.215	1.546	0.777	3.078
	ovarian reserve	0.111			
	DOR		1.000		
	NOR	0.148	1.664	0.835	3.315
	HOR	0.037	2.225	1.051	4.709
	Type of endometriosis	0.138			
	endometriosis cysts		1.000		
	adenomyosis	0.047	0.463	0.217	0.989
	co-occurrence	0.844	0.890	0.278	2.847
	Constant	0.068	1.860		
Cumulative live birth	GnRH protocol				
	antagonist		1.000		

(Continued)

10.3389/fendo.2023.1084927

TABLE 2 Continued

Reproductive outcome	variable	P	ORs	95% C.I.	
				Lower	Upper
	agonist	0.075	1.780	0.943	3.358
	ovarian reserve	0.065			
	DOR		1.000		
	NOR	0.144	1.609	0.850	3.045
	HOR	0.020	2.266	1.140	4.507
	Type of endometriosis	0.179			
	endometriosis cysts		1.000		
	adenomyosis	0.067	0.507	0.245	1.049
	co-occurrence	0.638	0.782	0.281	2.178
	Constant	0.826	1.073		

Odds ratios (ORs) and 95% confidence intervals (CIs) are based on the logistic regression analysis. OR and P value for ovarian reserve, GnRH protocol, and type of endometriosis using a binary logistic regression analysis for each reproductive outcome. P<0.05 was considered statistically significant. DOR, diminished ovarian reserve; NOR, normal ovarian reserve; HOR, high ovarian reserve.

outcomes were increased in patients with endometriosis with NOR or HOR than in those with DOR. Nevertheless, patients with endometriosis with DOR could still have an acceptable live birth rate (33.3%) and a cumulative live birth rate (56.1%) with available oocytes. Moreover, live birth was significantly increased in patients with NOR than in those with DOR choosing GnRH-ANTA, and no significant difference was found in clinical pregnancy, cumulative clinical pregnancy, and cumulative live birth among patients with different ovarian reserve with GnRH-ANTA or GnRH-AGO. This result indicated that in fresh embryo transfer cycles, patients with endometriosis with NOR would have a better live birth rate than those with DOR, and with sufficient available oocytes, these patients could reach a similar cumulative live birth rate in subsequent frozen cycles. A previous study also revealed that for patients with endometriosis with DOR, the GnRH-AGO protocol may achieve better clinical IVF-ET outcomes (13). However, another study reported that when combined with the frozen-embryo transfer strategy, the GnRH-ANTA protocol had comparable clinical pregnancy outcomes as the GnRH-AGO protocol in patients with DOR. Cohen et al. (14) found that women with diminished ovarian

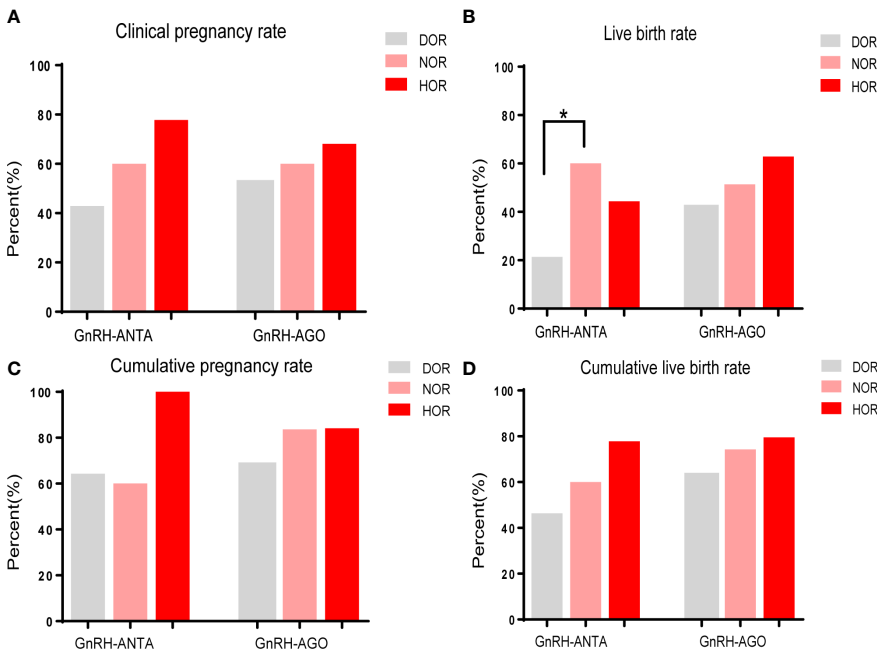


FIGURE 1
Subgroup analysis for reproductive outcomes among different ovarian reserve group by stratifying the women into GnRH-AGO and GnRH-ANTA subgroups: **(A)** Clinical pregnancy rate. **(B)** Live birth rate. **(C)** Cumulative pregnancy rate. **(D)** Cumulative live birth rate.

10.3389/fendo.2023.1084927

TABLE 3 Singleton perinatal outcomes.

Variable		GROUP			P value	
	DOR N=29	NOR N=84	HOR N=86	D VS N	D VS H	N VS H
Age, y	32.72 ± 3.95	29.71 ± 2.65	29.33 ± 3.20			
FSH, IU/L	8.14 ± 2.69	7.28 ± 1.58	6.88 ± 1.48			
AFC	8.10 ± 5.13	9.56 ± 1.98	15.46 ± 4.66			
AMH, ng/mL	2.59 ± 2.63	2.73 ± 0.90	7.67 ± 3.17			
BMI, kg/m^2	20.93 ± 2.52	21.61 ± 2.44	21.02 ± 2.18	ns	ns	ns
Type of endometriosis						
endometriosis cysts	22(75.9%)	73(86.9%)	80(93.0%)	ns	ns	ns
adenomyosis	6(20.7%)	10(11.9%)	4(4.7%)	ns	ns	ns
cysts co-occurrence with adenomyosis	1(3.4%)	1(1.2%)	2(2.3%)	ns	ns	ns
Gestational age, d	271.83 ± 8.89	270.46 ± 12.23	271.28 ± 9.44	ns	ns	ns
Birth weight, g	3356.55 ± 403.96	3281.67 ± 513.29	3283.20 ± 483.24	ns	ns	ns
Delivery mode						
Natural labor	23(79.3%)	66(78.6%)	58(67.4%)	ns	ns	ns
Cesarean delivery	6(20.7%)	18(21.4%)	28(32.6%)	ns	ns	ns
Gender						
Male	18(62.1%)	45(53.6%)	45(52.3%)	ns	ns	ns
Female	11(37.9%)	39(46.4%)	41(47.7%)	ns	ns	ns
Abnormal perinatal outcomes						
Preterm birth < 37 week	2(6.9%)	9(10.7%)	9(10.5%)	ns	ns	ns
Gestational hypertension	0	3(3.6%)	4(4.7%)	ns	ns	ns
Gestational diabetes mellitus	7(24.1%)	6(7.1%)	5(5.8%)	0.039	0.015	ns
Placenta previa	4(13.8%)	8(9.5%)	5(5.8%)	ns	ns	ns
Fetal malformation	0	0	4(4.7%)	ns	ns	ns
Abruptio placentae	0	3(3.6%)	0	ns	ns	ns
Macrosomia > 4,000 g	2(6.9%)	3(3.6%)	4(4.7%)	ns	ns	ns
Low birth weight < 2,500 g	0	7(8.3%)	4(4.7%)	ns	ns	ns

Continuous data are reported as mean ± standard deviation. Categorical data are reported as n (%). One-way analysis of variance test was used for the continuous data, and chi-square test was used for categorical data. P<0.05 was considered statistically significant. ns, not statistically significant; DOR, diminished ovarian reserve; NOR, normal ovarian reserve; HOR, high ovarian reserve; AFC, antral follicle count, FSH, follicle stimulating hormone; AMH, anti-Müllerian hormone.

reserves have low live birth rates after the first IVF-ICSI cycle. Yu Deng et al. (15) also found no statistically significant differences in the cumulative live birth rate in women with DOR and endometriosis. Van Rooij et al. (16) reported that when patients were defined as poor responders based on the number of AFC, the results of the comparison of poor and normal responders were similar because they could also have oocytes available. In our COS outcomes, we found that the oocyte maturation rate and normal fertilization rate were similar between the DOR and NOR groups, indicating that patients with endometriosis with DOR might still have available oocytes. Finally, with similar percentages of available oocytes, endometriosis patients with DOR achieved an acceptable cumulative live birth rate.

Another clinically relevant finding is the singleton perinatal outcome. In a recent study, women with endometriosis had an increased risk of adverse perinatal outcomes compared to those with other reproductive diseases, including miscarriage, preeclampsia, gestational hypertension, preterm deliveries, placenta previa, and caesarean section (17–21). A previous study found that women with high ovarian response had a higher risk of adverse obstetric outcomes of preterm deliveries and low birth weight (10). However, our study found that the risks of preterm birth, gestational hypertension, placenta previa, fetal malformation, abruptio placentae, macrosomia, and low birth weight did not differ significantly between patients with endometriosis with NOR or HOR and those with DOR. The risk of gestational diabetes mellitus

was extremely low in the NOR and HOR group than DOR group. One possible explanation is that women with infertility may be at greater risk for gestational diabetes mellitus overall, and the risk increases with age (22, 23). Patients in the NOR and HOR group were younger, which might therefore exhibit decreased risk of developing gestational diabetes mellitus than DOR group. Therefore, we found that endometriosis is associated with DOR, affecting quantity, but not embryo quality, and would not impact subsequent abnormal perinatal outcomes.

To our knowledge, this is the first study to evaluate abnormal perinatal outcomes in patients with endometriosis with different ovarian reserves. Our study has several limitations. As a retrospective study, although we corrected for several known confounders, the potential for unrecognized confounders still remains. Due to the more frequent application of GnRH-AGO protocol than GnRH-ANTA protocol in infertility females with endometriosis in China, and the strict inclusion and exclusion criteria, the sample size in the GnRH-ANTA group was relatively small, further prospective study with large sample size was necessary. Moreover, our results were based only on patients in their first fresh cycle, the effect of frozen-thawed cycle on perinatal outcomes need further investigation.

In conclusion, our study revealed that although patients with endometriosis with NOR and HOR had increased reproductive outcomes, patients with endometriosis with DOR had still an acceptable live birth rate and a similar cumulative live birth rate with available oocytes. Moreover, patients with NOR and HOR might not exhibit a decreased risk of abnormal perinatal outcomes, except for gestational diabetes mellitus. Multicenter prospective studies are needed to further clarify the relationship.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the ethical committee of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

LJ and FL conceived of and designed the study. SL and YG collected the data. SL analyzed the data and wrote the paper. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by the National Natural Science Foundation of China (No.81701521 to FL) and the National Key Research & Development Program of China (2021YFC2700603).

Acknowledgments

We acknowledge the contributions of whole staff of our reproductive medicine center and Editage (www.editage.cn) for English language editing.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Practice Committee of the American Society for Reproductive Medicine. Testing and interpreting measures of ovarian reserve: a committee opinion. *Fertil. Steril* (2020) 114(6):1151–7. doi: 10.1016/j.fertnstert.2020.09.134

2. Giudice LC. Endometriosis. *New Engl J Med* (2010) 362(25):2389–98. doi: 10.1056/NEJMcp1000274

3. Shafrir AL, Farland LV, Shah DK, Harris HR, Kvaskoff M, Zondervan K, et al. Risk for and consequences of endometriosis: a critical epidemiologic review. *Best Pract Res Clin obstetrics gynaecol* (2018) 51:1–15. doi: 10.1016/j.bpobgyn.2018.06.001

4. Borisova AV, Konnon SRD, Tosto V, Gerli S, . Radzinsky VE. Obstetrical complications and outcome in patients with endometriosis. *J maternal-fetal neonatal Med* (2022) 35(14):2663–77. doi: 10.1080/14767058.2020.1793326

5. Warzecha D, Pietrzak B, Szymusik I, Smiech Z, . Wielgos M. Should the patients with endometriosis be treated as a risk group of pregnancy complications? single center experience and literature review and literature review. *Ginekol. Pol* (2020) 91(7):383–8. doi: 10.5603/GP.a2020.0084

6. Kitajima M, Khan KN, Harada A, Taniguchi K, Inoue T, Kaneuchi M, et al. Association between ovarian endometrioma and ovarian reserve. *Front bioscience (Elite edition)* (2018) 10(1):92–102. doi: 10.2741/e810

7. Broekmans FJ, Kwee J, Hendriks DJ, Mol BW, Lambalk CB. A systematic review of tests predicting ovarian reserve and IVF outcome. *Hum Reprod Update* (2006) 12 (6):685–718. doi: 10.1093/humupd/dml034

8. Herman HG, Volodarsky-Perel A, Ton Nu TN, Machado-Gedeon A, Cui Y, Shaul J, et al. Diminished ovarian reserve is a risk factor for preeclampsia and placental malperfusion lesions. *Fertil. Steril.* (2023). doi: 10.1016/j.fertnstert.2023.01.029

9. Abdullah RK, Liu N, Zhao Y, Shuang Y, Shen Z, Zeng H, et al. Cumulative live-birth, perinatal and obstetric outcomes for POSEIDON groups after IVF/ICSI cycles: a

single-center retrospective study. *Sci Rep.-UK* (2020) 10(1):11822–2. doi: 10.1038/s41598-020-68896-1

10. Sunkara SK, La Marca A, Seed PT, Khalaf Y. Increased risk of preterm birth and low birthweight with very high number of oocytes following IVF: an analysis of 65 868 singleton live birth outcomes. *Hum Reprod (Oxford England)* (2015) 30(6):1473–80. doi: 10.1093/humrep/dev076

11. Jie QIAO CMJL. A consensus of poor ovarian response. *Reprod Contraception* (2015) 04):211–23. doi: 10.7669/j.issn.0253-357X.2015.04.0211

12. Practice Committee of the American Society for Reproductive Medicine. Testing and interpreting measures of ovarian reserve: a committee opinion. *Fertil. Steril* (2015) 103(3):e9–e17. doi: 10.1016/j.fertnstert.2014.12.093

13. Zhao F, Lan Y, Chen T, Xin Z, Liang Y, Li Y, et al. Live birth rate comparison of three controlled ovarian stimulation protocols for *in vitro* fertilization-embryo transfer in patients with diminished ovarian reserve after endometrioma cystectomy: a retrospective study. *J Ovarian Res* (2020) 13(1):23–3. doi: 10.1186/s13048-020-00622-x

14. Cohen J, Mounsambote L, Prier P, Mathieu d'ARGENT E, Selleret L, Chabbert-Buffet N, et al. Outcomes of first IVF/ICSI in young women with diminished ovarian reserve. *Minerva ginecologica* (2017) 69(4):315–21. doi: 10.23736/S0026-4784.16.04003-X

15. Deng Y, Ou Z, Yin M, Chen Z, Chen S, Sun L. Does current ovarian endometrioma increase the time for DOR patients to reach live birth in IVF? *BMC Pregnancy Childb.* (2022) 22(1):324. doi: 10.1186/s12884-022-04670-7

16. van Rooij IAJ, Bancsi LFJM, Broekmans FJM, Looman CWN, Habbema JDF, te Velde ER. Women older than 40 years of age and those with elevated follicle-stimulating hormone levels differ in poor response rate and embryo quality in *in vitro* fertilization. *Fertil. Steril.* (2003) 79(3):482–8. doi: 10.1016/S0015-0282(02)04839-2

17. Ribot E, Berbis J, Hamouda I, Cohen D, Agostini A, Courbiere B. Pregnancy outcomes after *in vitro* fertilization for moderate and severe endometriosis. a case-control study. *J gynecol obstetrics Hum Reprod* (2022) 51(2):102274. doi: 10.1016/j.jogoh.2021.102274

18. Frincu F, Carp-Veliscu A, Petca A, Badiu D, Bratila E, Cirstoiu M, et al. Maternal-fetal outcomes in women with endometriosis and shared pathogenic mechanisms. *Medicina (Kaunas Lithuania)* (2021) 57(11):1258. doi: 10.3390/medicina57111258

19. Wang J, Zhang J, Qian B. Adverse pregnancy outcomes for women with endometriosis: a systematic review and meta-analysis. *Ginekol. Pol* (2021). doi: 10.5603/GP.a2021.0081

20. Gasparri ML, Nirgianakis K, Taghavi K, Papadia A, Mueller MD. Placenta previa and placental abruption after assisted reproductive technology in patients with endometriosis: a systematic review and meta-analysis. *Arch Gynecol. Obstet.* (2018) 298 (1):27–34. doi: 10.1007/s00404-018-4765-x

21. Pérez-López FR, Villagrasa-Boli P, Muñoz-Olarte M, Morera-Grau Á., Cruz-Andrés P, Hernandez AV. Association between endometriosis and preterm birth in women with spontaneous conception or using assisted reproductive technology: a systematic review and meta-analysis of cohort studies. *Reprod Sci (Thousand Oaks Calif.)* (2018) 25(3):311–9. doi: 10.1177/1933719117749760

22. Farland LV, Prescott J, Sasamoto N, Tobias DK, Gaskins AJ, Stuart JJ, et al. Endometriosis and risk of adverse pregnancy outcomes. *Obstet. Gynecol.* (2019) 134 (3):527–36. doi: 10.1097/AOG.0000000000003410

23. Tobias DK, Chavarro JE, Williams MA, Buck Louis GM, Hu FB, Rich-Edwards J, et al. History of infertility and risk of gestational diabetes mellitus: a prospective analysis of 40,773 pregnancies. *Am J Epidemiol.* (2013) 178(8):1219–25. doi: 10.1093/aje/kwt110

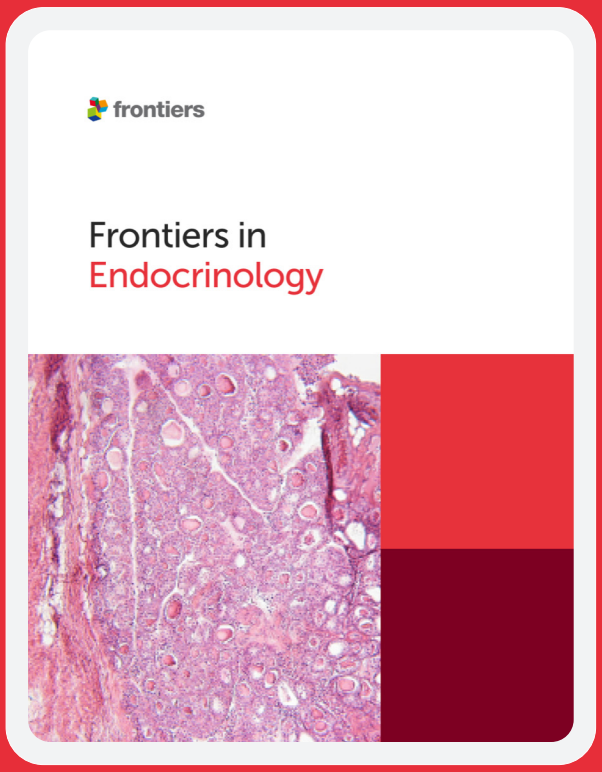
Frontiers in Endocrinology

Explores the endocrine system to find new therapies for key health issues

The second most-cited endocrinology and metabolism journal, which advances our understanding of the endocrine system. It uncovers new therapies for prevalent health issues such as obesity, diabetes, reproduction, and aging.

Discover the latest Research Topics

See more →



Frontiers

Avenue du Tribunal-Fédéral 34
1005 Lausanne, Switzerland
frontiersin.org

Contact us

+41 (0)21 510 17 00
frontiersin.org/about/contact