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Experimental Research Article

Identification of endometriosis molecular regulatory axes through bioinformatics analysis: insights into lncRNAs, miRNAs, and mRNAs

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ABSTRACT

Introduction: Endometriosis (EMS) is a highly prevalent gynecological disorder with substantial health consequences, affecting as many as 10% of women in their reproductive years. Although EMS is widespread, its intricate origin and pathophysiology are not well understood. This study investigated the molecular characteristics of EMS by examining the expression patterns of long noncoding RNAs (lncRNAs), microRNAs (miRNAs), and genes linked to the condition.

Methods: The study used publicly accessible datasets, namely RNA-seq data for long non-coding RNAs (GSE105764), miRNA expression data (GSE105765), and gene expression data (GSE12768), to compare samples of ectopic and eutopic endometrial samples. The study conducted differential expression analysis, pathway enrichment analyses, protein-protein interaction network creation, and hub gene identification to uncover the molecular markers linked to EMS.

Results: The investigation identified substantial dysregulation of lncRNAs, miRNAs, and genes in endometriotic tissues compared with the control eutopic endometrium. Hub genes, including CDH1, ESR1, GATA4, PGR, FOXA2, EPCAM, APOA1, BDNF, FGFR2, and PAX2, dominate the molecular landscape. miR-6500-3p has been identified as a pivotal regulator that influences the expression of seven essential genes. Pathway enrichment analysis identified biological processes, including embryonic organ morphogenesis, immunological response, and medication metabolism. Exploring lncRNA-mediated regulatory axes, particularly H19 and MIR600HG, suggests complex networks that may clarify the molecular underpinnings of EMS.

Conclusion: This thorough investigation offers significant insight into the molecular pathways underlying EMS. Hub genes and regulatory miRNAs, including miR-6500-3p, have been identified as prospective targets for therapeutic interventions. Pathway enrichment studies provide a more comprehensive understanding of the biological processes implicated in EMS, indicating new possibilities for therapeutic interventions.

Keywords:
Endometriosis
lncRNAs
miRNAs
Hub genes
Bioinformatics

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Introduction

Endometriosis (EMS) is a condition in which cells that resemble the endometrium, including secretory and stromal tissues, are found outside the uterus. It is associated with dysmenorrhea, persistent pelvic discomfort, and infertility (Borghese et al., 2017; Shafrir et al., 2018; Tomassetti and D'Hooghe 2018). EMS is a common gynecological illness that relies on estrogen. It affects around 10% of women who are of reproductive age and up to 50% of women who have pelvic discomfort and/or infertility (Giudice 2010; Shafrir et al., 2018). Although it is a non-threatening disorder, it displays aggressive biological characteristics, such as tissue infiltration, local dissemination, distant spread, and recurrence, which have a substantial influence on the physical and emotional well-being of women (Matias-Guiu and Stewart 2018). Regrettably, the absence of efficient therapies for EMS persists owing to its intricate pathophysiology and wide range of symptoms. It is widely believed that ectopic lesions originate from the retrograde parts of the endometrium that occur during menstruation (Sampson 1927). Nevertheless, although the majority of women of reproductive age demonstrate retrograde menstruation, not all of them have EMS (Halme et al., 1984). However, EMS is prevalent, and its etiology and pathogenesis are poorly understood. EMS is now acknowledged to be a complex illness influenced by several factors and genetic variations (Montgomery et al., 2008). Several molecular abnormalities may be seen between the ectopic endometrium (EC) (endometriotic lesions) and the eutopic endometrium (EU), which may help explain the aberrant growth of the EU outside the uterus.

Long noncoding RNAs (lncRNAs) are a significant group of ncRNAs that are longer than 200 nucleotides in length. They have the ability to control gene function at several levels, including the transcriptional, post-transcriptional, and epigenetic levels (Geisler and Coller 2013; Xiong et al., 2016). Multiple studies have shown that abnormally expressed lncRNAs are linked to many disorders, including cancer (Du et al., 2013), neurological disorders (Riva et al., 2016), and EMS (Panir et al., 2018). Additionally, significant interactions between lncRNAs and miRNAs further contribute to the complexity of the regulatory networks be in control of gene expression (Paraskevopoulou and Hatzigeorgiou 2016). The interaction between lncRNAs and miRNAs is essential for the precise regulation of cellular activities. This interaction controls the availability and function of miRNAs, which subsequently controls the expression of certain genes (Sebastian-delaCruz et al., 2021). Li et al. discovered that miR-27b-3p specifically targets lncRNA HOXA10, leading to significant effects on cell growth, motility, and invasion in EMS cells (Li et al., 2021). According to Wan et al., miR-340-5p in endometrial stromal cells specifically targets the lncRNA MAP3K2. This targeting action leads to the inhibition of cell migration, invasiveness, and epithelial-mesenchymal transition. The mechanism underlying this inhibition involves the deactivation of the MAPK/ERK signaling pathway (Wan et al., 2022).

This study aimed to conduct a thorough analysis of RNA-seq and microarray gene expression datasets to discover possible molecular participants, such as lncRNAs, miRNAs, and genes, involved in the development of EMS. Our thorough examination will provide insights into the complicated regulatory networks and molecular signatures linked to this delicate gynecological illness, enhancing our comprehension of its underlying causes. Figure 1. Represents the study flow chart (Figure 1).

Material and Methods

Data collection

Publicly available information was used to examine the expression patterns of lncRNAs, miRNAs, and genes associated with EMS. The datasets were chosen based on their relevance and comprehensive coverage of different RNA types associated with EMS. The selection criteria included the dataset's sample size, the platform used, and the availability of both case and control samples. Keywords such as "endometriosis," "lncRNA," "miRNA," "RNA-seq," and "gene expression" were employed for the GEO search to ensure that the most relevant and high-quality datasets were identified. In particular, RNA-seq data for lncRNAs were acquired using the Illumina HiSeq 4000 platform (GPL20301) from the GSE105764 dataset (Zhao et al., 2018), which included eight samples of EC compared to eight samples of normal EU. This dataset was selected due to its robust sample size and high-throughput sequencing platform, which provided comprehensive lncRNA expression profiles. Similarly, utilizing the Illumina HiSeq 2000 platform (GPL11154), miRNA expression data were collected from the GSE105765 dataset (Zhao et

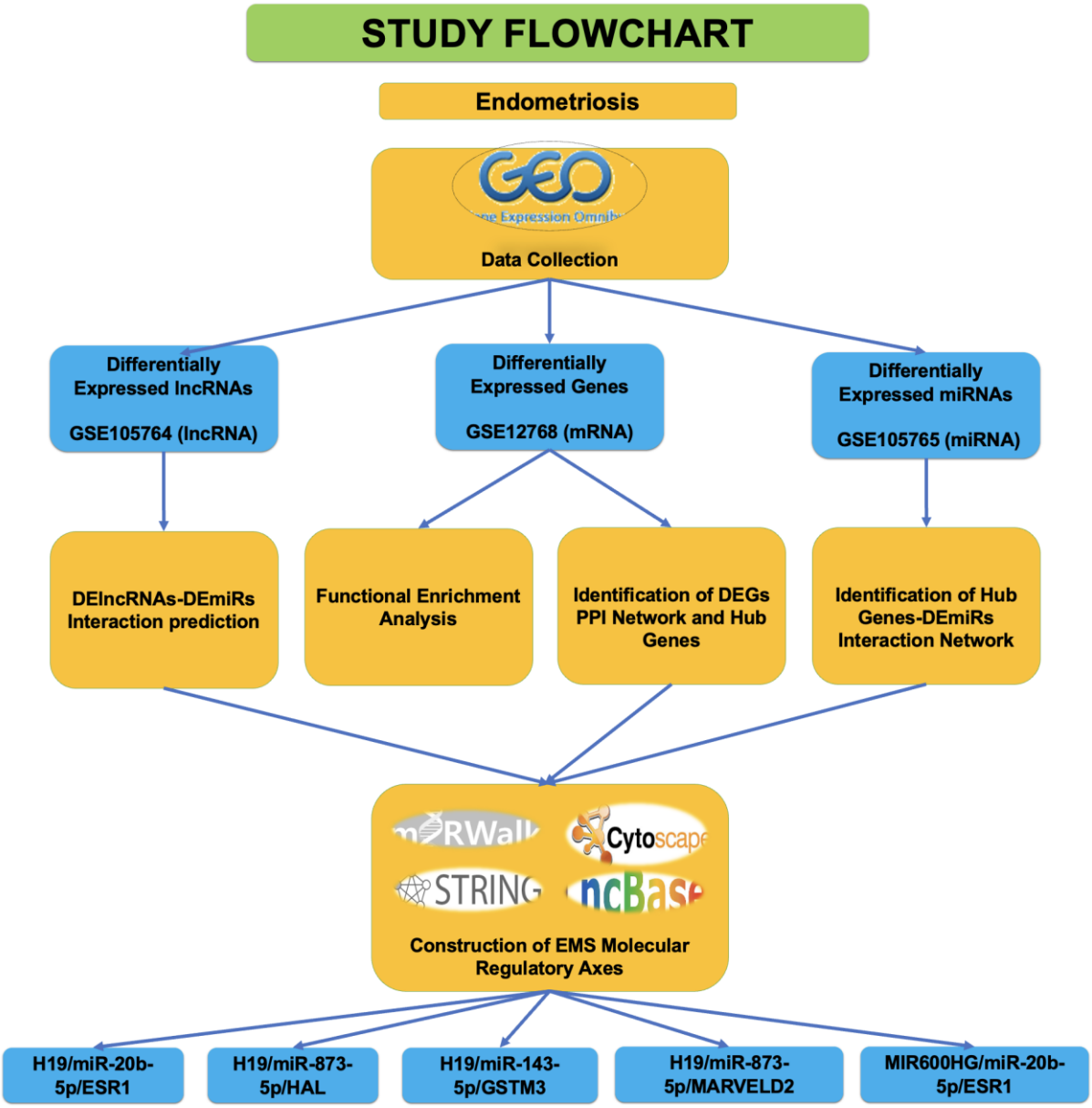

FIGURE 1. The figure presents a flowchart outlining the methodology used in the study to investigate the molecular mechanisms of endometriosis. The flowchart details the steps involved in data collection, processing, and analysis, ultimately leading to the identification of key regulatory networks and potential therapeutic targets.

al., 2018), which included eight EC samples compared to eight control EU samples. The choice of this dataset was driven by its high-quality miRNA sequencing data and the matched case-control design, allowing for a direct comparison of miRNA expression between EMS cases and controls. The GSE12768 MicroArray dataset (Borghese et al., 2008) was used for gene expression analysis, comparing two EC samples with two control EU samples using gene expression data obtained from the Institut Cochin HG18 60mer expression array 47 K platform (GPL7304). This dataset was included because of its focus on gene expression profiling in EMS.

Data Processing and Identification of Differentially Expressed Genes (DEGs)

Raw data collected from the above datasets were preprocessed to guarantee consistency and dependability. The raw read counts for the lncRNA and miRNA datasets (GSE105764 and GSE105765) were normalized, and differential expression analysis was performed using appropriate statistical techniques. The raw intensity values underwent preprocessing for the gene expression

dataset (GSE12768), including background correction and normalization. The DESeq2 package (Love et al., 2014) was used to identify DEGs in the RNA-seq datasets, whereas the limma package (Ritchie et al., 2015) was used for microarray datasets in R. The criteria for statistical significance were established as adj.P.Val < 0.05, |log2FC| > 4 for GSE12768, FDR < 0.05, and |log-2FC| > 1.5 GSE105764 and GSE105765. DEGs were identified, and further analyses were conducted specifically on those genes that showed substantial changes in expression between EC and EU samples. The thorough identification of DEGs across these datasets provides a basis for understanding the complex molecular landscape associated with EMS.

Pathway Enrichment Analysis

In order to further understand the functional significance of the discovered DEGs in the context of EMS, we conducted thorough pathway enrichment studies using the ClusterProfiler package (Yu et al., 2012). Kyoto Encyclopedia of Genes and Genomes (KEGG) (Kanehisa et al., 2016) pathway analysis, Gene Ontology (GO) (2019) analysis, and Gene Set Enrichment Analysis (GSEA) (Subramanian et al., 2005) were performed to determine the probable biological pathways and processes related to the DEGs. The KEGG database was used to ascertain the participation of DEGs in certain biological pathways, offering a methodical comprehension of their functional roles. A GO analysis was performed in order to classify DEGs into specific biological processes, which allowed for a more comprehensive understanding of the functional annotations associated with these genes. In addition, GSEA was used to detect enriched gene sets and pathways using pre-established gene sets, thereby improving our understanding of the molecular patterns linked to EMS.

Construction of DEGs protein-protein interaction (PPI) network and identification of hub genes

To understand the functional connections between the DEGs, we created a PPI network using the STRING database (Szklarczyk et al., 2023). The network was visualized and analyzed using the Cytoscape program (Shannon et al., 2003), which included the CytoHubba plugin (Chin et al., 2014) and degree ranking algorithm. Using these technologies, we aimed to clarify the complex relationships within DEGs, offering a complete understanding of the molecular landscape. Our approach focused on identifying hub genes that are essential nodes with significant connections within the PPI network. The use of CytoHubba's degree-ranking algorithm enabled us to precisely identify these hub genes by evaluating their degree centrality.

Exploration of hub genes-DEmiRs interaction network

In order to understand the regulatory connections between hub genes and miRNAs, we used the miRWalk database, a comprehensive tool for predicting the interactions between miRNAs and target genes. Our study aimed to determine the miRNAs interacting with the hub genes we found. This provides insight into the possible regulatory pathways and processes involved. After identifying the hub genes and their corresponding miRNAs, our objective was to evaluate the extent of overlap with Differentially Expressed miRNAs (DEmiRs) in our dataset. Using Venn diagrams, we carefully compared the DEMiRs with the miRNAs predicted by miRWalk (Sticht et al., 2018). Using this method, we were able to identify common miRNAs between the DEmiRs and projected miRNAs. This analysis provided valuable information on the regulatory connections between hub genes and miRNAs that displayed modified expression patterns under our experimental conditions.

Construction of EMS Molecular Regulatory Axes Involving lncRNAs, miRNAs, and Genes

To integrate, analyze, and visualize the expression data, various bioinformatics databases and tools were utilized. The diana-LncBase database (Paraskevopoulou et al., 2016) was used to identify the interactions between DElncRNAs and DEmiRs. Additionally, the miRwalk database was employed to identify interactions between DEGs and DEmiRs. The STRING database facilitated the analysis of DEGs interactions. The constructed networks from these databases were imported into Cytoscape, where they were merged using the merge command. To integrate expression data and obtain the final network, we applied two filters in Cytoscape: a degree filter above 5 and a betweenness centrality filter above 0.015. This approach helped in extracting a smaller, more significant network with specific components and stronger connections.

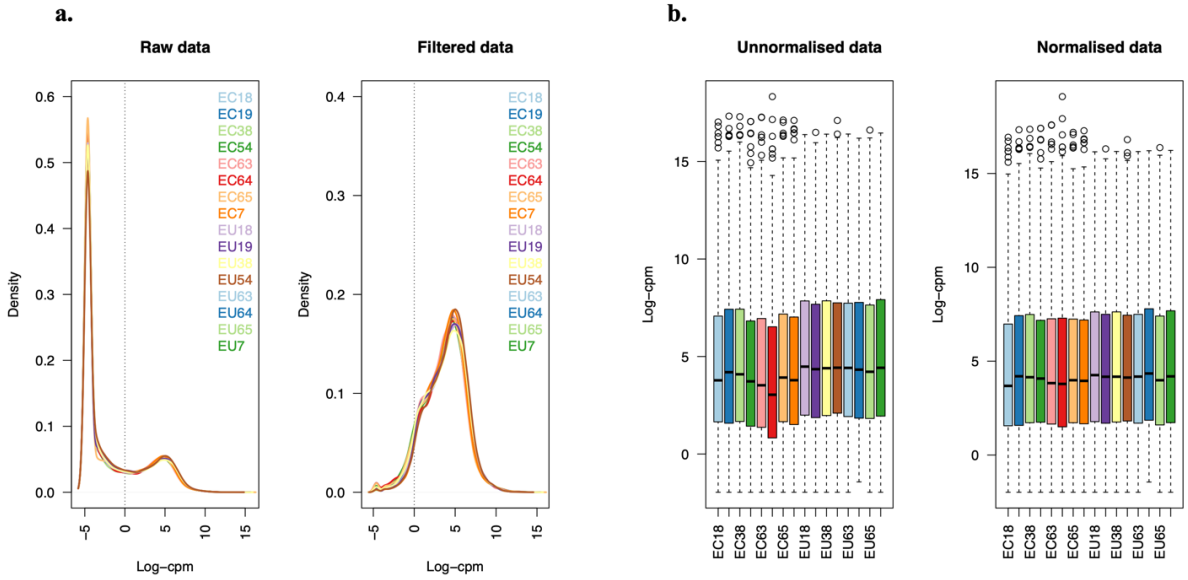

FIGURE 2. Quality control (QC for Raw & Filter) for datasets. a) Quality Control using the QCforRaw&Filter method for the lncRNAs dataset (GSE105764): The X-axis represents the Logcpm (logarithm counts per million) values, while the Y-axis depicts the density of the data. This quality control analysis assesses the distribution and abundance of lncRNAs across the samples, ensuring data reliability and integrity. b) Quality Control using the QCforRaw&Filter method for the miRNAs dataset (GSE105765): The X-axis corresponds to the individual samples, and the Y-axis displays the Logcpm values. This quality control assessment offers insights into the consistency and distribution of miRNA expression levels across the different samples, ensuring the robustness of the miRNA dataset.

Results

Dataset Quality Assessment

The gathered datasets underwent thorough quality evaluation to ensure their dependability and integrity, as shown in Figure 2. The QCforRaw and Filter techniques were used to analyze the lncRNA datasets. This approach ensured a comprehensive assessment of the quality of the raw data and applied essential filtering steps to improve the reliability of the dataset (Figure 2a). This quality control study evaluated the dispersion and prevalence of lncRNAs among samples, guaranteeing the dependability and soundness of the results. The BCV and logFC method, together with QC boxplots, was used for the miRNA datasets, as shown in Figure 2b. This quality control evaluation provides valuable information regarding the uniformity and dispersion of miRNA expression levels across many samples, ensuring the reliability and strength of the miRNA dataset. In addition, mean difference plots (Figure 3) were used to display the average log expression concerning the expression log ratio.

Identification of DEGs, DEmiRs, and DElncRNAs

Examination of RNA-seq datasets, particularly GSE105764, concentrated on lncRNAs and uncovered significant disruption. Specifically, 235 lncRNAs exhibited up-regulation, whereas 219 lncRNAs exhibited down-regulation in endometriotic tissue compared to normal EU. In the miRNA dataset GSE105765, RNA-seq analysis revealed that 140 miRNAs were up-regulated and 138 miRNAs were down-regulated in EC tissues compared to those in the control EU. Simultaneously, gene expression analysis performed on the microarray dataset GSE12768 revealed 150 up-regulated and 145 down-regulated genes in EC tissues compared to control EU. Tables 1–3 provide information on the top ten DEGs, DEmiRs, and DElncRNAs, both upregulated and downregulated. Supplementary File 1 contains the expression matrix for this investigation, which includes DEGs, DEmiRs, and DElncRNAs.

Pathway Enrichment Analysis

GSEA KEGG analysis provided further insight into pathways such as autoimmune thyroid disease, protein processing in the endoplasmic reticulum, rheumatoid arthritis, and cell adhesion molecules (Figure 4). GSEA GO analysis uncovered pathways related to uterine development, the mesenchymal cell apoptotic process, DNA damage response regulation, and skin barrier establishment, providing insights into the molecular

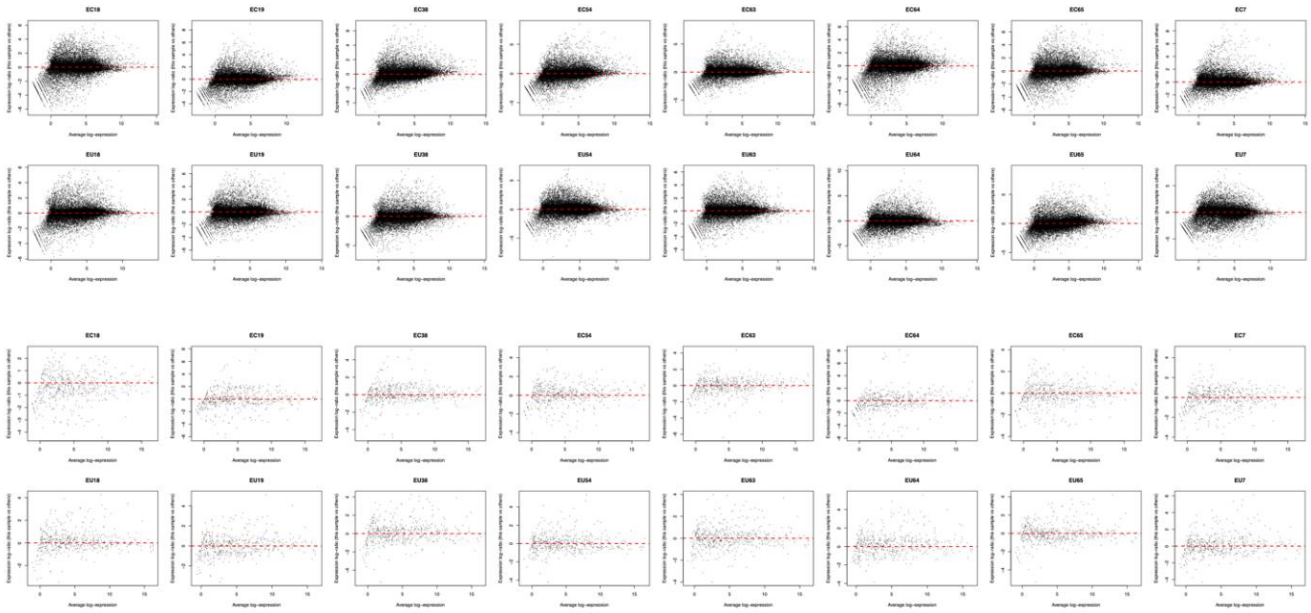

FIGURE 3. Mean Difference Plot. a) Mean Difference Plot for lncRNAs (GSE105764): X-axis, average log-expression; Y-axis, expression log ratio. Visualizing mean differences in lncRNA expression between ectopic and control samples. b) Mean Difference Plot for miRNAs (GSE105765): X-axis, average log-expression; Y-axis, expression log ratio. Illustrating mean differences in miRNA expression between ectopic and control samples.

TABLE 1: The top 10 up- and down-regulated DEGs in EMS

Up-regulated			Down-regulated		
DEGs	**FDR**	**Log2FC**	**DEGs**	**FDR**	**Log2FC**
LOC129293	0.00979	3.42565655	MMP23B	0.01008	-5.3337891
LOC129293	0.04478	3.42068866	CDH1	0.01961	-4.3368338
LOC129293	0.04062	3.41106916	CDH1	0.01587	-4.3336122
LOC129293	0.03585	3.40370173	RP13-347D8.3	0.0345	-4.133185
LOC440895	0.02288	3.12487774	ODZ4	0.02883	-4.0247805
LOC440895	0.04289	3.11501608	CDH23	0.02473	-3.6255573
LOC440895	0.03292	3.114742	NPAS3	0.00934	-3.6228451
LOC644150	0.04223	2.37981209	ACPP	0.02481	-3.2302626
LOC646201	0.01287	2.24224431	KCNC3	0.00411	-3.1154261
LOC644715	0.00998	1.84554	C1orf34	0.01944	-2.9331154

mechanisms underlying EMS pathophysiology. In addition, KEGG analysis revealed participation in pathways, such as drug metabolism via cytochrome P450 and tyrosine metabolism (Figure 5). In addition, targeted analysis of KEGG pathways was conducted to investigate the enrichment of genes involved in drug metabolism, specifically focusing on the cytochrome P450 pathway. This analysis revealed unique patterns of up-regulated genes (shown by red boxes) and down-regulated genes (indicated by green boxes), as shown in Figure 6.

TABLE 2: The top 10 up- and down-regulated DEmiRs in EMS

Up-regulated			Down-regulated		
DEmiRs	**FDR**	**Log2FC**	**DEmiRs**	**FDR**	**Log2FC**
hsa-miR-202-5p	5.08E-07	9.41804726	hsa-miR-375	6.12E-07	-7.6268627
hsa-miR-514a-3p	9.41E-05	7.78143529	hsa-miR-6507-5p	0.00028284	-7.4170666
hsa-miR-506-3p	0.00053775	6.65213028	hsa-miR-449b-5p	4.86E-08	-7.0010143
hsa-miR-509-3-5p	0.00040325	6.568136	hsa-miR-449c-5p	5.08E-07	-6.8937896
hsa-miR-509-3p	0.0001863	6.45048426	hsa-miR-767-5p	1.21E-07	-6.6692269
hsa-miR-513c-5p	0.00026443	6.38484987	hsa-miR-449a	3.01E-07	-6.367746
hsa-miR-509-5p	0.0006001	5.73367494	hsa-miR-141-3p	1.21E-07	-6.1157056
hsa-miR-202-3p	3.02E-06	5.6980702	hsa-miR-449b-3p	3.48E-05	-5.8583131
hsa-miR-216a-5p	7.54E-07	4.31582304	hsa-miR-200c-5p	3.29E-07	-5.4737347
hsa-miR-217	3.89E-06	4.13898703	hsa-miR-141-5p	2.54E-07	-5.3490226

TABLE 3: The top 10 up- and down-regulated DElncRNAs in EMS

Up-regulated			Down-regulated		
DElncRNAs	FDR	Log2FC	DElncRNAs	FDR	Log2FC
MIR202HG	7.73E-05	9.07420216	DLGAP1-AS3	0.00011418	-8.6044161
LINC01018	1.67E-06	7.49796386	LINC00261	1.24E-07	-8.5179006
CHL1-AS2	4.86E-06	6.44063108	LINC01502	8.84E-05	-7.9135697
LINC01638	1.10E-06	5.7564622	TUNAR	1.45E-06	-7.5616193
LAMP5-AS1	0.00015688	5.71388816	HNF1A-AS1	3.25E-07	-7.2579892
TARID	6.75E-06	5.6107729	LINC02532	0.00020564	-6.9647727
FLJ16779	0.00136155	5.47264596	LINC01541	2.14E-05	-6.7864271
LINC02381	5.45E-08	5.39263432	LINC02669	5.63E-05	-6.6064079
NALCN-AS1	2.16E-05	5.19886588	UCA1	2.35E-05	-6.3918879
CHL1-AS1	4.54E-05	5.10598725	LINC00645	9.59E-05	-6.0449589

PPI network and hub genes

The PPI network for DEGs, built using the STRING database, was complicated, with 407 nodes and 1169 edges, suggesting substantial functional interactions among the DEGs (Figure 7). The network was visualized and analyzed using Cytoscape software and the CytoHubba plugin with the degree ranking algorithm, revealing deep relationships between the DEGs. The Degree ranking approach was used to identify hub genes, which were critical nodes with solid connectivity, resulting in a more extensive network with 27 nodes and 55 edges. CDH1, ESR1, GATA4, PGR, FOXA2, EPCAM, APOA1, BDNF, FGFR2, and PAX2 were identified as key participants in the molecular landscape, indicating their critical involvement in the functional connections within the DEG network.

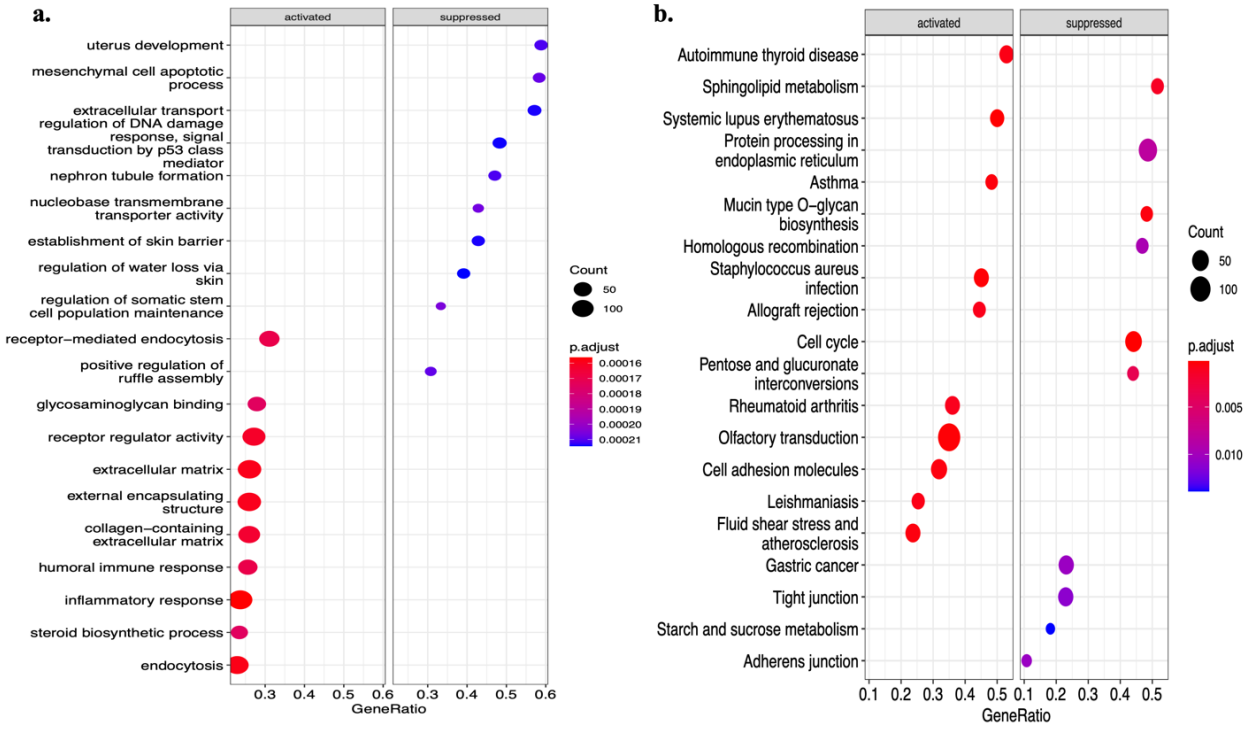

FIGURE 4. GSEA Enrichment Analysis. a) GO Enrichments: The X-axis displays gene set count, and the Y-axis shows gene set functions. The dot color indicates the adjusted P-value (dark blue to red), and the size represents GeneRatio. Two columns show activated and suppressed gene sets. b) KEGG Enrichments: Similar to the GO plot, the X-axis shows gene set count, and the Y-axis displays gene set functions. The dot color denotes the adjusted P-value, and the size reflects GeneRatio. Two columns distinguish activated and suppressed gene sets in KEGG pathways.

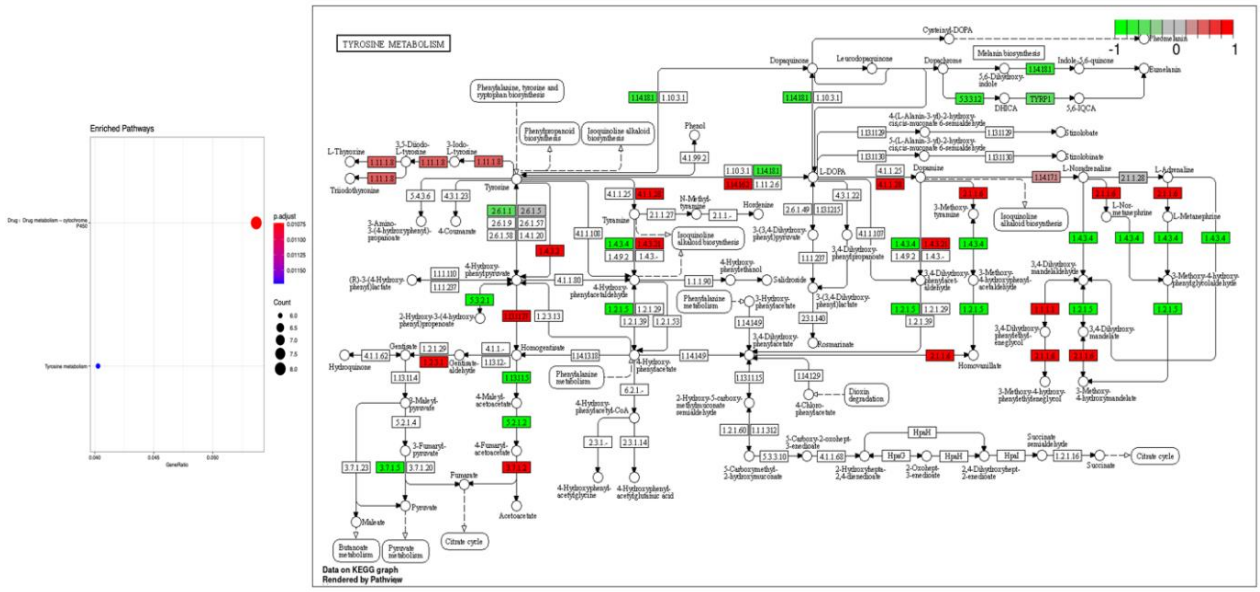

FIGURE 5. KEGG Pathway Enrichment - Tyrosine Metabolism. Green boxes indicate down-regulated genes, and red boxes denote up-regulated genes. The figure depicts the differential expression of genes associated with Tyrosine Metabolism.

Hub-genes-DEmiRs interaction network

With 235 nodes and 1177 edges, the resultant Hub-gene-DEmiR interaction network demonstrated exceptional intricacy, emphasizing the broad interplay between hub genes and DEmiRs. Interestingly, miR-6500-3p emerged as a critical factor in this network, interacting with seven hub genes including FGFR2, BDNF, ESR1, PGR, GATA4, CDH1, and PAX2 (Figure

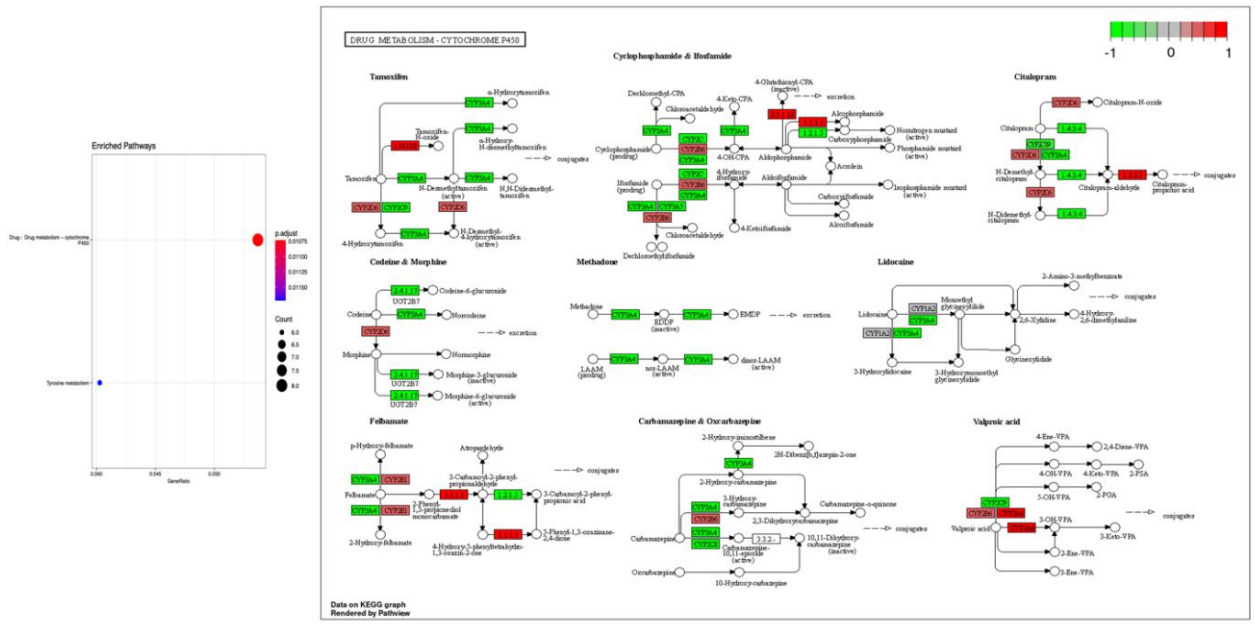

FIGURE 6. DRUG METABOLISM - CYTOCHROME P450 KEGG Pathway. Green boxes represent down-regulated genes, while red boxes signify up-regulated genes. The figure illustrates the altered expression of genes related to DRUG METABOLISM - CYTOCHROME P450.

8). This key location emphasizes miR-6500-3p's critical significance in altering the regulatory connections among the identified hub genes.

Refined Network of EMS Molecular Regulatory Axes Involving lncRNAs, miRNAs, and Genes

The initial network constructed from integrating lncRNA, miRNA, and gene expression data comprised 897 nodes and 3,165 edges, representing a complex web of molecular interactions. After applying specific filters in Cytoscape, namely a degree filter above 5 and a betweenness centrality filter above 0.015, the network was refined to a more focused and manageable size, resulting in 59 nodes and 128 edges. This refined network, shown in Figure 9, highlights the most significant and strongly connected components, providing a clearer view of the key regulatory axes involving lncRNAs, miRNAs, and genes associated with EMS. Notable features of the network include the emergence of ESR1 as the central hub, suggesting its crucial role in coordinating various molecular pathways in EMS. Key up-regulated genes include GATA4, CYP17A1, and NR5A1, while down-regulated genes include FOX11, SPAG6, and DLX5. Several miRNAs, such as hsa-miR-200-5p, hsa-miR-30c-3p, and hsa-miR-873-5p, suggest potential regulatory roles in EMS. The inclusion of lncRNAs like H19 and MIR6004G indicates their involvement in the regulatory landscape of EMS. Furthermore, the network analysis revealed several important lncRNA-associated regulatory axes, including H19/miR-20b-5p/ESR1, H19/miR-873-5p/HAL, H19/miR-143-5p/GSTM3, H19/miR-873-5p/MARVELD2, and MIR600HG/miR-20b-5p/ESR1. These axes highlight the potential regulatory roles of lncRNAs, particularly H19 and MIR600HG, in modulating gene expression through interactions with miRNAs and key genes involved in EMS pathogenesis.

Discussion

EMS is a multifaceted gynecological condition that has a substantial effect on women's well-being. It presents with dysmenorrhea, persistent pelvic pain, and infertility (Zhang et al., 2018). Despite being widespread and having significant effects, there is still a lack of identified therapies that are most effective, which reflects the complexities in understanding the causes and physiological processes of the condition. This study examined the complex factors involved in EMS and included molecular knowledge by investigating the roles of lncRNAs, miRNAs, and disease-related genes.

Identifying DEGs provided a foundational understanding of the molecular disparities between ectopic and eutopic endometria. A comprehensive PPI network was

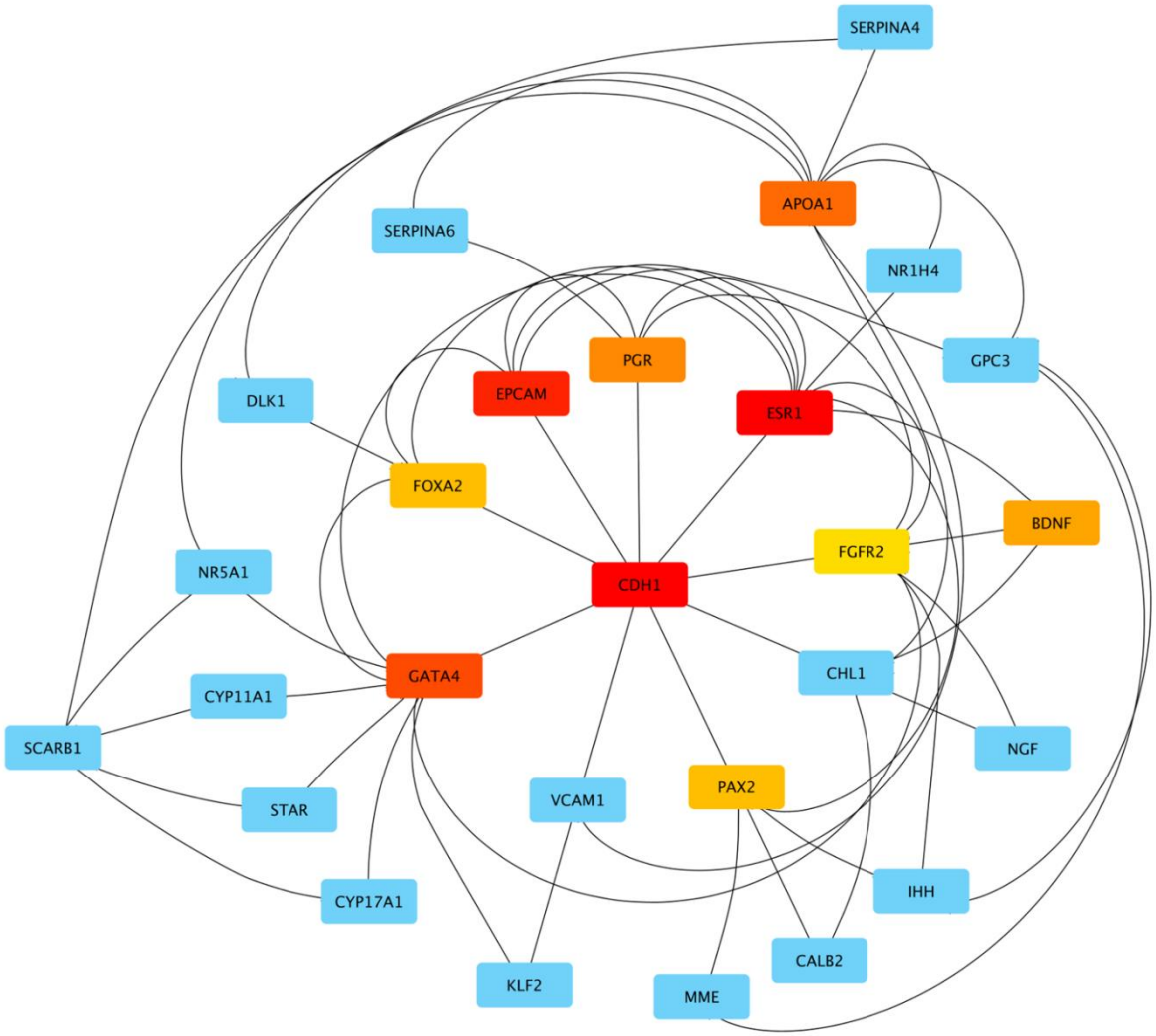

FIGURE 7. Expanded Hub Gene Network. Utilizing Cytoscape software and the CytoHubba plugin with the Degree ranking method, the expanded network showcases 27 hub genes with high connectivity, forming key nodes in the molecular landscape. Notable hub genes, including CDH1, ESR1, GATA4, PGR, FOXA2, EPCAM, APOA1, BDNF, FGFR2, and PAX2, emerge as central players.

constructed through rigorous analysis, which revealed intricate functional relationships within the DEGs. The identification of hub genes, including CDH1 (Chen et al., 2020), ESR1 (Marla et al., 2021; Zhao et al., 2016), GATA4 (Fouquet et al., 2016), PGR (Kim et al., 2021; Zhang and Wang 2023), FOXA2 (Lin et al., 2018), EPCAM (Liu et al., 2020), APOA1 (Brosens et al., 2010; Ferrero et al., 2007), BDNF (Dwiningsih et al., 2022; Wang et al., 2022), FGFR2 (Xu et al., 2022), and PAX2 (Yun et al., 2022), illuminated central players within the molecular landscape of EMS. These hub genes, known for their pivotal roles in various cellular processes, may serve as potential targets for therapeutic intervention.

Analysis of the relationships between hub genes and miRNAs using miRWalk revealed an intricate regulatory network. At the center of this network, miR-6500-3p was identified as a crucial controller that delicately affects the expression of seven key genes. This discovery highlights the important role of miR-6500-3p in regulating EMS and suggests that it has the capacity to control critical genes related to illness. miR-6500-3p, a recently discovered miRNA whose function in disease is not entirely understood, has been found to be involved in new interactions between mRNA and microRNA. In particular, Liang et al. (Liang et al., 2016) identified interactions between KIF-14/miR-6500-3p and NCAPG/miR-6500-3p. These findings suggest that targeting miR-6500-3p may be a potential therapeutic approach for pediatric high-grade gliomas. Based on its interaction with seven essential hub genes in this study, miR-6500-3p has

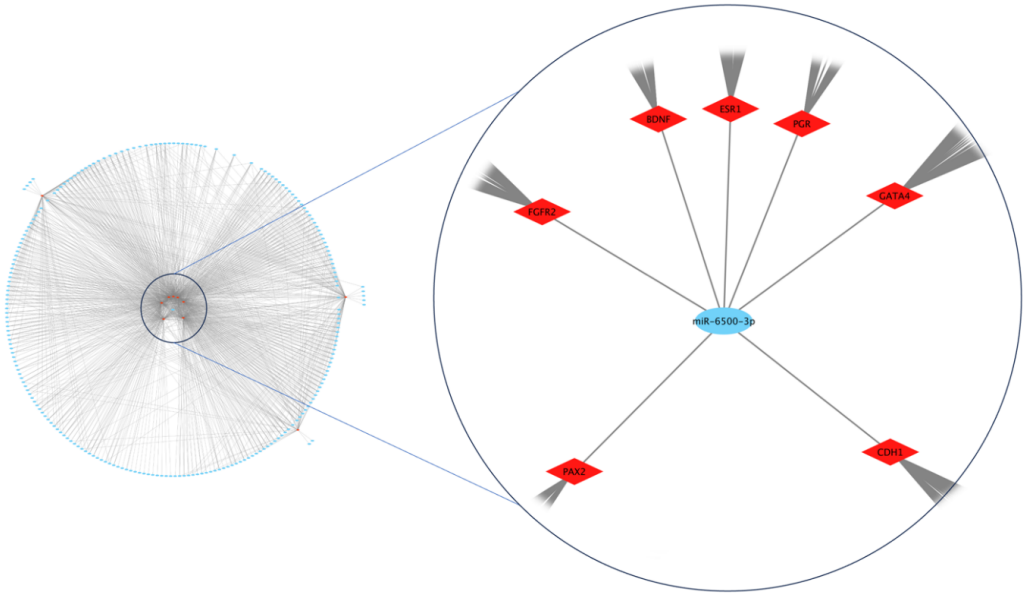

FIGURE 8. Hub-Genes-DEmiRs Interaction Network. The network is characterized by remarkable complexity, comprising 235 nodes and 1177 edges, emphasizing the extensive interplay between hub genes and DEmiRs. Notably, miR-6500-3p emerges as a central player within the network, occupying a core position and interacting with seven hub genes: FGFR2, BDNF, ESR1, PGR, GATA4, CDH1, and PAX2.

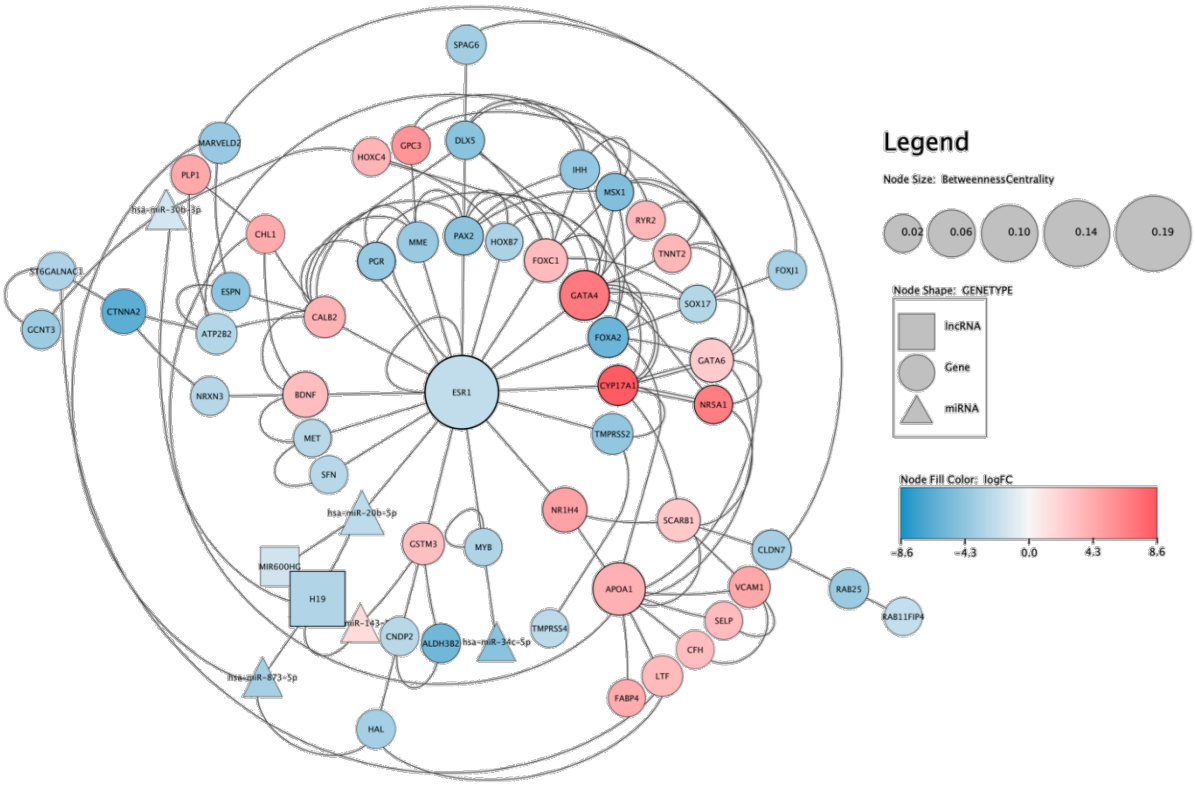

FIGURE 9. Refined network of EMS molecular regulatory axes involving lncRNAs, miRNAs, and genes. This network was constructed by integrating expression data from lncRNAs, miRNAs, and genes, and applying filters for degree above 5 and betweenness centrality above 0.015 in Cytoscape. Nodes representing lncRNAs (squares), miRNAs (triangles), and genes (circles) are interconnected, illustrating the complex regulatory relationships in EMS. The size of each node corresponds to its betweenness centrality, with larger nodes indicating higher centrality and thus greater importance in the network. Node color represents differential expression: the spectrum from deep blue to white indicates decreasing levels of down-regulation, while white to deep red shows increasing levels of up-regulation. ESR1 emerges as the central hub, connecting multiple regulatory pathways. Key up-regulated genes (e.g., GATA4, CYP17A1) and down-regulated genes (e.g., FOX11, SPAG6) are highlighted. The network also features important lncRNA-associated axes, such as H19/miR-20b-5p/ESR1 and MIR600HG/miR-20b-5p/ESR1, underscoring the role of non-coding RNAs in EMS regulation. The final network, comprised of 59 nodes and 128 edges, provides a focused view of the most significant interactions and key regulatory components in EMS pathogenesis.

emerged as a potential target for future studies in EMS. A comprehensive comparison of DEmiRs with anticipated miRNAs yielded valuable insights into common regulatory pathways and elucidated the complex interplay of molecular components.

Network analysis also found several lncRNA-associated regulatory axes, including lncRNA-miRNA-gene axes that required further attention. For instance, the axis composed of lncRNA H19, miR-20b-5p, and ESR1 reflected important regulatory interplay among the molecules concerning EMS. Axes such as H19/miR-873-5p/HAL, H19/miR-143-5p/GSTM3, and H19/miR-873-5p/MARVELD2 suggested some possible regulatory roles of H19 in the regulation of the expression of a few important genes through interaction with specific miRNAs. By inhibiting the modulation of the process of the EMT, H19 enables the development of EMS (Zhao et al., 2017). It has been shown that H19 expression is extinguished in mononuclear cells isolated from peritoneal fluid in patients with EMS (Liu et al., 2019). Activation of the H19/miR-216a-5p/ACTA2 pathway stimulates fibrotic tissue formation in patients with EMS (Xu et al., 2019). H19 silencing significantly reduced the incidence of EMS in nude mice in in vivo studies (Liu et al., 2021). Additionally, the MIR600HG/miR-20b-5p/ESR1 axis highlights the involvement of another lncRNA, MIR600HG, in the regulatory landscape of EMS. Although the expression of MIR600HG remained unexplored in EMS, several recent studies reported the association of MIR600HG with the survival outcome of pancreatic cancer patients (Qian et al., 2023). Yao et al. showed that MIR600HG retarded tumor invasion by targeting ALDH1A3 to promote chemoresistance in colorectal cancer (Yao and Li 2020). Similarly, MIR600HG participated in modulating the occurrence and progression of oropharyngeal squamous cell carcinoma through its regulation of autophagy-related pathways (Jiang et al., 2021). These results implicated an important role for MIR600HG in cancer biology and possible involvement during EMS. Elucidation of mechanisms by which MIR600HG interacts with miRNAs/target genes may provide novel insights into the pathogenesis of EMS and its relationship with tumor biology, which could allow novel therapeutic strategies to be developed. These lncRNA-mediated regulatory axes provide insights into the complex interplay between non-coding RNAs and protein-coding genes, and suggest that lncRNAs, particularly H19 and MIR600HG, may act as important molecular regulators in the pathogenesis of EMS.

Pathway enrichment studies provide an essential biological context for the functional roles and implications of DEGs, lncRNAs, and miRNAs in EMS. Several crucial pathways have been emphasized, providing insights into significant disease-related processes. The study of GO biological pathways showed a notable enrichment of DEGs in pathways related to the development of embryonic organs. The presence of the endometrial gland and stroma growing outside the uterus in an anatomically incorrect manner suggests that deregulation of genes involved in organ development and morphogenesis is likely to be involved in the development of EMS (Vercellini et al., 2014). Pathways relevant to the immune system, such as the humoral immune response facilitated by circulating immunoglobulins and complement activation, have also been shown to be enriched. This indicates that abnormal immune activation may play a role in forming and continuing ectopic lesions, which aligns with the existing theories of EMS as an inflammatory condition (Giacomini et al., 2021). Other significant pathways identified were epithelial cell growth (Matsuzaki and Darcha 2013) and programmed cell death (Agic et al., 2009), indicating that the disturbed processes of cell growth and cell survival play a role in the formation of endometriotic lesions.

In addition to processes directly linked to tissue development and inflammatory responses, enrichment analysis has also revealed associations between EMS and pathways that enhance our comprehension of disease causes (Trabert et al., 2011). Interestingly, the presence of an increased number of genes related to autoimmune thyroid disease suggests similarities to other autoimmune diseases. This aligns with the proposed idea that immunological malfunctions play a role in EMS. A recent study discovered a substantial statistical relationship between the size of endometrioma and the levels of anti-thyroid peroxidase antibodies, which suggests a possible connection between more advanced or severe EMS and autoimmune thyroid conditions (Şerifoğlu et al., 2023). Together, these pathway studies provide important biological information about the participation of many molecular mechanisms in EMS, enhancing our understanding of its pathophysiology. Additionally, they proposed new potential approaches for preclinical research in treating EMS, such as investigating substances

that may modify medication metabolism and immunoregulation.

Despite the significant findings of this study, there are some limitations to consider. The variability in the platforms and datasets used for analysis posed challenges. Specifically, this variability prevented the possibility of thoroughly analyzing the correlation between the components of the competing endogenous RNA (ceRNA) axes and their biological functions. Nevertheless, the study provides valuable insights and a strong foundation for future research in this area.

Conclusion

This study thoroughly examined lncRNAs, miRNAs, and genes linked to EMS, uncovering the complex molecular differences between abnormal and normal endometrial tissues. CDH1, ESR1, GATA4, PGR, FOXA2, EPCAM, APOA1, BDNF, FGFR2, and PAX2 are recognized as hub genes, serving as key participants and presenting potential targets for therapeutic interventions. MiR-6500-3p has been identified as a key regulator that influences the activity of seven central genes, highlighting its essential role in regulation. Pathway enrichment analysis establishes connections between dysregulated genes and processes such as embryonic organ morphogenesis and immune response. Identification of lncRNA-associated regulatory axes, especially H19, together with MIR600HG, points to the need to investigate their molecular mechanisms. Should that be known—namely, how these lncRNAs influence miRNA activity and target gene expression—it may turn out to yield new biomarkers and therapeutic targets and thus open up all-new avenues in treatment strategies concerning EMS.

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