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Identification of common hub genes and miRNAs between blood and granulosa cells in polycystic ovary syndrome

Abstract

Polycystic ovary syndrome (PCOS) is a common endocrine and metabolic disorder affecting women of reproductive age globally. The importance of aberrant immune response dysregulation in PCOS has emerged as a prominent subject of discussion. Non-invasive approaches utilizing important blood biomarkers not only provide valuable information about a patient's biological profile but also present a promising opportunity for developing new diagnostic and prognostic tools. In the present study, PCOS data sets, including GSE34526 from granulosa cell (GC) samples and GSE54248 from blood samples, were investigated, and shared differentially expressed mRNA (DE-mRNAs) between the two data sets were identified. In the next step, DE-mRNA enrichment analysis of the shared mRNAs has been performed using the Kyoto Encyclopedia of Genes and Genomes (KEGG) and the FunRich tool. Finally, protein-protein interaction (PPI) and miRNA-mRNA networks were constructed to identify the most significant DE-mRNAs and their corresponding targets. In total, hub miRNA-mRNA was retrieved from 20 nodes and 24 edges, including 9 mRNAs: *CXCL8, IL1B, TLR4, PTPRC, AIF1, CXCR2, TLR6, CD86,* and *ILIRN*, and 11 predicted miRNAs included; has-miR-155-5p, has-miR-126-3p, has-miR-146a-5p, has-miR-21-5p, has-miR-19b-3p, has-miR-19a-3p, has-miR-106b-5p, has-miR-212-3p, has-miR-93-5p, has-miR-20a-5p, and has-miR-17-5p. Top-up-regulated genes were enriched in the immune system, immune response, and top-down-regulated genes were enriched in carbohydrate metabolism and sphingolipid metabolism pathways. The findings of the current study might help researchers shine a spotlight on the role of immune biomarkers in the pathogenesis and development of PCOS.

Keywords: *Polycystic ovary syndrome, inflammatory immune response, hub genes, hub miRNAs, bioinformatics analysis*

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Introduction

PCOS is a common endocrine and metabolic disorder that affects women of reproductive age globally, with reported incidence rates varying between 5% and 26%, depending on the diagnostic criteria utilized [1]. PCOS has been linked to various health conditions, such as ovulatory infertility, hypertension, type 2 diabetes, obesity, cardiovascular disease, and metabolic syndrome [2]–[4]. Investigations into genetic biomarkers have the potential to facilitate prompt detection, molecular categorization of diseases in medical circumstances, and comprehension of the fundamental molecular pathways implicated in diseases.

Granulosa cells (GCs) have gained increasing significance in recent years in the investigation of the pathophysiology of PCOS. GC dysfunction and/or proliferative dysregulation are thought to cause PCOS and an inadequate ovarian response [5], [6]. Therefore, GCs represent a good cellular model for studying PCOS and poor ovarian response (POR). Peripheral blood mononuclear cells (PBMCs) have emerged as valuable non-invasive tissue for detecting interactive factors that may contribute to PCOS.

Biomarkers in PBMC could potentially reflect an individual's physiological and pathological health status in response to changes taking place in diverse tissues throughout the body [7], [8]. mRNAs and miRNAs, two important molecular components, have a significant impact on the control of gene expression. mRNAs and miRNAs have been utilized to identify specific gene expression patterns associated with disease and assist in diagnosis and prognosis [9], [10]. Targeting miRNAs may result in better treatment outcomes for cancer and other disorders, according to several studies. Overall, the use of mRNAs and miRNAs in the diagnosis and management of diseases constitutes a dynamic field of study with the potential to transform personalized medicine.

The present study involved the comparison of two Gene Expression Omnibus (GEO) studies, GSE34526 about GCs and GSE54248 pertaining to PBMCs, in order to find overlapped and key possible biomarkers in PCOS patients. The aberrant DE-mRNA transcripts that successfully underwent

screening were subjected to prediction analysis for potential target miRNAs. The study conducted a functional enrichment analysis and Gene Ontology (GO) annotation to explore the possible functions of genes. Furthermore, in order to elucidate the physical associations among targets, a network of protein-protein interactions was established for further scrutiny. The primary aim of this investigation was to identify core and common DE-mRNAs between two vital tissues in the pathogenesis of PCOS. Further, in vitro and in vivo investigations may validate these findings, thereby facilitating prompt identification and enhanced management of PCOS.

Methods

Acquisition of microarray data

We obtained the gene expression data for GSE34526 from NCBI-GEO, which is a public database that provides gene expression profiles (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE34526; accessed November 6, 2012) and GSE54248 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE54248; accessed December 31, 2022) from PCOS paired with normal controls for bioinformatics analysis.

Data processing

Background correction and normalization were performed. DE-mRNAs were identified between PCOS and normal specimens with log2FC ≥ 1, log2FC ≤ -0.5, and a p-value < 0.05 after using the GEO2R online tools available on the NCBI-GEO website [11]. To visualize the correlation heat map, DEGs heat map, volcano plot, and PCA plot, the GEOexplorer (https://geoexplorer.rosalind.kcl.ac.uk; accessed July 5, 2022), web server, and R package were employed [12]. The Venn online software was then used to identify common DE-mRNAs among the two datasets.

KEGG and gene enrichment analysis

ShinyGO v0.77 is an online tool (http://bioinformatics.sdstate.edu/go/; accessed April 19, 2022) that visualizes and conducts gene ontology pathway enrichment analysis [13]. Through ShinyGO, KEGG pathways were enriched (FDR cutoff 0.05). FunRich v3.1.4 is an open-access software for functional enrichment analysis (http://funrich.org/index.html; accessed December 2, 2017) that integrates the functions of genes and biological pathway enrichment analysis [14]. Using FunRich, we identified the unique biological properties of the overlapped up- and down-regulated DE-mRNAs. DE-mRNAs were enriched in molecular function (MF), cellular components (CC), and biological processes (BP) (p-value < 0.05).

PPI network construction

The search tool for the retrieval of interacting genes, STRING v11.5 (https://string-db.org/; accessed August 12, 2021), an online tool was used to evaluate PPI information [15]. Thereafter, Cytoscape software v3.7.2 was utilized to visualize the potential correlation and interaction between these DE-mRNAs (maximum number of interactors = 0 and confidence score ≥ 0.4) [16]. The 30 hub targets in the network were selected using the CytoHubba plugin [17].

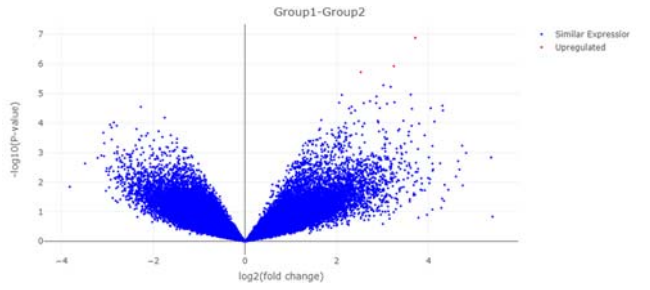
miRNAs prediction and network construction

The next objective was to identify potential target miRNAs for the DE-mRNAs. The miRNA targets, which were predicted and validated based on the miRTarBase and TargetScan, were retrieved using the miRNet v2.0 online tool (https://www.mirnet.ca/miRNet/home.xhtml; accessed on January 13, 2023). with a degree cutoff of 0.0 and a betweenness cutoff of 0.0 [18]. The validated databases in miRNet were filtered for the human organism and the specific tissue of interest, peripheral blood, following the study objectives.

Results

Identification of DE-mRNAs in PCOS

Using the GEO2R online tool, we identified 2912 and 1447 DEGs from GSE34526 and GSE54248, respectively (p-value < 0.05). The results were visualized using a volcano plot and a PCA plot (**Fig 1a-d**). The classification of mRNAs was illustrated by a hierarchical clustering heat map (**Fig 1e-f**). Additionally, the top 100 and top 10 DEGs were identified using a DEG heat map (**Fig 2a-d**). We further used a Venn diagram to identify overlapped DE-mRNAs (**Fig 2e-g**), which resulted in 232 DE-mRNAs. Among these, we selected 81 up-regulated DEGs (log2FC ≥1) and 46 down-regulated DEGs (log2FC≤ -0.5).



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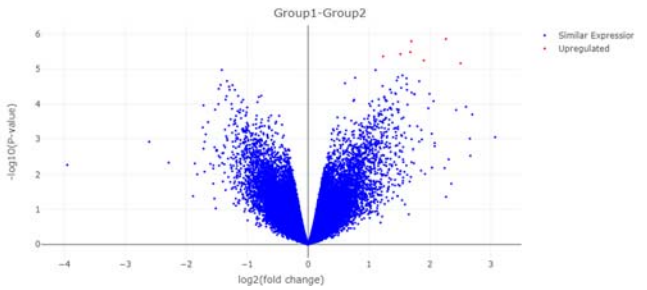

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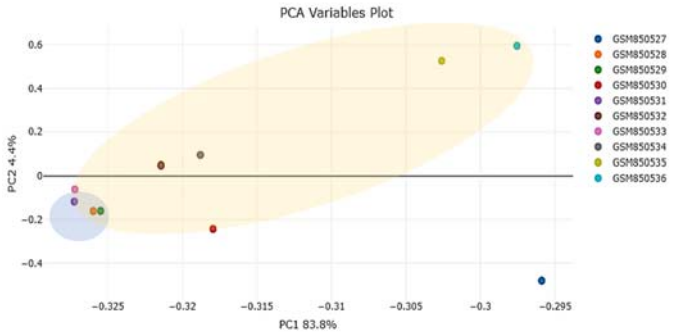

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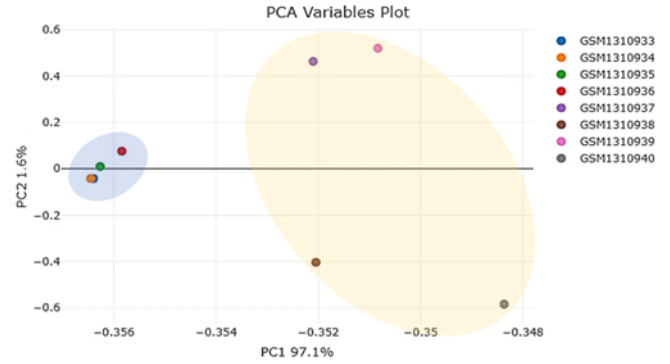

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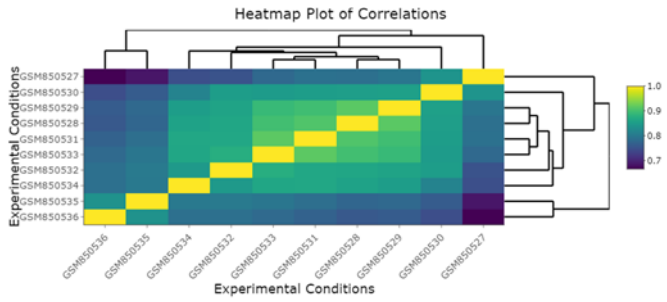

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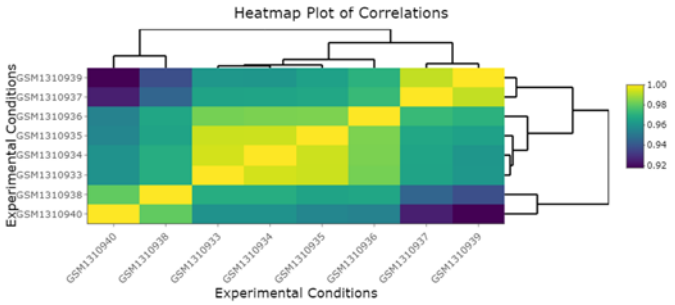

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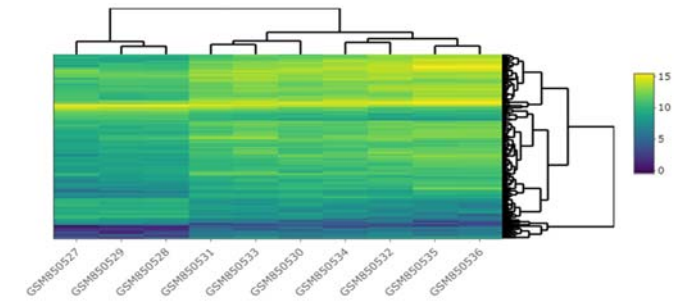
Fig 1 An overview of the mRNA gene expression profile is illustrated; (a, b) volcano plots have been shown a visual identification of genes with significant fold changes and p-value; (c, d) principal component analyses (PCA) plots display groups of data based on the similarities between them; (e, f) correlation heatmaps that are color-coded and present matrixes illustrating the correlation between different variables in GSE34526 and GSE54248.



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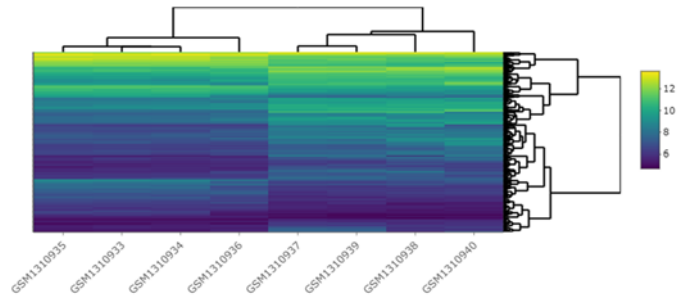

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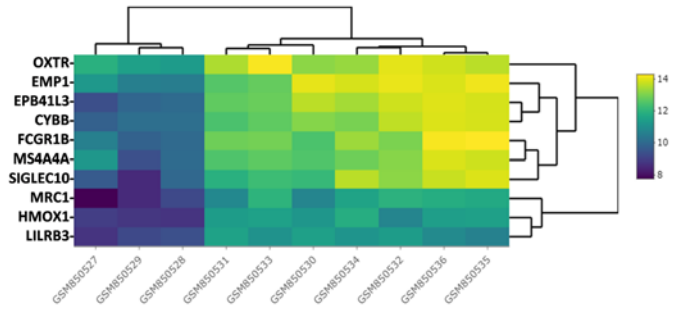

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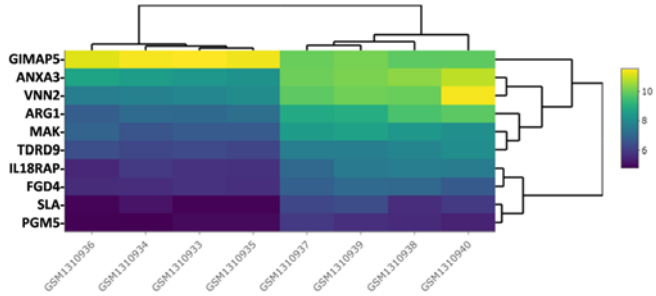

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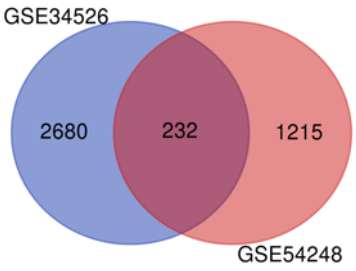

Log2FC ≥ 1, log2FC ≤ -0.5, p-value < 0.05

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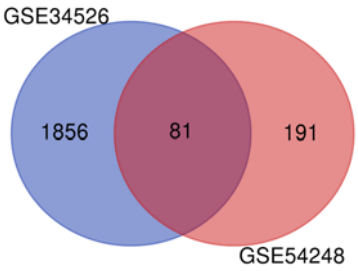

Log2FC ≥ 1, p-value < 0.05

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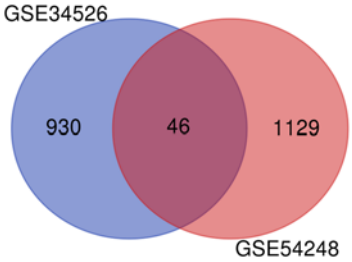

Log2FC ≤ -0.5, p-value < 0.05

(**g**)

Fig 2 (a, b) a heat map plot of the top 100 DE-mRNAs in PCOS compared to normal is displayed in a grid, and rows indicate the specific differentially expressed genes and samples represented in each column; (c, d) a heat map plot of the top 10 DE-mRNAs in PCOS compared to normal; (e) total overlapped DE-mRNAs in GSE34526 and GSE54248; (f) up-regulated and overlapped DE-mRNAs in GSE34526 and GSE54248; (g) down-regulated and overlapped DE-mRNAs in GSE34526 and GSE54248.

Functional enrichment analyses

To examine the biological properties and gain an overview of the overlapped DE-mRNAs, all 127 common genes were first submitted to ShinyGO and then KEGG pathway analysis was conducted. The results of the KEGG pathway analysis identified the top two significant diseases: legionellosis and leishmaniasis which are related to PCOS (**Fig 3**). In the second step, we conducted separate analyses for up- and down-regulated DE-mRNAs using FunRich software (**Table 1-3**).

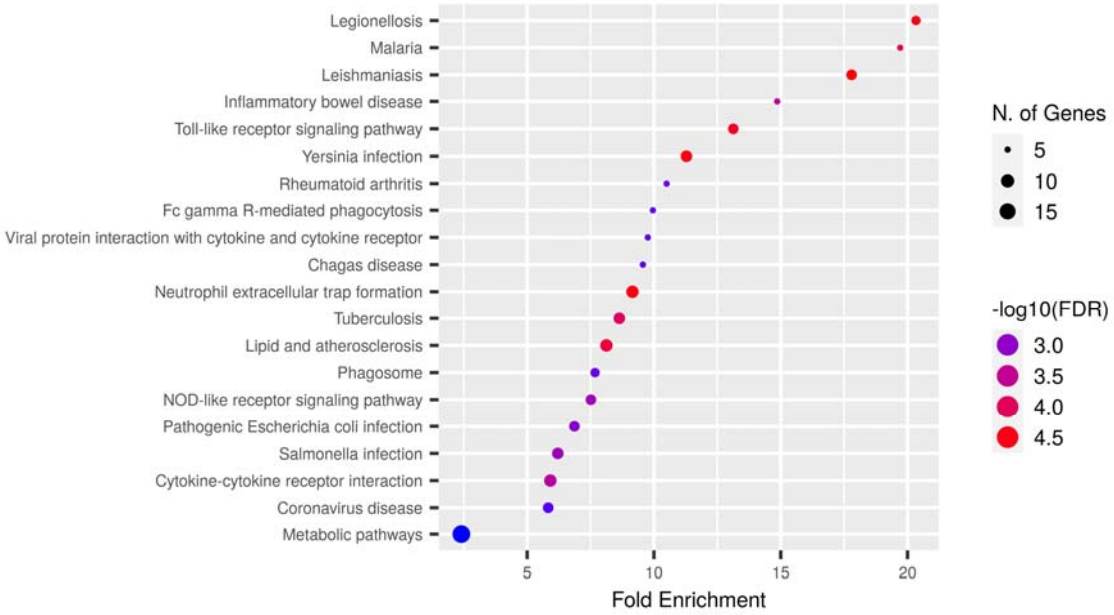

Fig 3 Visualization of the top and most relevant disease pathways that are enriched in the Kyoto Encyclopedia of Genes and Genomes (KEGG) with an FDR cutoff 0.05.

Table 1 Biological Process for up- and down-regulated DE-mRNAs.

Biological process	**Fold enrichment**	**P-value**	**Bonferroni method**
Up-regulated			
* Immune response	5.116315	1.32E-06	0.000235
* Cell communication	1.76983	0.000794	0.141338
* Signal transduction	1.727987	0.000924	0.16455
* Protein localization	76.00898	0.013184	1
* Innate immune response	57.05412	0.017541	1
Cell migration	15.24231	0.064227	1
Energy pathway	1.527244	0.103205	1
Metabolism	1.481872	0.120605	1
Anti-apoptosis	7.147361	0.132104	1
Regulation of cell cycle	4.013103	0.223181	1
Down-regulated			
* Carbohydrate metabolism	98.84748	0.000175	0.031106
* Mitochondrial transport	79.41192	0.012627	1
* Protein metabolism	2.087166	0.047782	1
RNA metabolism	15.29618	0.063981	1
Lipid metabolism	12.05252	0.080511	1
Regulation of gene expression, epigenetic	6.027174	0.154669	1
Biological process unknown	1.249767	0.19881	1
Cell growth and/or maintenance	1.404074	0.319202	1
Transport	1.30007	0.372105	1
Energy pathways	1.208512	0.40064	1
an asterisk indicated a p-value < 0.05			

Table 2 Cellular Component for up- and down-regulated DE-mRNAs.

Cellular component	Fold enrichment	P-value	Bonferroni method
Up-regulated			
* Plasma membrane	2.2870084	9.26E-09	7.26E-06
* Integral to the plasma membrane	2.4947392	0.002796	1
* External side of mitochondrial outer membrane	194.01426	0.005154	1
* Mast cell granule	194.01426	0.005154	1
* Exosomes	1.805283	0.006584	1
* Barr body	65.101132	0.015382	1
* Interleukin-6 receptor complex	65.101132	0.015382	1
* Exocytic vesicle	65.101132	0.015382	1
* Extracellular space	2.8861309	0.017776	1
* Extracellular	1.7020007	0.021977	1
Down-regulated			
* Isoamylase complex	393.21832	0.002542	1
* ER to Golgi transport vesicle	197.58731	0.005079	1
* UBC13-MMS2 complex	197.58731	0.005079	1
* Mitochondrion	2.5017107	0.012272	1
* Axin-APCbeta-catenin-GSK3B complex	66.081614	0.015161	1
* ESC/E(Z) complex	44.078857	0.022657	1
* Cytoplasmic membrane-bounded vesicle	20.891662	0.047245	1
* Clathrin-coated vesicle	20.891662	0.047245	1
Membrane	3.3815809	0.05875	1
Cytoplasmic vesicle	4.9394964	0.062278	1
an asterisk indicated a p-value < 0.05			

Table 3 Molecular Function for up- and down-regulated DE-mRNAs.

Molecular function	Fold enrichment	P-value	Bonferroni method
Up-regulated			
* Receptor activity	6.28129	4.14E-06	0.000928
* Transmembrane receptor activity	17.46592	8.11E-05	0.018164
* Receptor signaling complex scaffold activity	4.931541	0.000548	0.122751
* Cytokine activity	8.48895	0.001287	0.288206
* Complement receptor activity	45.66859	0.021877	1
* Oxidoreductase activity	4.234944	0.03438	1
Inward rectifier channel	16.33117	0.060072	1
Hydrolase activity	3.358792	0.060776	1
Receptor signaling protein tyrosine phosphatase activity	14.29105	0.068358	1
Peroxidase activity	11.43427	0.084713	1
down-regulated			
* Protein domain-specific binding	66.20228	0.015133	1
* Cytoskeletal protein binding	5.438963	0.017823	1
* Glucosidase activity	44.15934	0.022616	1
* Translation regulator activity	7.838947	0.027142	1
Oxidoreductase activity	4.917782	0.062946	1

Molecular function	Fold enrichment	P-value	Bonferroni method
Ubiquitin-specific protease activity	3.145138	0.070351	1
Protein serine/threonine phosphatase activity	9.040574	0.105906	1
Transporter activity	2.058555	0.17926	1
Receptor signaling complex scaffold activity	2.458967	0.19671	1
Serine-type peptidase activity	4.059542	0.220968	1
an asterisk indicated a p-value < 0.05			

Construction of the PPI network

To analyze the PPI information of the 127 DE-mRNAs, we used the STRING online database and Cytoscape software to construct a PPI network. A total of 127 DE-mRNAs were included in the PPI network, which comprised 63 nodes, and 280 edges, and consisted of 54 up-regulated and 9 down-regulated DE-mRNAs. We then constructed PPI networks based on the maximal clique centrality (MCC) module, including the top 30 DE-mRNAs with and without neighbors, and expanded the network.

miRNA-mRNA network construction

To predict miRNAs and related hub miRNA-mRNA network, we first submitted the 16 DE-mRNAs identified in the previous step to the miRNet online web tool. We then filtered the expressed tissue for miRNAs in peripheral blood, according to the study objective. The miRNet web tool retrieved a total of 20 nodes, 24 edges, including 9 mRNAs and 11 miRNAs (**Table 4**). It is important to note that six DE-mRNAs were not present in the hub miRNA-mRNA network according to tissue-specific network analyses.

Table 4 miRNA-mRNA network interactions ranked based on correlation degrees.

Components	Gene symbol	Degree	Betweenness
mRNA	*CXCL8*	9	105.1667
miRNA	has-miR-155-5p	5	69.16667
miRNA	has-miR-126-3p	5	44.66667
miRNA	has-miR-146a-5p	4	28
miRNA	has-miR21-5p	3	6.166667
mRNA	*IL1B*	3	13.16667
mRNA	*TLR4*	3	9.833333
mRNA	*PTPRC*	3	35
mRNA	*AIF1*	2	3.83333
miRNA	has-miR-93-5p	1	0
miRNA	has-miR-19a-3p	1	0
miRNA	has-miR-106b-5p	1	0
miRNA	has-miR-17-5p	1	0
miRNA	has-miR-20a-5p	1	0
miRNA	has-miR-212-3p	1	0
mRNA	*CD86*	1	0
mRNA	*IL1RN*	1	0
mRNA	*TLR6*	1	0
mRNA	*CXCR2*	1	0

Discussion

In recent years, there has been an increasing focus on the role of GCs in the pathophysiology of PCOS. GCs play a crucial role in follicular development. Their dysfunction can lead to abnormal folliculogenesis, characterized by the presence of small antral follicles that fail to mature into dominant ones, which is a hallmark of PCOS. Studying GCs requires invasive procedures. To address this challenge, we utilized two publicly available GEO studies, namely GSE34526 and GSE54248, which examined GCs and PBMCs, respectively. By analyzing the data from these studies computationally, we aimed to identify potential biomarkers that are shared between GCs and blood samples, offering a non-invasive approach to studying PCOS. Our in-silico analyses identified 9 core common DE-

mRNAs in GCs and PBMCs between PCOS patients and healthy controls. These DE-mRNAs include *CXCL8, IL1B, TLR4, PTPRC, AIF1, CXCR2, TLR6, CD86*, and *ILIRN*. Additionally, we predicted the involvement of 11 miRNAs, namely has-miR-155-5p, has-miR-126-3p, has-miR-146a-5p, has-miR-21-5p, has-miR-19b-3p, has-miR-19a-3p, has-miR-106b-5p, has-miR-212-3p, has-miR-93-5p, has-miR-20a-5p, and has-miR-17-5p, in the regulation of these DE-mRNAs. This integrative analysis provides valuable insights into potential molecular biomarkers that may contribute to the understanding of PCOS pathogenesis. Furthermore, the use of blood-based biomarkers offers a non-invasive and easily accessible means of studying PCOS. However, further experimental validation is required to confirm the functional relevance and diagnostic potential of these identified DE-mRNAs and miRNAs in PCOS.

Notably, *IL1B* emerged as a significant DE-mRNA associated with PCOS etiology. *IL1B* is involved in the immune system and has been linked to type 2 diabetes [19] and PCOS [20]. Additionally, *CXCL8*, a protein regulating acute inflammation [21], showed elevated levels in adipose tissue [22] and may contribute to the development of PCOS [23]. These findings highlight *IL1B* and *CXCL8* as potential therapeutic targets for PCOS treatment, given their involvement in inflammation and immune regulation. CX-C Motif Chemokine Receptor 2 (*CXCR2*) is a protein-coding gene that belongs to the G-protein-coupled receptor family and is involved in cell communication. It acts as a receptor for interleukin-8 and is associated with neutrophil chemotaxis. Clinical trials are currently assessing *CXCR2* antagonists for their potential in treating cancer [24] and inflammatory diseases [25]. Additionally, *CXCR2* deficiency in mice has been linked to protection against diet-induced insulin resistance and diabetes, which are risk factors for PCOS [26], [27]. Thus, targeting *CXCR2* may hold promise as a therapeutic approach for PCOS.

miRNAs are conserved molecules that originate from longer hairpin precursors and are typically 22 nucleotides in length. They regulate gene expression by degrading mRNA or inhibiting mRNA translation. These molecules play important roles in the post-transcriptional regulation of various cellular processes, including development, differentiation, and signaling. Abnormal expression of miRNAs has been associated with diseases such as cancer and immunological dysfunction. miRNAs primarily target the 3' untranslated regions of mRNA, leading to the inhibition of gene expression at the post-transcriptional level [28]. Additionally, miRNA recognition elements (MREs) can also target protein-coding sequences (CDS) [29]. Based on miRNA-mRNA network analysis, 11 miRNAs were predicted. Among them, has-miR-126-3p, has-miR-155-5p, has-miR-146a-5p, and has-miR-21-5p are known as master regulators. Has-miR-155-5p is a pro-inflammatory master regulator, that regulates macrophage M1 polarization and apoptosis [30], and down-regulation of miR-155-5p in blood leukocytes associated with gestational diabetes in PCOS women [31]. In addition, down-regulation of miR-155-5p was confirmed in ovarian cancer's (OC) advanced stage compared with the early stage [32]. Has-miR-155-5p has been associated with gestational diabetes [33], and follicular dysplasia of GCs in PCOS [34], as well as inflammation and insulin resistance biomarkers [35]. Interestingly, C-X-C Motif Chemokine Ligand 8 (*CXCL8*), has been shown highest connections in our in-silico analyses and may have potential regulatory roles in the PCOS development through the Cytokine-cytokine receptor interaction and inflammatory pathways. *CXCL8* is a gene that codes for a protein and plays a big part in regulating acute inflammation [21]. Lower serum levels of has-miR-126-3p and has-miR-146a-5p were observed in PCOS patients compared to controls [36], suggesting their potential as biomarkers for PCOS prognosis and treatment. Moreover, has-miR-126-3p has been linked to wound healing in diabetes patients [37], while its regulatory role in endometrial receptivity has been validated [38]. Has-miR-146a-5p has shown lower expression in ovarian tumor tissue and insulin-resistant PCOS rat ovaries [39], and its modulation improves therapeutic efficacy in ovarian cancer [40]. Hsa-miR-21-5p is a circulating miRNA that could be found in various extracellular fluids, including blood, plasma, and CSF [41]. Has-miR-21-5p has been implicated in various biological processes, including angiogenesis and inflammatory responses [42]. Hence, it is a circulating biomarker in the blood of patients with thymic malignancies [43], or an independent prognostic biomarker in pancreatic ductal adenocarcinoma [44]. Has-miR-21-5p plasma expression level was reported as an independent diagnostic biomarker in the OC [45]. Also, hsa-miR-21-5p has been recognized as a diagnostic biomarker in infertile women with dysregulated AMH levels [46]. Also, down-regulation of has-miR-21-5p in PCOS affects cell apoptosis and may serve as a diagnostic biomarker for treatment and identifying infertile women [47].

Conclusion

PCOS is a complex endocrine disorder with an unknown cause that affects a substantial proportion of women of reproductive age. Immune system abnormalities are believed to exert a substantial impact on the development and progression of PCOS. In the current study, the GSE34526 dataset derived from GC samples and the GSE54248 dataset derived from blood samples were analyzed using multiple bioinformatics approaches. Subsequently, a pathway enrichment analysis was performed on the DE-mRNAs that were common to both datasets. Interestingly, top-up-regulated genes based on GO were enriched in the immune system and immune response

pathways, and top-down-regulated genes were enriched in carbohydrate metabolism and sphingolipid metabolism pathways. The results of the present study provide evidence for the substantial and influential involvement of the immune system and interleukin-mediated inflammatory responses in the pathogenesis and advancement of PCOS.

Credit Authorship Contribution Statement

Roozbeh Heidarzadehpilehrood: Conceptualization, Methodology, Investigation, Software, Writing – original draft, Visualization. **Maryam Pirhoushiaran**: Software, Formal analysis, Writing – review & editing, Resources, Data curation. **Malina Binti Osman**: Software, Validation, Resources. **King-Hwa Ling**: Project administration, Writing – review & editing. **Habibah Abdul Hamid**: Supervision, Funding acquisition, Resources, Project administration.

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References

[1] A. N. Elasam *et al.*, "The prevalence and phenotypic manifestations of polycystic ovary syndrome (PCOS) among infertile Sudanese women: a cross-sectional study," *BMC Women's Health*, vol. 22, no. 1, pp. 1–7, Dec. 2022, doi: 10.1186/S12905-022-01762-6/TABLES/3.

[2] S. M. Sirmans and K. A. Pate, "Epidemiology, diagnosis, and management of polycystic ovary syndrome," *Clinical Epidemiology*, vol. 6, no. 1, pp. 1–13, Dec. 2013, doi: 10.2147/CLEP.S37559.

[3] O. Osibogun, O. Ogunmoroti, and E. D. Michos, "Polycystic ovary syndrome and cardiometabolic risk: Opportunities for cardiovascular disease prevention," *Trends in Cardiovascular Medicine*, vol. 30, no. 7, pp. 399–404, Oct. 2020, doi: 10.1016/J.TCM.2019.08.010.

[4] W. Chen, Y. Pang, J. W. Hill, and P. Meikle, "Metabolic Syndrome and PCOS: Pathogenesis and the Role of Metabolites," *Metabolites 2021, Vol. 11, Page 869*, vol. 11, no. 12, p. 869, Dec. 2021, doi: 10.3390/METABO11120869.

[5] M. Cozzolino and E. Seli, "Mitochondrial function in women with polycystic ovary syndrome," *Current opinion in obstetrics & gynecology*, vol. 32, no. 3, pp. 205–212, Jun. 2020, doi: 10.1097/GCO.0000000000000619.

[6] B. O. Yildiz, G. Bozdag, Z. Yapici, I. Esinler, and H. Yarali, "Prevalence, phenotype and cardiometabolic risk of polycystic ovary syndrome under different diagnostic criteria," *Human reproduction (Oxford, England)*, vol. 27, no. 10, pp. 3067–3073, 2012, doi: 10.1093/HUMREP/DES232.

[7] S. Yin *et al.*, "Peripheral blood circulating microRNA-4636/−143 for the prognosis of cervical cancer," *Journal of Cellular Biochemistry*, vol. 121, no. 1, pp. 596–608, Jan. 2020, doi: 10.1002/JCB.29305.

[8] E. Shabani Varaki, G. D. Gargiulo, S. Penkala, and P. P. Breen, "Peripheral vascular disease assessment in the lower limb: a review of current and emerging non-invasive diagnostic methods," *BioMedical Engineering OnLine 2018 17:1*, vol. 17, no. 1, pp. 1–27, May 2018, doi: 10.1186/S12938-018-0494-4.

[9] L. Heidari *et al.*, "Reverse expression pattern of sirtuin-1 and histone deacetylase-9 in coronary artery disease," *https://doi.org/10.1080/13813455.2020.1797100*, vol. 129, no. 1, pp. 46–53, 2020, doi: 10.1080/13813455.2020.1797100.

[10] A. Rahimpour *et al.*, "Bioinformatics Analysis of MicroRNA Profiles Unveils Novel Biological Markers of Alzheimer's Disease," *Neurochemical Journal 2022 16:3*, vol. 16, no. 3, pp. 334–342, Oct. 2022, doi: 10.1134/S1819712422030096.

[11] D. Sean and P. S. Meltzer, "GEOquery: a bridge between the Gene Expression Omnibus (GEO) and BioConductor," *Bioinformatics*, vol. 23, no. 14, pp. 1846–1847, Jul. 2007, doi: 10.1093/BIOINFORMATICS/BTM254.

[12] G. P. Hunt *et al.*, "GEOexplorer: a webserver for gene expression analysis and visualisation," *Nucleic Acids Research*, vol. 50, no. W1, pp. W367–W374, Jul. 2022, doi: 10.1093/NAR/GKAC364.

[13] S. X. Ge, D. Jung, D. Jung, and R. Yao, "ShinyGO: a graphical gene-set enrichment tool for animals and plants," *Bioinformatics*, vol. 36, no. 8, pp. 2628–2629, Apr. 2020, doi: 10.1093/BIOINFORMATICS/BTZ931.

[14] P. Fonseka, M. Pathan, S. V. Chitti, T. Kang, and S. Mathivanan, "FunRich enables enrichment analysis of OMICs datasets," *Journal of molecular biology*, vol. 433, no. 11, May 2021, doi: 10.1016/J.JMB.2020.166747.

[15] D. Szklarczyk *et al.*, "The STRING database in 2023: protein-protein association networks and functional enrichment analyses for any sequenced genome of interest," *Nucleic acids research*, vol. 51, no. D1, pp. D638–D646, Jan. 2023, doi: 10.1093/NAR/GKAC1000.

[16] P. Shannon *et al.*, "Cytoscape: A Software Environment for Integrated Models of Biomolecular Interaction Networks," *Genome Research*, vol. 13, no. 11, pp. 2498–2504, Nov. 2003, doi: 10.1101/GR.1239303.

[17] C. H. Chin, S. H. Chen, H. H. Wu, C. W. Ho, M. T. Ko, and C. Y. Lin, "cytoHubba: Identifying hub objects and sub-networks from complex interactome," *BMC Systems Biology*, vol. 8, no. 4, pp. 1–7, Dec. 2014, doi: 10.1186/1752-0509-8-S4-S11/TABLES/4.

[18] L. Chang and J. Xia, "MicroRNA Regulatory Network Analysis Using miRNet 2.0," *Methods in Molecular Biology*, vol. 2594, pp. 185–204, 2023, doi: 10.1007/978-1-0716-2815-7_14/COVER.

[19] T. Cirelli *et al.*, "Genetic polymorphisms in the Interleukins IL1B, IL4, and IL6 are associated with concomitant periodontitis and type 2 diabetes mellitus in Brazilian patients," *Journal of Periodontal Research*, vol. 55, no. 6, pp. 918–930, Dec. 2020, doi: 10.1111/JRE.12784.

[20] R. Herman, M. Jensterle, A. Janež, K. Goričar, and V. Dolžan, "Genetic Variability in Antioxidative and Inflammatory Pathways Modifies the Risk for PCOS and Influences Metabolic Profile of the Syndrome," *Metabolites 2020, Vol. 10, Page 439*, vol. 10, no. 11, p. 439, Oct. 2020, doi: 10.3390/METABO10110439.

[21] S. Thompson *et al.*, "Nitration of chemokine CXCL8 acts as a natural mechanism to limit acute inflammation," *Cellular and Molecular Life Sciences*, vol. 80, no. 1, pp. 1–14, Jan. 2023, doi: 10.1007/S00018-022-04663-X/FIGURES/8.

[22] R. S. Lima *et al.*, "CXCL8 expression and methylation are correlated with anthropometric and metabolic parameters in childhood obesity," *Cytokine*, vol. 143, p. 155538, Jul. 2021, doi: 10.1016/J.CYTO.2021.155538.

[23] M. Jenabi, P. Khodarahmi, F. Tafvizi, and S. Z. Bostanabad, "Evaluation of expression CXCL8 chemokine and its relationship with oocyte maturation and embryo quality in the intracytoplasmic sperm injection method," *Molecular Biology Reports*, vol. 49, no. 9, pp. 8413–8427, Sep. 2022, doi: 10.1007/S11033-022-07660-2/METRICS.

[24] Y. Cheng *et al.*, "Targeting CXCR2 inhibits the progression of lung cancer and promotes therapeutic effect of cisplatin," *Molecular Cancer*, vol. 20, no. 1, pp. 1–21, Dec. 2021, doi: 10.1186/S12943-021-01355-1/FIGURES/9.

[25] H. Zhao *et al.*, "Blocking the CXCL1-CXCR2 axis enhances the effects of doxorubicin in HCC by remodelling the tumour microenvironment via the NF-κB/IL-1β/CXCL1 signalling pathway," *Cell Death Discovery 2023 9:1*, vol. 9, no. 1, pp. 1–10, Apr. 2023, doi: 10.1038/s41420-023-01424-y.

[26] J. G. Neels, L. Badeanlou, K. D. Hester, and F. Samad, "Keratinocyte-derived chemokine in obesity. Expression, regulation, and role in adipose macrophage infiltration and glucose homeostasis," *Journal of Biological Chemistry*, vol. 284, no. 31, pp. 20692–20698, Jul. 2009, doi: 10.1074/JBC.M109.018556/ATTACHMENT/D81E0D7B-83DF-4481-AA7C-355A1F713F4E/MMC1.PDF.

[27] C. Chavey *et al.*, "CXC Ligand 5 Is an Adipose-Tissue Derived Factor that Links Obesity to Insulin Resistance," *Cell Metabolism*, vol. 9, no. 4, pp. 339–349, Apr. 2009, doi: 10.1016/J.CMET.2009.03.002.

[28] S. Gu, L. Jin, F. Zhang, P. Sarnow, and M. A. Kay, "The biological basis for microRNA target restriction to the 3' untranslated region in mammalian mRNAs," *Nature structural & molecular biology*, vol. 16, no. 2, p. 144, Feb. 2009, doi: 10.1038/NSMB.1552.

[29] M. Thomas, J. Lieberman, and A. Lal, "Desperately seeking microRNA targets," *Nature Structural & Molecular Biology 2010 17:10*, vol. 17, no. 10, pp. 1169–1174, Oct. 2010, doi: 10.1038/nsmb.1921.

[30] G.-S. Li, L. Cui, and G.-D. Wang, "miR-155-5p regulates macrophage M1 polarization and apoptosis in the synovial fluid of patients with knee osteoarthritis," *Experimental and Therapeutic Medicine*, vol. 21, no. 1, pp. 1–1, Jan. 2021, doi: 10.3892/ETM.2020.9500.

[31] M. Hocaoglu *et al.*, "Identification of miR-16-5p and miR-155-5p microRNAs differentially expressed in circulating leukocytes of pregnant women with polycystic ovary syndrome and gestational diabetes," *https://doi.org/10.1080/09513590.2020.1843620*, vol. 37, no. 3, pp. 216–220, 2020, doi: 10.1080/09513590.2020.1843620.

[32] S. N. Chasanah *et al.*, "The Expression of microRNA-155 and mRNA Hypoxia Inducible Factor Alpha (HIF1A) in the early and advanced stages of ovarian cancer patients blood plasma," *Journal of the Medical Sciences (Berkala Ilmu Kedokteran)*, vol. 48, no. 4, pp. 22–22, Feb. 2017, doi: 10.19106/JMEDSCIESUP004804201620.

[33] M. Hocaoglu *et al.*, "Identification of miR-16-5p and miR-155-5p microRNAs differentially expressed in circulating leukocytes of pregnant women with polycystic ovary syndrome and gestational diabetes," *https://doi.org/10.1080/09513590.2020.1843620*, vol. 37, no. 3, pp. 216–220, 2020, doi: 10.1080/09513590.2020.1843620.

[34] J. Cao *et al.*, "Follicular fluid-derived exosomal miR-143-3p/miR-155-5p regulate follicular dysplasia by modulating glycolysis in granulosa cells in polycystic ovary syndrome," *Cell Communication and Signaling*,

vol. 20, no. 1, pp. 1–17, Dec. 2022, doi: 10.1186/S12964-022-00876-6/FIGURES/8.

[35] Y. Qin, Y. Wang, H. Zhao, Z. Yang, and Y. Kang, "Aberrant miRNA-mRNA regulatory network in polycystic ovary syndrome is associated with markers of insulin sensitivity and inflammation," *Annals of Translational Medicine*, vol. 9, no. 18, pp. 1405–1405, Sep. 2021, doi: 10.21037/ATM-21-1288.

[36] R. Li *et al.*, "Genetic Variants miR-126, miR-146a, miR-196a2, and miR-499 in Polycystic Ovary Syndrome," *British Journal of Biomedical Science*, vol. 79, p. 7, Jan. 2022, doi: 10.3389/BJBS.2021.10209/BIBTEX.

[37] L. Wang *et al.*, "Effects of Intensive Glycemic Control on Serum Exosome miR-126-3p and miR-125b-1-3p Levels and Wound Healing in Patients with Diabetic Ulcers," *Evidence-based Complementary and Alternative Medicine*, vol. 2023, 2023, doi: 10.1155/2023/2523245.

[38] W. Wang *et al.*, "Mechanism of human chorionic gonadotropin in endometrial receptivity via the miR-126-3p/PI3K/Akt/eNOS axis," *The Kaohsiung Journal of Medical Sciences*, Mar. 2023, doi: 10.1002/KJM2.12672.

[39] C. Zhang, C. Yu, Z. Lin, H. Pan, K. Li, and H. Ma, "MiRNAs expression profiling of rat ovaries displaying PCOS with insulin resistance," *Archives of Gynecology and Obstetrics 2020 302:5*, vol. 302, no. 5, pp. 1205–1213, Aug. 2020, doi: 10.1007/S00404-020-05730-Z.

[40] X. Li, Y. Jin, Z. Mu, W. Chen, and S. Jiang, "MicroRNA-146a-5p enhances cisplatin-induced apoptosis in ovarian cancer cells by targeting multiple anti-apoptotic genes," *International Journal of Oncology*, vol. 51, no. 1, pp. 327–335, Jul. 2017, doi: 10.3892/IJO.2017.4023/HTML.

[41] T. A. Petrova, S. A. Kondratyev, A. A. Kostareva, R. V. Rutkovskiy, I. A. Savvina, and E. A. Kondratyeva, "miR-21, miR-93, miR-191, miR-let-7b, and miR-499 Expression Level in Plasma and Cerebrospinal Fluid in Patients with Prolonged Disorders of Consciousness," *Neurology International 2023, Vol. 15, Pages 40-54*, vol. 15, no. 1, pp. 40–54, Dec. 2022, doi: 10.3390/NEUROLINT15010004.

[42] F. J. Sheedy, "Turning 21: Induction of miR-21 as a key switch in the inflammatory response," *Frontiers in Immunology*, vol. 6, no. JAN, p. 19, Jan. 2015, doi: 10.3389/FIMMU.2015.00019/BIBTEX.

[43] T. Bellissimo *et al.*, "Circulating miR-21-5p and miR-148a-3p as emerging non-invasive biomarkers in thymic epithelial tumors," *Cancer Biology and Therapy*, vol. 17, no. 1, pp. 79–82, Jan. 2016, doi: 10.1080/15384047.2015.1108493/SUPPL_FILE/KCBT_A_1108493_SM0956.PDF.

[44] P. Karasek *et al.*, "Pre-operative Plasma miR-21-5p Is a Sensitive Biomarker and Independent Prognostic Factor in Patients with Pancreatic Ductal Adenocarcinoma Undergoing Surgical Resection," *Cancer Genomics & Proteomics*, vol. 15, no. 4, pp. 321–327, Jul. 2018, doi: 10.21873/CGP.20090.

[45] D. N. P. Oliveira *et al.*, "Diagnostic plasma miRNA-profiles for ovarian cancer in patients with pelvic mass," *PLOS ONE*, vol. 14, no. 11, p. e0225249, Nov. 2019, doi: 10.1371/JOURNAL.PONE.0225249.

[46] I. Belguith *et al.*, "Diagnostic value of miR-199a and miR-21 in the plasma of infertile women with dysregulated AMH levels," *https://doi.org/10.1080/14647273.2020.1750715*, vol. 25, no. 1, pp. 154–165, 2020, doi: 10.1080/14647273.2020.1750715.

[47] G. De Nardo Maffazioli, E. C. Baracat, J. M. Soares, K. C. Carvalho, and G. A. R. Maciel, "Evaluation of circulating microRNA profiles in Brazilian women with polycystic ovary syndrome: A preliminary study," *PLOS ONE*, vol. 17, no. 10, p. e0275031, Oct. 2022, doi: 10.1371/JOURNAL.PONE.0275031.