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Gene Expression Profiling and Pathway Analysis of the Effect of Dienogest on Ovarian Endometriosis: A Comparative Study

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Purpose: Endometriosis affects about 10% of reproductive-age women and can be managed through medical treatments, surgical intervention, or both. Approximately 40%–50% of patients experience recurrence within 5 years after surgery. Therefore, medical treatments, especially progestins, play a major role in the management of endometriosis. Dienogest has been used to treat endometriosis; however, its effects on endometriosis remain unclear. This study aimed to evaluate the molecular effects of dienogest on endometriosis.

Materials and Methods: The enrolled patients were aged ≥20 years, premenopausal, and had pathologically confirmed endometriosis. The study participants consisted of four women who had received dienogest treatment before surgery and four women who had not received any medical treatment before surgery. This study compared the RNA profiles of the dienogest-treated and untreated groups before surgery.

Results: We identified 406 differentially expressed genes by comparing the dienogest-treated and untreated groups and conducted enrichment analysis with an adjusted p-value of <0.05. We identified pathways such as regulation of immune effector processes, leukocyte activation involved in the immune response, cell activation involved in the immune response, and leukocyte cell-cell adhesion.

Conclusion: Comparison of the dienogest-treated and untreated groups before endometriotic ovarian cyst surgery revealed pathways related to immune system function, inflammatory response, cell signaling and adhesion, and metabolic regulation. These findings suggest that dienogest may play a role in controlling inflammation and immune regulation, potentially alleviating endometriosis-related symptoms and delaying recurrence. Although further studies are required for validation, our preliminary findings suggest that dienogest may contribute to delaying the progression from endometriosis to carcinoma.

Key Words: Endometriosis, differentially expressed genes, pathway analysis, inflammation, immune response

INTRODUCTION

Endometriosis is a benign disease characterized by the implantation of endometrial tissue, glands, and stroma outside the uterine endometrium.[1] Because pathological diagnosis is challenging, the incidence of endometriosis remains unclear.[2] Approximately 10% of women of reproductive age have endometriosis, and approximately 50% of women with endometriosis are at a higher risk of developing ovarian cancer than those without endometriosis.[3] However, its etiology remains unclear.[4-6] Most young women seek clinical evaluation for symptoms; however, due to the wide range of symptoms, such as pelvic pain, infertility, dyspareunia, dysuria, dyschezia, and dysmenorrhea,[6-8] further assessment is often required. Despite being benign, endometriosis shares several features with malignant diseases, such as the capacity to invade locally, spread, and damage affected tissues.[9,10] Given these characteristics, lap-

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aroscopic ovarian cystectomy is commonly used to treat endometriomas, as it can reduce the risk of recurrence.[11-14] However, its role remains controversial due to concerns about a potential reduction in ovarian reserve following surgery[14-16] and a recurrence rate of 40%–50% within 5 years,[17] prompting physicians to evaluate the optimal timing of the procedure. Medical treatment is often considered to alleviate pain associated with endometriosis, prevent postoperative recurrence, and delay the need for surgery. Among the available treatments, progestins are the first-line option for relieving endometriosis-related pain.[6] Dienogest is a fourth-generation orally active progestogen with high specificity for the progesterone receptor.[18,19] It indirectly controls endometriosis by suppressing ovulation, thereby reducing serum estrogen levels.[20] Despite the ability of dienogest to control endometriosis, its effect on endometriotic ovarian tissue at the molecular level remains unknown. This study aimed to analyze the effects of dienogest in the treatment of endometriosis and to establish a foundational understanding of dienogest as a potential method for delaying the malignant transition from endometriosis to carcinoma at the molecular level by identifying differentially expressed genes (DEGs) and conducting pathway analysis.

MATERIALS AND METHODS

Data collection

Among the patients diagnosed with endometriosis at Severance Hospital between January 1, 2023, and December 1, 2024, we selected four samples based on the inclusion criteria of being aged ≥20 years, premenopausal, and having undergone surgery for endometriotic ovarian cysts. Patients without pathologically confirmed endometriosis were excluded. Additionally, we selected four samples using the same criteria, except for those treated with dienogest before the surgical procedure.

Study design

Gene expression profiling was performed to assess gene expression in endometriosis, with and without dienogest treatment, and RNA sequencing was performed on selected samples for comparative analysis. In collaboration with the Bioinformatics Unit at Severance Hospital, we conducted DEG analysis of the raw gene expression data, which underwent quality control, mapping, counting, and normalization. Principal component analysis (PCA) was used to examine gene expression patterns across samples, and heatmaps and volcano plots were used to visualize changes in gene expression. After identifying the DEGs, we conducted pathway analysis to explore the biological pathways associated with dienogest treatment for endometriotic ovarian cysts.

Statistical analysis

Statistical analysis of patient demographic characteristics was performed using IBM SPSS Statistics for Windows version 28 (IBM Corp., Armonk, NY, USA). Due to the small sample size, Fisher's exact test and the Mann–Whitney U test were used, with two-tailed *p*-values of <0.05 considered significant.

RNA processing

We extracted total RNA from the ovarian samples, removed DNA contamination using DNase and the Ribo-Zero rRNA Removal Kit (Illumina, San Diego, CA, USA) to purify the RNA, and then randomly fragmented the purified RNA for short-read sequencing. Reverse transcription was performed to synthesize complementary DNA (cDNA). We then ligated adapters to both ends of the cDNA fragments, followed by polymerase chain reaction (PCR) amplification of the ligated fragments. We prepared samples with insert sizes between 200 and 400 bp for size selection. After sequencing, we conducted quality control of the raw reads to assess the read quality, total bases, total reads, and Guanine-Cytosine content (%). RNA quantity was measured using fluorescence-based quantification. The Quant-iT™ RiboGreen RNA Assay (Invitrogen, Cat#R11490, Thermo Fisher Scientific, Eugene, OR, USA) was performed on a Victor Nivo Multimode Microplate Reader (PerkinElmer Informatics, Part#HH35000500, Waltham, MA, USA). Quality control metrics, including read depth, sequence quality (Q-scores), percentage of mapped reads, and duplication rates, are provided in Supplementary Table 1 (only online). All samples exceeded 116 million reads and had an average mapping rate above 98%.

Because all eight samples were processed within the same batch, no additional batch correction was performed. The DESeq2 normalization method was employed to account for sample-to-sample variability. To minimize data processing bias, we applied preprocessing steps to remove low-quality data and artifacts, such as adaptor sequences, contaminant DNA, and PCR duplicates. Furthermore, we mapped the preprocessed data to reference genomes using Hierarchical Indexing for Spliced Alignment of Transcript 2 (HISAT2) version 2.1.0 (Johns Hopkins University, Baltimore, MD, USA) to produce aligned reads. Transcript assembly was performed using StringTie version 2.1.3b (National Institute of Allergy and Infectious Diseases, Bethesda, MD, USA). Expression profiles were reported as fragments per kilobase of transcript per million mapped reads, reads per kilobase of transcript per million mapped reads, and transcripts per kilobase million.

Expression processing

We pre-filtered the raw counts based on the following criterion: genes with zero read counts in more than three samples within a group were excluded to increase the statistical power of further analysis. Pre-filtered data were subsequently analyzed using DESeq2 (R package v. 1.36.0; R Foundation for Statistical Computing, Vienna, Austria). Outlier samples were not removed from the analysis. A fold change >2 was considered significant for evaluation, and an adjusted *p*-value of <0.05 was used as the

threshold for statistical significance. Multiple test correction was performed using the Benjamini–Hochberg method. To explore the relevant biochemical processes, we conducted pathway analysis of the detected genes using clusterProfiler (R package, v3.17.1), and visualization was performed using an enrichment plot (R package, v1.9.1). Pathway analysis was performed based on the Gene Ontology Biological Process (GOBP).

Ethical approval and consent to participate

Ethical approval for data collection was obtained from the Ethics Committee of Yonsei University Health System, Severance Hospital, Institutional Review Board (Approval No. 4-2023-1539). All participants provided written informed consent.

RESULTS

Basic characteristics of participants

Each participant had different characteristics, as shown in Table 1. The average characteristics of the groups are presented in Table 2. The average age of the participants in the dienogest-treated group before surgery was 41 years, whereas the average age of the patients in the untreated group before surgery was 42 years (p>0.99). The average body mass index of the dienogest-treated group was 21.45 kg/m^2, while that of the untreated group was 21.97 kg/m^2 (p>0.99). With the exception of one participant in the untreated group, all patients were classified as having stage four endometriosis (p<0.69). Two individuals in the dienogest-treated group had a history of endometriosis, whereas none of those in the untreated group had a history of endometriosis-related surgery. These two patients, who had previously undergone surgery for endometriosis, began taking dienogest post-surgery for several months but discontinued it more than 5 years ago. The dienogest-treated group received dienogest from the time of diagnosis until they were able to undergo surgical treatment. One patient was prescribed dienogest for 2 years, whereas the other received it for 2 months. The other two patients received dienogest for 5 and 3 months, respectively. All participants were premenopausal, and none had a history of hormone replacement therapy or gynecological malignancy. A 2 mg dose of dienogest reaches a peak plasma concentration of 47 ng/mL approximately 1.5 hours after a single ingestion. Stable drug concentrations are achieved after 2 days of treatment.[21] Other studies have demonstrated that 48 hours of incubation with dienogest can significantly alter mRNA expression profiles.[22] Based on these findings, for participants in the dienogest-treated group, more than 1 month of treatment may be sufficient to induce changes in DEGs. Although the study was not directly related to dienogest, previous research suggests that the duration of hormone replacement therapy does not significantly affect molecular expression profiles,[23] implying that variations in the duration of dienogest treatment may have limited impact on gene expression diversity. Nevertheless, the cumulative effect of gene expression may influence the overall magnitude of transcriptomic changes.

DEGs of endometriosis treated with dienogest

We performed PCA to analyze the DEGs in the dienogest-treated group, which revealed distinct differences between the dienogest-treated and untreated groups. We compared the two groups using an adjusted p-value of <0.05 and identified 406 DEGs (Supplementary Table 2, only online). These 406 DEGs demonstrated significant changes (adjusted p<0.05) in re-

Table 2. Average Characteristics of Dienogest-Treated and Untreated Groups

	Dienogest-treated group before surgery (n=4)	Untreated group before surgery (n=4)	p
Age (yr)	41	42	>0.99
BMI (kg/m^2)	21.45	21.97	>0.99
Endometriosis stage	4.00	3.75	0.69
Past EMS history			0.43
None	2	4	
Surgical history	2	0	
Direction			>0.99
Unilateral ovary	1	2	
Bilateral ovaries	3	2	
OP method			>0.99
Enucleation/cystectomy	3	4	
Oophorectomy	1	0	

BMI, body mass index; EMS, endometriosis; OP, operation.

Table 1. Participant Characteristics of Dienogest-Treated and Untreated Groups

	Participant	Age	BMI	EMS stage	Past EMS history	Direction	OP method
Dienogest-treated group before surgery	1	43	25.78	4	None	Bilateral	Enucleation
	2	34	18.37	4	None	Bilateral	Enucleation
	3	44	21.88	4	Yes	Unilateral	Oophorectomy
	4	43	19.78	4	Yes	Bilateral	Cystectomy
Untreated group before surgery	1	36	18.90	4	None	Bilateral	Enucleation
	2	43	20.90	4	None	Bilateral	Enucleation
	3	41	28.46	3	None	Unilateral	Cystectomy
	4	48	19.62	4	None	Unilateral	Cystectomy

BMI, body mass index; EMS, endometriosis; OP, operation.

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sponse to dienogest treatment (Fig. 1). The top 20 identified DEGs are listed in Table 3. The top five genes were major histocompatibility complex class II DO beta (HLA-DOB), elongation of very long-chain fatty acid protein 2 (ELOVL2), N-ethylmaleimide-sensitive factor pseudogene 1 (NSFP1), glutathione S-transferase mu 1 (GSTM1), and leucine-rich repeat-containing protein 15 (LRRC15). As illustrated in Table 3 and Fig. 1, all genes, except GSTM1, lactotransferrin, and lipocalin 12, were downregulated. The expression trends of these DEGs were visualized using a volcano plot (Fig. 1) and a heatmap (Fig. 2). As shown in the heatmap, the genes upregulated in the dienogest-treated group tended to be downregulated in the untreated group (Fig. 2).

Pathways involved in dienogest treatment in endometriosis

We performed a signaling pathway enrichment analysis of DEGs using the clusterProfiler package (R version 3.17.1). Visualization was performed using the enrichplot package (R version 1.9.1), focusing on GOBP terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) terms. Enrichment analysis of the 406 DEGs in GOBP terms identified pathways related to immune response regulation, regulation of immune effector processes, leukocyte-mediated immunity, negative regulation of immune system processes, and positive regulation of cytokine production (Fig. 3 and Supplementary Table 3, only online). KEGG term analysis revealed associations with phagosomes, osteoclast differentiation, cytokine-cytokine receptor interactions, cell adhesion molecules, and chemokine signaling pathways in the dienogest-treated group compared to those in the untreated group (Fig. 4 and Supplementary Table 4, only online).

DISCUSSION

Abnormal chronic inflammation is a hallmark feature of endometriosis.[24] Consequently, elevated levels of inflammatory mediators, such as chemokines, cytokines, and prostaglandins, are commonly observed.[25,26] Dienogest, a selective progesterone receptor agonist, effectively alleviates endometriosis-related pain.[27-29] Animal studies have shown that dienogest can reduce plasma estradiol levels by inducing apoptosis in granulosa cells of the ovaries.[30] Additionally, dienogest inhibits progesterone receptor-mediated cell proliferation and reduces the production of inflammatory factors, including prostaglandins

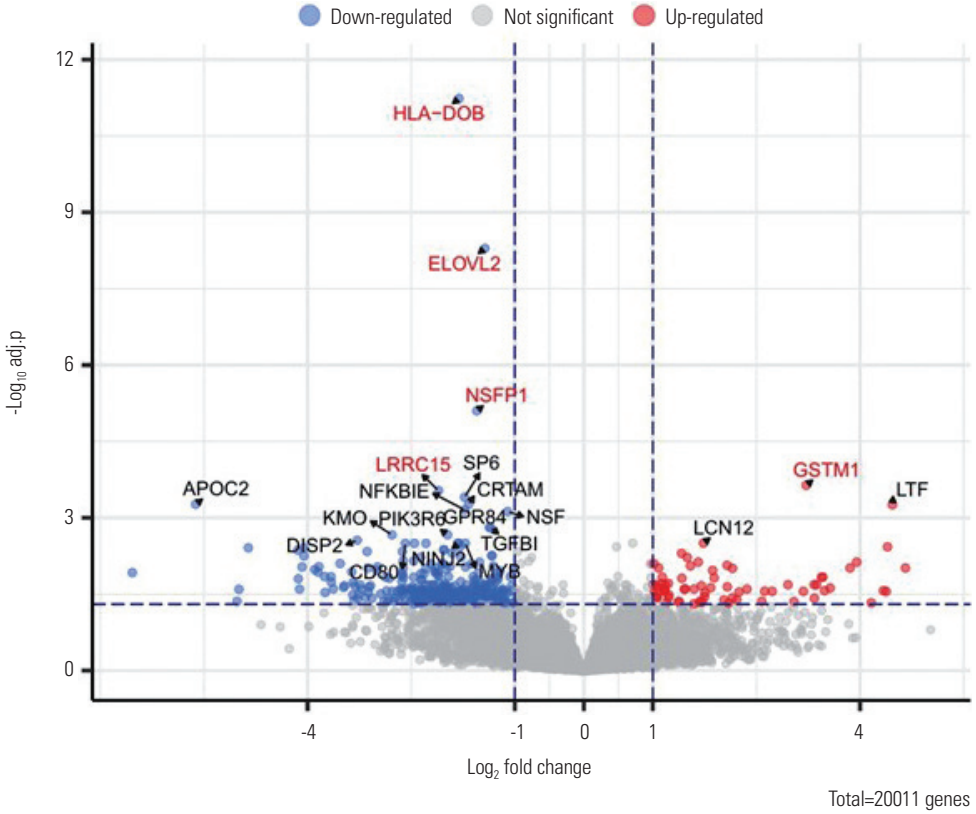

Fig. 1. Volcano plot of dienogest-treated versus untreated groups. The expression patterns of differentially expressed genes (DEGs) were visualized using a volcano plot, with downregulated genes shown in blue and upregulated genes in red. The x-axis represents $\log_2$ fold changes, while the y-axis represents the $-\log_{10}$ adjusted *p*-values. A fold change >2 was considered significant for evaluation, and an adjusted *p*-value<0.05 was used as the threshold for statistical significance. Dashed lines indicate the thresholds, which are approximately 1.30 on the y-axis and -1 and +1 on the x-axis. The top five identified DEGs are highlighted in a red box.

Table 3. Top 20 DEGs in the Dienogest-Treated and Untreated Groups

Gene	Base mean	Log_2 fold change	lfcSE	Stat	*p*	Adjusted *p*
HLA-DOB	213.20	-1.81	0.22	-8.18	<0.001	<0.001
ELOVL2	523.70	-1.43	0.20	-7.22	<0.001	<0.001
NSFP1	1291.99	-1.55	0.25	-6.08	<0.001	<0.001
GSTM1	1027.44	3.22	0.59	5.47	<0.001	<0.001
LRRC15	3044.28	-2.10	0.39	-5.38	<0.001	<0.001
SP6	137.80	-1.73	0.33	-5.30	<0.001	<0.001
APOC2	1473.63	-5.62	1.08	-5.20	<0.001	<0.001
CRTAM	141.36	-1.67	0.32	-5.18	<0.001	<0.001
LTF	2699.12	4.47	0.87	5.16	<0.001	<0.001
NFKBIE	546.37	-1.72	0.34	-5.09	<0.001	<0.001
NSF	5180.98	-1.10	0.22	-5.06	<0.001	<0.001
TGFBI	21052.24	-1.35	0.28	-4.91	<0.001	<0.01
GPR84	135.36	-1.37	0.28	-4.89	<0.001	<0.01
KMO	616.78	-2.78	0.58	-4.80	<0.001	<0.01
PIK3R6	946.44	-1.97	0.41	-4.80	<0.001	<0.01
DISP2	105.96	-3.28	0.69	-4.73	<0.001	<0.01
NINJ2	290.92	-1.81	0.39	-4.67	<0.001	<0.01
LCN12	51.22	1.73	0.37	4.68	<0.001	<0.01
CD80	196.95	-2.59	0.56	-4.65	<0.001	<0.01
TRAV9-2	19.60	-2.45	0.53	-4.62	<0.001	<0.01

DEGs, differentially expressed genes; lfcSE, standard error of the Log_2 fold change estimate; Stat, Wald statistic.

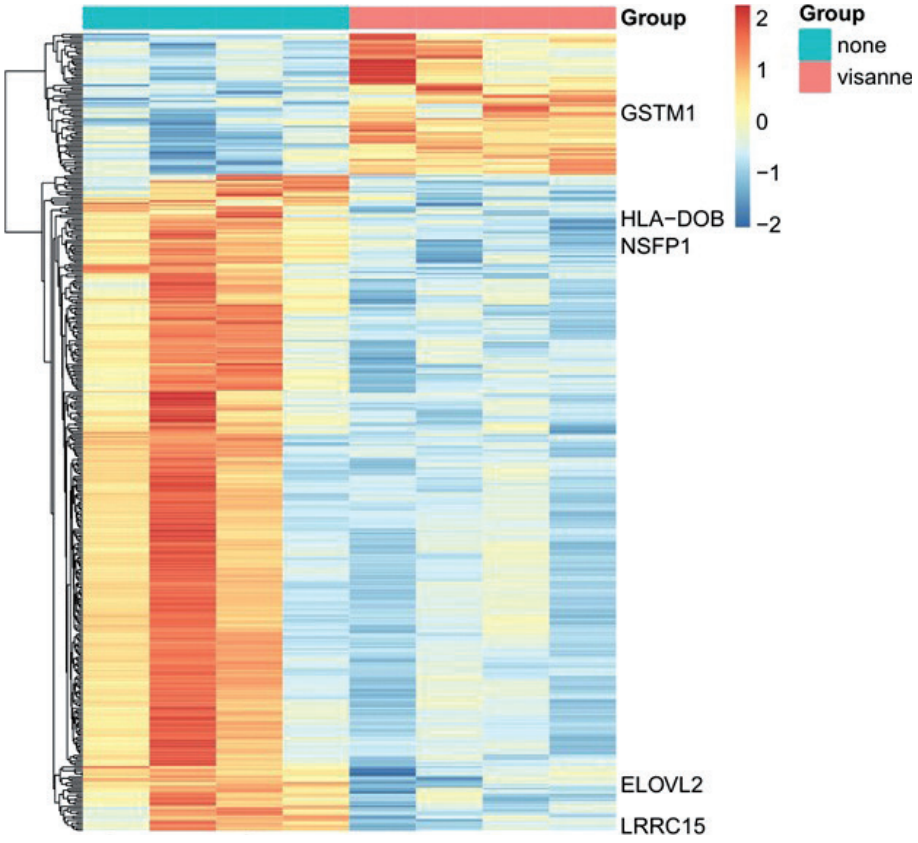

Fig. 2. Heatmap of dienogest-treated versus untreated groups. The four samples on the left, labeled in blue at the top bar, represent untreated participants, whereas the four samples on the right, labeled in pink, represent the dienogest-treated group. Upregulated genes are shown in red, and downregulated genes are shown in blue. Genes upregulated in the dienogest-treated group tended to be downregulated in the untreated group. The top five DEGs (GSTM1, HLA-DOB, NSFP1, ELOVL2, and LRRC15) are annotated on the right side of the heatmap.

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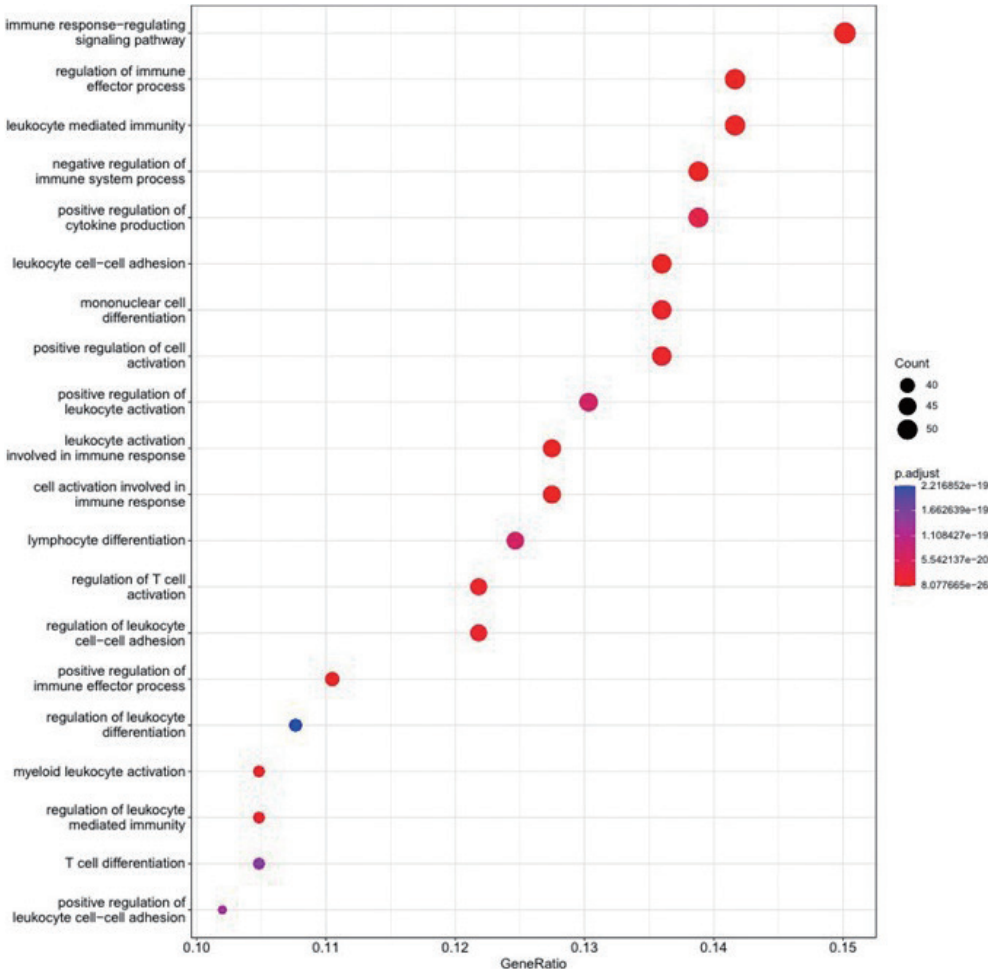

Fig. 3. Dot plot of enrichment analysis results in Gene Ontology Biological Processes. The pathways are shown on the y-axis, and the gene ratio is shown on the x-axis. Adjusted *p*-values and gene counts are represented by the color and size of the dots, respectively. An adjusted *p*-value<0.05 was used as the threshold for statistical significance.

and estradiol.[22,31-34] These findings support the validity of our results.

A total of 406 DEGs were associated with immune system regulation, inflammatory responses, cell signaling, and metabolic regulation. Among the 406 DEGs identified, we focused on the top five genes ranked according to the Wald test, a parametric statistical measure used to assess the significance of independent variables in the model. Based on previous studies, the connections between the top five genes and endometriosis were identified as follows. The top five DEGs were HLA-DOB, ELOVL2, NSFP1, GSTM1, and LRRC15. HLA-DOB, located in intracellular vesicles, suppresses peptide loading of major histocompatibility complex class II molecules, thus inhibiting immune recognition.[35] A previous study indicated the involvement of HLA-DOB in the etiology of endometriosis.[36] Since HLA-DOB was downregulated in this study, dienogest may delay the progression of endometriosis and enhance immune function. This is likely why HLA-DOB is frequently implicated in pathways associated with leukocyte cell-cell adhesion, leukocyte-mediated immunity, and MHC class II antigen processing, among others. ELOVL2 is involved in polyunsaturated fatty acid elongation and plays a key role in modulating inflammation, metabolic regulation, and maintaining cell membrane integrity.[37] Since endometriosis is strongly associated with inflammatory conditions, ELOVL2 may be linked to the development and progression of endometriosis. In the presence of dienogest, ELOVL2 expression was downregulated, potentially slowing the progression of inflammation. ELOVL2 is recognized in pathways related to the biosynthesis of monocarboxylic acid, organic acid, and carboxylic acids, as well as unsaturated fatty acid metabolism and long-chain fatty acid metabolic processes. NSFP1, a pseudogene with unknown functions, is downregulated in pregnant women with rheumatoid arthritis and systemic lupus erythematosus.[38] Thus, NSFP1 may be related to inflammation and hormonal changes. GSTM1 is conjugated to glutathione and is involved in detoxifying electrophilic compounds, including carcinogens and oxidative stress.[39] Previous research has indicated that the GSTM1 null allele is not associated with endometriosis susceptibility but may increase the risk of malignant transition to endometrioid or clear cell

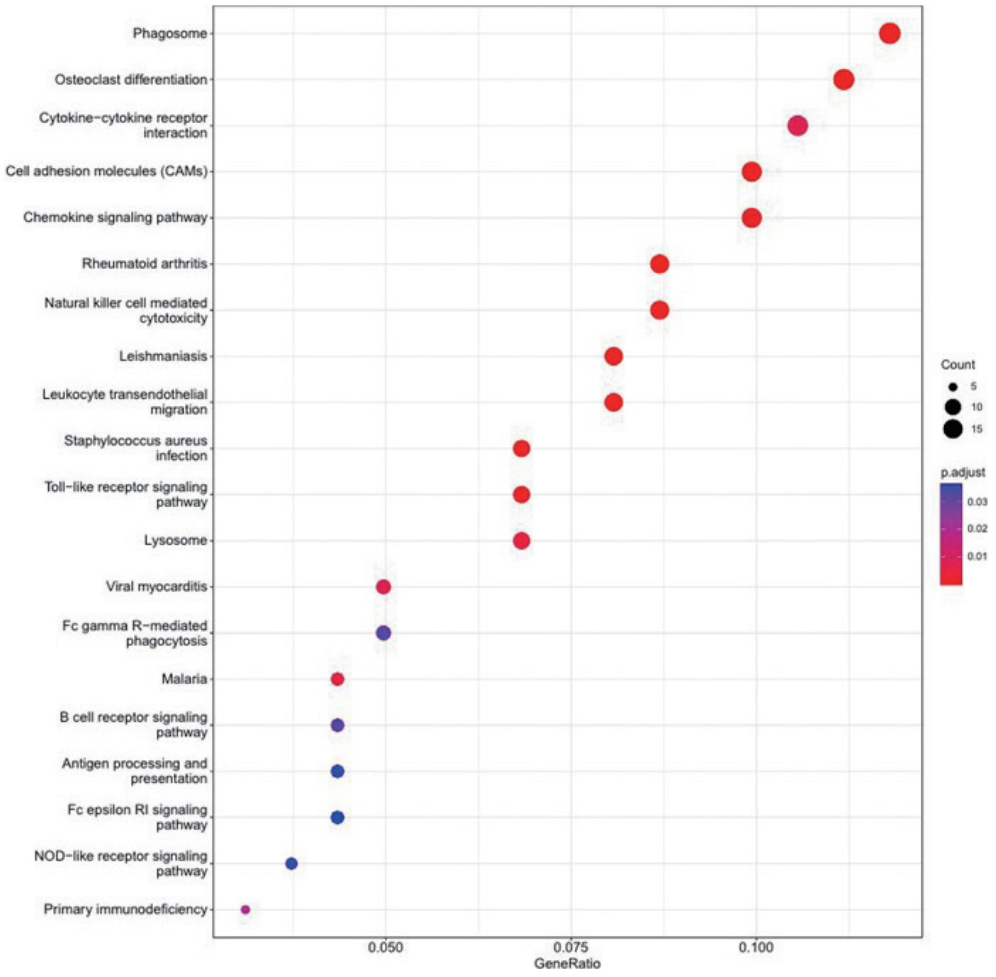

Fig. 4. Dot plot of enrichment analysis results in the Kyoto Encyclopedia of Genes and Genomes. The pathways are shown on the y-axis, and the gene ratio is shown on the x-axis. Adjusted *p*-values and gene counts are represented by the color and size of the dots, respectively. An adjusted *p*-value<0.05 was used as the threshold for statistical significance.

carcinoma.[40] GSTM1 was upregulated in the dienogest-treated group, suggesting that dienogest may help delay the transition from endometriosis to endometrioid or clear cell carcinoma. GSTM1 is recognized in pathways related to the biosynthesis of monocarboxylic acids, carboxylic acids, and organic acids, as well as the metabolism of unsaturated fatty acids, prostanoids, prostaglandins, and long-chain fatty acids. LRRC15 is involved in cell adhesion and interactions with the extracellular matrix and is a potential target for metastatic ovarian cancer.[41] Some studies have reported that LRRC15 expression is increased in endometriosis and is associated with cell migration.[42] LRRC15 expression was downregulated in the dienogest-treated group, suggesting that dienogest prevented endometrial tissue from migrating beyond the endometrium. LRRC15 is involved in pathways related to the adhesion of symbionts to the host and the biological processes involved in symbiotic interactions. To validate the relevance of the top five genes—HLA-DOB, ELOVL2, NSFP1, GSTM1, and LRRC15—and their connection to dienogest action in endometriosis, further studies such as quantitative reverse transcription PCR or immunohistochemistry are necessary to substantiate these interpretations.

Enrichment analysis of the 406 DEGs revealed key pathways related to the immune system function, inflammation, cell signaling, adhesion, and metabolic regulation in dienogest-treated endometriosis. Specifically, pathways involved in T cell proliferation, leukocyte migration, and the differentiation of T cells, lymphocytes, and leukocytes were significantly enriched, as shown in Supplementary Tables 3 and 4 (only online). Furthermore, other highly significant DEGs were grouped by biological functions, such as cytokine production, chemokine activity, and cell adhesion, to better illustrate mechanistic insights into the effects of dienogest in endometriosis treatment. These findings suggest that dienogest treatment may delay the progression of endometriosis by inhibiting the adhesion and migration of endometrial tissue, while alleviating endometriosis-related pain through the modulation of immune and inflammatory responses. In previous research, compared with other progestogens, dienogest has been shown to reduce endometriosis by inhibiting ovulation, reducing inflammation, increasing apoptosis, and decreasing angiogenesis.[20,43] Moreover, endometriomas

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are associated with inflammatory pathways such as interleukin-6, tumor necrosis factor, and nuclear factor kappa-light-chain-enhancer of activated B cells, as well as immune factors and oxidative stress in the epithelial layer due to trapped blood.[3] A limitation of this study is the small sample size. However, given that the results align with previously established endometriosis-related pathways and show suppression of these mechanisms by dienogest, this study offers meaningful inferences. Another limitation of this study is that the DEGs and pathways influenced by dienogest in endometriosis treatment were identified based solely on gene expression data. Consequently, additional research is required to validate whether these genes, proteins, and cytokines are altered in endometriotic stromal cells treated with dienogest. Nevertheless, despite the lack of experimental validation, this study remains valuable as it highlights potential genes and pathways associated with dienogest in endometriosis treatment. Although further research with larger prospective sample sizes and longer follow-up durations is necessary to evaluate the long-term safety, efficacy, and underlying mechanisms of dienogest, this study underscores its potential role in regulating inflammation and immune responses in endometriosis, which may contribute to disease resolution. According to previous studies, individuals with endometriosis have a higher risk of endometrioid and clear-cell ovarian cancers; however, the underlying mechanism is not well understood.[5,9,43] Although not all cases of endometriosis progress to malignancy, particularly endometrioid or ovarian clear cell carcinoma, the results of this study suggest the possibility of delaying malignant transformation or metastasis. However, this remains a hypothesis, and further molecular-level analysis is required to elucidate the relationship between endometriosis and ovarian clear cell carcinoma.

In conclusion, we identified 406 DEGs by comparing the dienogest-treated and untreated groups prior to endometriotic ovarian cyst surgery. Enrichment analysis revealed that these 406 DEGs were strongly associated with immune system regulation, inflammatory responses, cell signaling and adhesion, and metabolic regulation. Although larger prospective sample sizes are needed to establish the efficacy and mechanistic effects of dienogest in endometriosis, the findings of this study provide a foundation for identifying potential genes and pathways associated with dienogest in endometriosis treatment. As a top priority before conducting any further molecular-level studies to validate whether the 406 DEGs are truly associated with dienogest treatment, such as immunohistochemistry or quantitative reverse transcription PCR, we plan to validate the top five genes (HLA-DOB, ELOVL2, NSFP1, GSTM1, and LRRC15) using immunohistochemistry. Successful validation may provide insights into whether one or more of these key DEGs could serve as biomarkers of reponse to dienogest. Although not all cases of endometriosis progress to malignancy and further studies are warranted, drawing definitive conclusions at this stage remains premature. Our findings suggest that dienogest may play a role in slowing or potentially preventing malignant transformation; however, this hypothesis should be validated in larger, independent cohorts.

AVAILABILITY OF DATA AND MATERIALS

The raw data supporting the conclusions of this article will be made available by the authors without undue reservation. The raw data analyzed during the current study are available in the Gene Expression Omnibus GSE283755, at https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE283755.

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AUTHOR CONTRIBUTIONS

Conceptualization: Seok Kyo Seo. Data curation: Jung Eun Shim. Formal analysis: Jung Eun Shim. Funding acquisition: Seok Kyo Seo. Investigation: Yun Soo Chung. Methodology: Yun Soo Chung, Jin Kyung Baek, Euna Choi, and Seok Kyo Seo. Project administration: Seok Kyo Seo. Resources: Seok Kyo Seo. Software: Yun Soo Chung and Jung Eun Shim. Supervision: Seok Kyo Seo. Validation: Heeyon Kim and Bo Hyon Yun. Visualization: Jung Eun Shim. Writing—original draft: Yun Soo Chung. Writing—review & editing: Yun Soo Chung, Jin Kyung Baek, Euna Choi, Heeyon Kim, Bo Hyon Yun, and Seok Kyo Seo. Approval of final manuscript: all authors.

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