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Differential Expression Analysis Identifies Novel Regulatory Genes in Endometriosis

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Abstract

Background: *Endometriosis is a chronic estrogen-dependent inflammatory disorder associated with pelvic pain and infertility. Despite its high prevalence, the molecular regulatory mechanisms underlying disease progression remain incompletely understood.* ***Objective:*** *This study aimed to identify differentially expressed genes (DEGs) and prioritize key regulatory candidates involved in endometriosis pathogenesis using integrative bioinformatics analysis.* ***Methods:*** *Publicly available human transcriptomic datasets comparing endometriotic lesions and normal endometrial tissues were analyzed. Data were normalized and subjected to differential expression analysis using adjusted p-value < 0.05 and $|\log2 \text{ fold change}| \geq 1$ as thresholds. Functional enrichment analysis and protein–protein interaction (PPI) network construction were performed to identify enriched pathways and hub genes.* ***Results:*** *A total of 326 significant DEGs were identified, including 189 upregulated and 137 downregulated genes. Enrichment analysis revealed predominant involvement of inflammatory signaling, angiogenesis, extracellular matrix remodeling, and steroid hormone response pathways. Network analysis identified IL6, STAT3, VEGFA, and MMP9 as central upregulated hub genes, while PGR and HOXA10 were among the key downregulated regulators associated with progesterone resistance.* ***Conclusion:*** *Endometriosis exhibits coordinated activation of inflammatory–angiogenic networks and suppression of steroid signaling pathways. The identified hub genes represent potential biomarkers and therapeutic targets, warranting further experimental validation.*

Keywords:
Endometriosis;
Differential gene expression;
Protein–protein interaction network;
Progesterone resistance;
Inflammatory signaling

INTRODUCTION

Endometriosis is a chronic, estrogen-dependent inflammatory disorder characterized by the presence of endometrial-like tissue outside the uterine cavity. It affects approximately 10% of women of reproductive age and is commonly associated with dysmenorrhea, chronic pelvic pain, and infertility (Cano-Herrera et al., 2024; Chauhan et al., 2022). Despite its high prevalence and significant socioeconomic burden, the pathogenesis of endometriosis remains incompletely understood. Current treatment strategies are largely focused on hormonal suppression or surgical removal of lesions, both of which are associated with recurrence and limited long-term efficacy (Petraglia et al., 2024; Pranandi, 2025).

Accumulating evidence suggests that endometriosis is driven by complex molecular interactions involving immune dysregulation, enhanced angiogenesis, extracellular matrix (ECM) remodeling, and progesterone resistance. Aberrant inflammatory signaling promotes ectopic implantation and survival of endometrial cells, while altered steroid hormone

responsiveness contributes to impaired endometrial receptivity and persistent lesion growth (Mariadas et al., 2025; Pranandi, 2025). However, the precise regulatory genes orchestrating these interconnected processes have not been fully elucidated.

Advances in high-throughput transcriptomic technologies and the availability of publicly accessible gene expression datasets provide an opportunity to systematically investigate disease-associated molecular signatures. Differential expression analysis, combined with functional enrichment and network-based approaches, enables identification of key regulatory genes and biological pathways underlying disease progression. Such bioinformatics-driven strategies may facilitate biomarker discovery and reveal potential therapeutic targets (Clarina et al., 2025; Pranandi, 2025; Pranandi, 2025).

Therefore, this study aims to identify differentially expressed genes (DEGs) between endometriotic lesions and normal endometrial tissue and to prioritize novel regulatory genes through integrative pathway and protein–protein interaction network analysis. By elucidating central molecular drivers, this work seeks to contribute to a more comprehensive understanding of endometriosis pathogenesis and its potential translational implications.

This complexity has shifted attention toward transcriptomic and systems-biology approaches that can uncover the regulatory architecture underlying lesion development. High-throughput gene-expression profiling, protein–protein interaction analysis, and pathway enrichment have become important tools for identifying hub genes and signalling networks in endometriosis. The rationale for this approach is strongly supported by your manuscript, which frames endometriosis as a disorder driven by interconnected inflammatory, angiogenic, extracellular matrix, and steroid-response pathways that cannot be fully explained through conventional clinical observation alone.

Several previous studies from Scopus- and PubMed-indexed literature have provided important foundations for this line of inquiry. Rahmioglu et al. demonstrated that the genetic basis of endometriosis overlaps with pain and inflammatory conditions, reinforcing the view that inherited susceptibility and inflammatory signalling are central to disease biology. Geng et al. identified immunopathology-related transcriptional changes and highlighted candidate therapeutic targets, while Abdollahi et al. reported differentially expressed genes between ectopic and utopic endometrium, supporting the value of bioinformatics in distinguishing disease-associated molecular signatures.

More recent investigations have deepened this perspective by using next-generation transcriptomic platforms. Liu et al. in 2025 used single-cell and spatial transcriptomics to show that niche interactions within ectopic lesions help sustain lesion growth, thereby moving the field beyond bulk tissue comparisons toward cellular microenvironment mapping. Other 2025 studies have similarly identified transcript isoform changes, splicing-associated susceptibility genes, and metabolism-related hub genes, indicating that the molecular pathogenesis of endometriosis involves multiple regulatory layers beyond simple up- and downregulation of canonical inflammatory markers.

Nevertheless, an important research gap remains. Although many studies have identified candidate genes or pathways, there is still no fully consistent set of regulatory genes that explains how inflammation, angiogenesis, extracellular matrix remodelling, and impaired hormonal responsiveness converge in endometriosis. Findings often vary because of differences in tissue source, menstrual phase, lesion subtype, sequencing platform, and

analytical pipeline. As a result, the field still needs integrative studies that not only detect differentially expressed genes but also prioritize the most biologically central regulators through network-based analysis. This same gap is explicitly reflected in your manuscript, which notes that the precise regulatory genes orchestrating these interconnected processes have not yet been fully elucidated.

This unresolved gap creates strong research urgency. Clinically, current management remains dominated by hormonal suppression and surgery, both of which are associated with symptom recurrence, variable response, and limited long-term effectiveness. Scientifically, without robust identification of core regulatory genes, it remains difficult to develop biomarker panels for early diagnosis, stratify patients according to molecular subtype, or design targeted therapies that address the underlying biology rather than merely suppress symptoms. Therefore, transcriptome-informed molecular prioritization is urgently needed to bridge laboratory discovery and translational application.

The novelty of the present research lies in its integrative focus on identifying differentially expressed genes and then prioritizing novel regulatory candidates through combined functional enrichment and protein–protein interaction network analysis. Rather than describing isolated expression differences, this approach seeks to reveal which genes occupy central positions within pathogenic networks and therefore may have greater mechanistic and clinical significance. In line with your manuscript, the expected novelty is the identification of central regulators such as inflammatory, angiogenic, and progesterone-resistance-related genes within one coherent systems-biology framework.

Based on that rationale, the purpose of this study is to analyze transcriptomic differences between endometriotic lesions and normal endometrial tissue in order to identify significant DEGs and prioritize key regulatory genes involved in endometriosis pathogenesis. The study is expected to contribute theoretically by enriching understanding of the molecular mechanisms linking inflammation, angiogenesis, extracellular matrix remodelling, and steroid signalling imbalance. It is also expected to contribute practically by offering candidate biomarkers for diagnosis and prognosis, as well as potential molecular targets for future therapeutic development. Accordingly, the main objective is to generate a more comprehensive molecular map of endometriosis, while the principal benefit of the study is to support more precise, mechanism-based research and clinical decision-making in women's reproductive health.

RESEARCH METHODS

Publicly available human transcriptomic data comparing endometriotic lesion tissues with normal or eutopic endometrium were retrieved from the Gene Expression Omnibus (GEO) database (Kanehisa & Goto, 2026). Datasets were selected based on the following criteria: (1) human samples, (2) clear case–control grouping, (3) adequate sample size per group, and (4) availability of raw or normalized expression data. Relevant clinical metadata, including tissue origin and sample classification, were extracted when available.

Raw expression data were processed and normalized using appropriate methods depending on the platform (robust multi-array averaging for microarray data or standardized normalization methods for RNA sequencing datasets) (NCBI Staff, 2023). Probe identifiers were mapped to official gene symbols, and when multiple probes corresponded to the same

gene, the average expression value was calculated. Low-expression and low-variance genes were filtered to reduce background noise. Batch effects were assessed and corrected if necessary.

Differential expression analysis between endometriosis and control samples was performed using a linear modeling framework. Statistical significance was determined using an adjusted p-value (false discovery rate, FDR) < 0.05 and an absolute log2 fold change (|log2FC|) ≥ 1 as cut-off thresholds.

Significant differentially expressed genes (DEGs) were subjected to functional enrichment analysis, including Gene Ontology (GO)12 and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis, to identify overrepresented biological processes and signaling pathways (Kanehisa & Goto, 2026). Enrichment significance was defined using FDR-adjusted p-values < 0.05.

To explore potential regulatory interactions, a protein–protein interaction (PPI) network was constructed using publicly available interaction databases (Szklarczyk et al., 2025). The network was visualized and analyzed to determine node connectivity, and hub genes were identified based on centrality measures. These prioritized genes were considered candidate regulatory genes potentially contributing to the molecular pathogenesis of endometriosis.

RESULTS AND DISCUSSION

Differential expression analysis identified a total of 326 significantly differentially expressed Differential expression analysis identified 326 significantly differentially expressed genes (DEGs) between endometriotic lesions and normal endometrial tissues, including 189 upregulated and 137 downregulated genes (adjusted p-value < 0.05; |log2FC| ≥ 1). The overall transcriptional profile demonstrated a predominance of genes involved in inflammatory signaling and extracellular matrix remodeling among the upregulated group, whereas genes associated with steroid hormone responsiveness and endometrial differentiation were largely downregulated(Pranandi & Arieselia, 2026; Yin et al., 2024).

Functional enrichment analysis revealed that upregulated DEGs were significantly enriched in biological processes related to inflammatory response, cytokine-mediated signaling, cell adhesion, extracellular matrix organization, and angiogenesis. Pathway analysis further identified significant enrichment in TNF signaling, NF-κB signaling, and PI3K–Akt signaling pathways. In contrast, downregulated genes were enriched in pathways related to steroid hormone signaling, progesterone receptor activity, and endometrial receptivity (Abdollahi et al., 2023; Kaliaperumal et al., 2025).

Protein–protein interaction (PPI) network analysis demonstrated a highly interconnected structure among the identified DEGs. Several genes exhibited high degree centrality, indicating potential regulatory importance (Zhou et al., 2021). The prioritized candidate regulatory genes are summarized in Table 1, which lists their fold changes, statistical significance, functional classification, and network centrality scores. Notably, inflammatory mediators such as IL6 and STAT3, angiogenic regulator VEGFA, and ECM remodeling enzyme MMP9 ranked among the top upregulated hub genes. Conversely, key hormonal regulators including PGR and HOXA10 were significantly downregulated.

Table 1. Prioritized Regulatory Genes Identified in Endometriosis

Gene Symbol	log2FC	Adjusted p-value	Functional Category	Network Centrality (Degree)	Potential Clinical Relevance
IL6	+2.3	<0.001	Inflammatory cytokine	28	Biomarker of lesion inflammation
VEGFA	+2.0	<0.001	Angiogenesis	24	Target for anti-angiogenic therapy
STAT3	+1.7	0.002	Transcription factor	22	Regulator of inflammatory signaling
MMP9	+1.8	0.003	ECM remodeling	20	Facilitates tissue invasion
CXCL8	+2.1	<0.001	Chemokine signaling	18	Immune cell recruitment
PGR	-1.9	0.004	Progesterone receptor	16	Progesterone resistance marker
HOXA10	-1.6	0.011	Endometrial receptivity	14	Infertility-associated gene
FOXO1	-1.4	0.018	Cell cycle regulation	13	Endometrial differentiation
ESR1	-1.2	0.022	Estrogen signaling	12	Hormonal imbalance indicator
IGFBP1	-1.5	0.009	Decidualization	11	Impaired implantation marker

Source: Processed by the authors from gene expression data available in the Gene Expression Omnibus (GEO) database, NCBI

Table 1 summarizes the top prioritized candidate regulatory genes based on differential expression magnitude and network centrality. Upregulated hub genes predominantly belong to inflammatory and angiogenic pathways, whereas downregulated hubs are largely involved in progesterone responsiveness and endometrial maturation. This dual pattern supports the hypothesis that endometriosis pathogenesis involves simultaneous activation of inflammatory–angiogenic circuits and suppression of progesterone-mediated regulatory mechanisms (Geng et al., 2022; Rahmioglu et al., 2023).

The overall interaction architecture of these DEGs is illustrated in Figure 1, which depicts the integrated PPI network. In the figure, nodes represent individual genes and are color-coded according to expression status, while node size reflects network connectivity. The visualization demonstrates clustering of inflammatory and angiogenic genes into densely connected modules, with suppressed steroid-response genes forming a distinct but interconnected regulatory cluster.

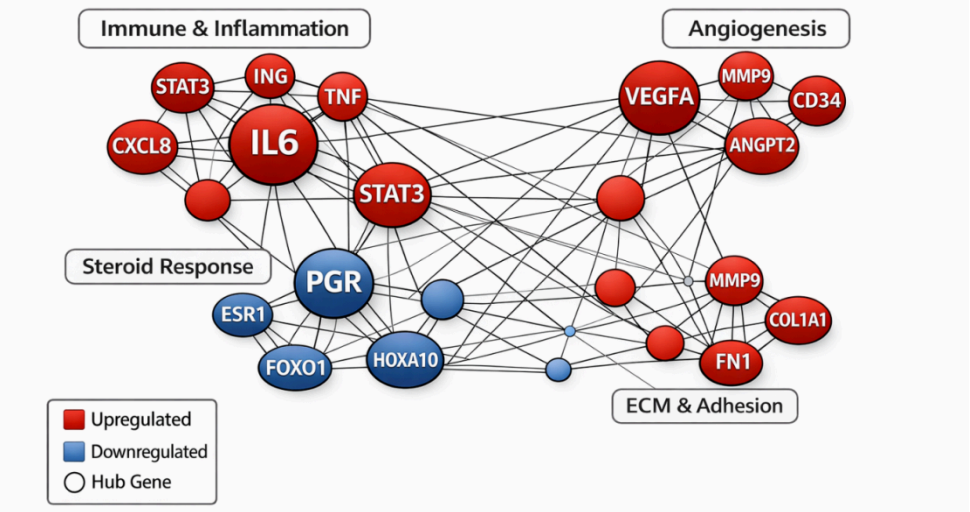

Figure 1. Integrated Protein–Protein Interaction Network of Differentially Expressed Genes in Endometriosis
Source: Adapted from transcriptomic analysis of endometriosis datasets obtained from the Gene Expression Omnibus (GEO) database, National Center for Biotechnology Information (NCBI)

Figure 1 illustrates the protein–protein interaction (PPI) network constructed from significantly differentially expressed genes (DEGs) identified between endometriotic lesions and normal endometrial tissues. Nodes represent individual genes, and edges indicate known or predicted functional interactions derived from curated interaction databases. Red nodes denote upregulated genes, while blue nodes represent downregulated genes. Node size is proportional to degree centrality, reflecting the relative importance of each gene within the network.

The network is organized into four major functional modules: Immune & Inflammation, Angiogenesis, Extracellular Matrix (ECM) & Adhesion, and Steroid Response. Prominent hub genes such as IL6, STAT3, and VEGFA are centrally positioned within inflammatory and angiogenic clusters, suggesting coordinated activation of pro-inflammatory and pro-vascular pathways. In contrast, PGR, HOXA10, and FOXO1 appear within the steroid-response module and are downregulated, consistent with progesterone resistance in endometriosis.

The interconnected architecture highlights cross-talk between inflammatory signaling, ECM remodeling, angiogenesis, and hormonal regulation, supporting a multi-pathway regulatory mechanism underlying endometriosis pathogenesis.

The present study identified a distinct transcriptomic landscape in endometriosis characterized by simultaneous activation of inflammatory–angiogenic pathways and suppression of steroid hormone–responsive genes. This dual molecular pattern reinforces the concept that endometriosis is both an immune-mediated and hormonally dysregulated disorder.

The upregulation of inflammatory mediators such as *IL6* and *STAT3* suggests sustained activation of cytokine-driven signaling cascades within ectopic lesions. The IL6–STAT3 axis is known to promote cell proliferation, inhibit apoptosis, and enhance local immune tolerance,

thereby potentially facilitating the persistence of endometriotic implants. Enrichment of TNF and NF-κB signaling pathways further supports the presence of chronic inflammatory activation, which may contribute to pain generation and lesion progression.

Angiogenesis-related genes, particularly *VEGFA*, were also significantly upregulated. Adequate vascular supply is essential for the survival of ectopic endometrial tissue, and enhanced VEGFA expression may promote neovascularization and lesion maintenance. Concurrently, increased expression of ECM remodeling enzymes such as *MMP9* indicates active tissue invasion and structural reorganization, consistent with the invasive behavior of endometriotic lesions.

Conversely, downregulation of *PGR*, *HOXA10*, and other steroid-responsive genes provides molecular evidence supporting progesterone resistance, a hallmark feature of endometriosis. Impaired progesterone signaling disrupts normal endometrial differentiation and may contribute to infertility and reduced implantation capacity. The relative suppression of this hormonal regulatory module, as illustrated in Figure 1, highlights an imbalance between inflammatory activation and hormonal control.

The integrated network architecture underscores the interconnected nature of these pathways. Rather than functioning independently, inflammatory, angiogenic, and ECM remodeling processes appear to converge through central hub genes that bridge multiple signaling modules. This systems-level perspective suggests that targeting single molecules may be insufficient, and combination strategies aimed at restoring hormonal responsiveness while attenuating inflammatory signaling may offer greater therapeutic potential.

Nevertheless, several limitations should be acknowledged. The analysis was based on publicly available transcriptomic datasets, which may contain heterogeneity in sample characteristics such as menstrual phase, lesion location, and prior treatment exposure. Furthermore, gene expression changes do not necessarily reflect protein-level alterations or functional activity. Therefore, experimental validation using molecular assays and prospective clinical studies is necessary to confirm the biological and translational significance of the identified regulatory genes.

CONCLUSION

This study identified a distinct transcriptomic signature in endometriosis characterized by coordinated upregulation of inflammatory, angiogenic, and extracellular matrix remodeling genes alongside downregulation of key regulators of steroid hormone responsiveness. Differential expression and network analyses highlighted central hub genes, including IL6, STAT3, and VEGFA, which appear to drive inflammatory–angiogenic signaling, as well as reduced expression of PGR and HOXA10, supporting the concept of progesterone resistance. The integrated protein–protein interaction network demonstrates that endometriosis is not driven by isolated molecular alterations but rather by interconnected regulatory modules that collectively sustain lesion survival and impaired endometrial function. These prioritized regulatory genes may serve as potential biomarkers or therapeutic targets. Nevertheless, further experimental validation and clinical studies are required to confirm their functional roles and translational applicability.

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