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Biological Network Analysis of Genes and Non-coding RNAs in Polycystic Ovary Syndrome

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Abstract

BACKGROUND/AIMS: Polycystic ovary syndrome (PCOS) is a neuroendocrine and metabolic disorder. This study aimed to explore interactions among differentially expressed genes (DEGs) and Food and Drug Administration (FDA)-approved drugs relevant to PCOS using bioinformatics approaches. The relationships between top-ranked hub genes, DE-microRNAs (miRNAs), and DE-long non-coding RNAs (lncRNAs) within the competing endogenous RNA (ceRNA) network framework were also investigated.

MATERIALS AND METHODS: Expression profiles for miRNA (GSE138572), lncRNA (GSE159466), and mRNA (GSE84958, GSE156067) were obtained from the GEO database. Data analysis was performed using R Project and relevant annotation packages. Drug-gene interactions (DGI) were explored using DGIdb, and Cytoscape software was employed to construct DEG-drug interaction networks.

RESULTS: Analysis of GSE84958 and GSE156067 datasets identified 71 common DEGs. Moreover, 11 DE-miRNAs and 203 DE-lncRNAs were DE in PCOS samples compared to controls. DGIdb analysis showed that FDA-approved drugs could potentially target the identified DEGs. Nine genes-*PDE5A, ETFB, COL11A1, SLC25A20, DAPK1, ICAM1, FABP5, GBE1*, and *SLC2A3*-were identified as hub genes within the DGI network and were considered promising therapeutic targets. Dysregulated miRNAs were significantly enriched in the ceRNA network. This suggests their potential role in PCOS therapeutic strategies. gene ontology and kyoto encyclopedia of genes and genomes enrichment analyses yielded hub genes primarily associated with secretory granule membranes, extracellular exosomes, and postsynaptic density.

CONCLUSION: Based on a combined analysis of DEGs, DE-miRNAs and DE-lncRNAs, the present study sheds light on the molecular mechanisms underlying PCOS. The findings may help to identify potential therapeutic targets and contribute to the understanding of the pathogenesis of PCOS.

Keywords: PCOS, biological network analysis, DEGs

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a common disease, with estimated prevalence varying from 5% to 20%.[1] Recent literature suggests the involvement of dysregulation of noncoding RNAs, such as microRNAs (miRNAs), along with long non-coding RNAs (lncRNAs) in the development of PCOS.[2] For example, a balance between "coding" RNAs (mRNAs) and non-coding RNAs is necessary to control gene transcription and cellular activity.[3] Other comparative studies have shown particular non-coding and coding RNAs that are DE in PCOS, enabling the

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development of strategies for clinical diagnosis and treatment of this syndrome.[4] Moreover, a better understanding of the complexity of interrelations between coding and non-coding RNAs might help to build targeted methods of treatment, including the use of medications like metformin, ethinyl estradiol, and levonorgestrel, that have Food and Drug Administration (FDA)-approved. These drugs have been demonstrated to address a number of PCOS symptoms, including insulin sensitivity and hormone regulation, with well-established mechanisms of action. These mechanisms of action that call for additional research using bioinformatic techniques. Significant alterations in mRNA, miRNA, and lncRNA expression among PCOS patients relative to healthy individuals have been used to explain the pathophysiology of the disease.[5] From these studies, genes and regulatory networks that could be potential therapeutic targets have been identified. Nevertheless, there are still unanswered questions about the initial targets and modes of action of FDA-approved medications in relation to PCOS. By addressing the research gaps and emphasizing the novelty of this work, the current study aims to concentrate on the communication between different miRNAs, lncRNAs, and mRNAs in PCOS patients and how these molecular pathways might be implicated in the action of FDA-approved medications.

MATERIALS AND METHODS

We obtained the expression profiles of mRNA (GSE84958 and GSE156067), miRNA (GSE138572), and lncRNA (GSE159466) from the Gene Expression Omnibus database (https://www.ncbi.nlm.nih.gov/geo/).[1] We then processed and analyzed these data using R Project (version 4.0.1). Due to the limited sample sizes, potential batch effects were addressed by applying the remove Batch Effect function from the limma package (version 3.46.0). Differentially expressed genes (DEGs) were subsequently identified by employing the edgeR (version 3.32.1) and limma (version 3.46.0) packages. The normalization of read counts was performed using the trimmed mean of M-values method to correct for compositional biases between libraries. Normalized counts were then transformed into log counts per million values via the voom function from the limma package. The selection of DEGs was based on a threshold of $|\log_2 \text{fold change}| \geq 1$ and an adjusted p-value false discovery rate (FDR) <0.05. Overlapping DEGs between the two mRNA datasets (GSE84958 and GSE156067) were identified for further analysis. To analyze regulatory interactions, a ceRNA network was constructed by integrating mRNA, miRNAs, and long non-coding RNA expression data. Experimentally validated interactions between miRNAs and mRNAs were extracted from TarBase (http://www.grnpedia.org/tarbase)[2], miRTarBase (http://mirtarbase.mbc.nctu.edu.tw)[3], and miRecords (http://mirecords.umn.edu), while the miRNA-lncRNA interactions were obtained from starBase (https://rnasysu.com/encori/index.php)[4] and DIANA-LncBase (https://diana.e-ce.uth.gr/lncbasev3).[5] The ceRNA network was visualized using Cytoscape (https://cytoscape.org).[6] For the elaboration of the possible relationships between a drug and a gene, the DGIdB (https://www.dgidb.org)[7] was used, and then these interactions were illustrated by network diagrams constructed using Cytoscape software. For Gene Ontology (GO) and KEGG pathway enrichment analysis of both DEGs and hub genes, the database for annotation, visualization, and integrated discovery database was used (https://david.ncifcrf.gov)[8] with a FDR cutoff of 0.05. For Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis, R packages DOSE[9], Hs.egENSEMBL, and clusterProfiler[10] were used. Ethical approval was granted by the Institutional Review Board (NEU/2024/128-1900).

RESULTS

Four datasets were analyzed to detect DEGs, miRNAs, and lncRNAs related to PCOS. In GSE84958, composed of 22 normal, and 15 PCOS samples, 2054 genes had differential expression, with 1036 overexpressed ($\log_2$ fold change ≥1) and 1018 underexpressed ($\log_2$ fold change ≥1). GSE156067, with nine control samples and nine PCOS samples, revealed 796 DEGs, including 341 overexpressed and 455 underexpressed genes. A comparative analysis revealed 37 commonly upregulated, and 34 commonly downregulated genes between two datasets. Furthermore, our analysis of miRNAs expression from GSE138572 (five control and five PCOS samples) identified 11 DE-miRNAs. GSE159466, which includes three control and three PCOS samples, identified 203 DE-lncRNAs. GO and KEGG pathway analyses revealed the involvement of biological processes such as signal transduction, cell-cell adhesion. Mapped genes were predominantly located in the plasma membrane, mitochondria, and sarcoplasmic reticulum. Enrichment analysis showed that the genes were involved in hydrolase activity, focal adhesion, metabolic regulation, and cell-matrix adhesion. KEGG pathway analysis identified associations with viral myocarditis, Cushing's syndrome, and NK cell-mediated cytotoxicity (Figure 1A). A ceRNA network was constructed to examine interactions among lncRNAs, miRNAs, and mRNAs. Network analysis identified hsa-miR-561-5p, hsa-miR-1299, and hsa-miR-19b-1-5p as key miRNAs with the highest degree of interactions. GSE159466, composed of three control and three PCOS samples, identified 203 DE-lncRNAs. GO and KEGG pathway analyses determined the involvement of biological processes such as signal transduction, cell-cell adhesion. The mapped genes were found to be primarily located in the plasma membrane, mitochondria, and sarcoplasmic reticulum. Enrichment analysis showed molecular functions related to hydrolase activity and focal adhesion, as well as pathways involved in metabolic regulation and cell-matrix adhesion. KEGG pathway analysis identified associations with viral myocarditis, Cushing's syndrome, and NK cell-mediated cytotoxicity. To examine interactions among lncRNAs, miRNAs, and mRNAs, a ceRNA network was constructed. Network analysis pinpointed hsa-miR-561-5p, hsa-miR-1299, and hsa-miR-19b-1-5p as key miRNAs with the highest degree of interactions. NEAT1, H19, and KCNQ1OT1 presented high connectivity with genes linked to PCOS (see Figure 1B). The drug-gene interaction (DGI) database analysis revealed 71 DEGs of potential therapeutic relevance, and nine hub genes were identified: PDE5A, ETFB, COL11A1, SLC25A20, DAPK1, ICAM1, FABP5, GBE1, and SLC2A3 (Figure 1C). Furthermore, the functional annotation of these hub genes via GO and KEGG analyses indicated their localization to secretory granule membranes, extracellular vesicles, and postsynaptic density (Figure 1D). Further analysis integrated hub genes from the DGI network into the ceRNA regulatory framework. Several hub genes, including PDE5A, COL11A1, and ICAM1, were found to be regulated by multiple miRNAs. For instance, hsa-miR-767-5p targeted lncRNAs H19, LINC00840, and NEAT1, which in turn regulated DAPK1, COL11A1, and ICAM1. Similarly, hsa-miR-940 was associated with SLC25A20 through interactions with H19 and NEAT1. Additionally, hsa-miR-19b-1-5p was linked to lncRNAs H19, DANCR, and KCNQ1OT1, affecting the expression of PDE5A (Figure 1E).

DISCUSSION

The current study uncovers key molecular pathways and networks associated with PCOS pathogenesis. This result is in line with prior work that implicated these pathways involved in ovarian dysfunction and metabolic abnormalities in PCOS.[11-13] KEGG pathway analysis has

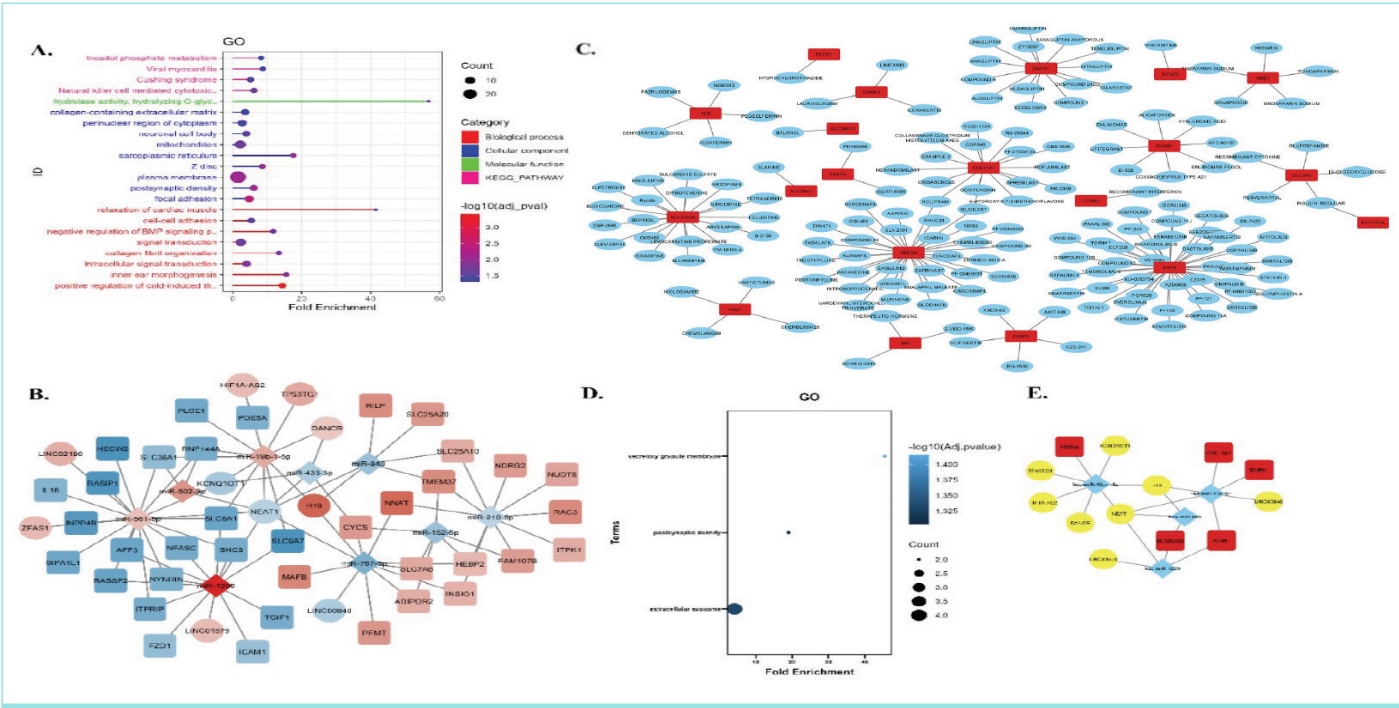

Figure 1. A) KEGG pathway and GO enrichment analysis for DEGs. B) ceRNA network: prism nodes indicate miRNAs. Quadrilateral nodes indicate the mRNA. Circles nodes that lncRNAs. C) Drug gene interaction network: The orange octagonal nodes indicate medications for PCOS, while the blue oval nodes represent FDA-approved drugs. The red rectangles are hub genes, and the prism nodes indicate miRNAs. The quadrilateral nodes indicate mRNA, while the circles indicate lncRNAs. The blue prism nodes indicate miRNAs, the red square nodes indicate genes, and the yellow circle nodes indicate lncRNAs. D) KEGG pathway and GO enrichment analysis for hub genes. E) The lncRNA-miRNA-mRNA network: blue prism nodes indicate miRNAs. Red square nodes indicate genes. Yellow circle nodes indicate lncRNAs

lncRNAs: Long non-coding RNAs, miRNA: MicroRNAs, PCOS: Polycystic ovary syndrome, FDA: Food and Drug Administration, DEGs: Differentially expressed genes, GO: Gene Ontology, KEGG: Kyoto Encyclopedia of Genes and Genomes, DEGs: Differentially expressed genes, GO: Gene Ontology, KEGG: Kyoto Encyclopedia of Genes and Genomes, DEGs: Differentially expressed genes

been shown to have an association with viral myocarditis, Cushing's syndrome, and NK cell-mediated cytotoxicity. Previous studies have established a link between these conditions and chronic inflammation and hormonal imbalance in PCOS.[14,15]

These findings reinforce the multifactorial pathogenesis of PCOS with both immune and metabolic dysregulation. To further characterize the post-transcriptional regulatory network, we performed a ceRNA network analysis and found several key lncRNAs (NEAT1, H19, and KCNQ1OT1) and miRNAs (hsa-miR-767-5p and hsa-miR-19b-1-5p) as central regulatory players. These non-coding RNAs have already been demonstrated to be involved in ovarian follicle development and insulin signaling[16-18], which supports their functional relevance in the pathophysiology of PCOS. Additionally, hub genes identified through DGI analysis, including PDE5A, DAPK1, and COL11A1, were found to be involved in vascular function, apoptosis, and extracellular matrix remodeling. PDE5A, in particular, is associated with metabolic pathways linked to nitric oxide signaling, a pathway frequently impaired in PCOS-related endothelial dysfunction.[19-21] We also predict that DAPK1 interacts with the antidiabetic drug sitagliptin. This may be a therapeutic target; however, experimental evidence supporting this will need validated. Integrating ceRNA networks with DGIs provides a novel perspective on post-transcriptional regulation in PCOS. Additionally, hsa-miR-767-5p was found to regulate DAPK1 and ICAM1 via H19 and NEAT1, which offers a potential mechanism by which non-coding RNAs modulate immune and metabolic pathways. Similarly, it was predicted that hsa-miR-19b-1-5p influences PDE5A expression through its interactions with H19 and KCNQ1OT1, suggesting a regulatory pathway involved in vascular homeostasis and glucose metabolism. While these findings are primarily derived from computational analyses, they align with previous experimental reports demonstrating miRNA-mediated regulation of insulin sensitivity and steroidogenesis in PCOS models. The findings are significant because they revealed the hub genes, including SLC2A3 and COL11A1, that play key roles in glucose transport and extracellular matrix remodeling.[22,23]

Study Limitations

While many datasets have been analyzed, some groups may have insufficient sample sizes to provide conclusive evidence regarding the patterns of expression and regulatory mechanisms in PCOS. Secondly, empirical validation of bioinformatic predictions with respect to biological relevance is warranted. Laboratory studies, such as qRT-PCR, western blot, or in vivo studies, are needed.

CONCLUSION

In conclusion, this study explores the cellular pathophysiology of PCOS by analyzing DEGs, miRNAs, and lncRNAs across multiple datasets. Additionally, investigations of the KEGG and GO pathways highlighted important biological functions such as cell adhesion and signal transmission. Significantly, hub genes were found to be located in important signaling-related cellular areas, highlighting their possible

involvement in the pathophysiology of PCOS,With core miRNAs emerging as major regulatory nodes and their interactions with lncRNAs and mRNAs, the development of a ceRNA network revealed important interactions among miRNAs, lncRNAs, and mRNAs. Furthermore, the discovery of 71 DEGs as therapeutic targets provided information on FDA-approved medications that might be useful for the treatment of PCOS.

MAIN POINTS

- MicroRNAs and long non-coding RNAs were shown to affect the polycystic ovary syndrome (PCOS) related RNA expression.
- Food and Drug Administration-approved drugs could potentially target these identified differentially expressed genes.
- Enrichment analyses showed that the hub genes in PCOS were predominantly linked to functions related to secretory granule membranes, extracellular exosomes, and postsynaptic density.

ETHICS

Ethics Committee Approval: Ethical approval was granted by the Near East University Institutional Review Board (approval number: 128-1900, date: 2024).

Informed Consent: Not available.

Footnotes

Authorship Contributions

Concept: P.T., Design: P.T., Analysis and/or Interpretation: B.O.H., Z.L.S., P.O.H., Literature Search: P.O.H., P.T., Writing: B.O.H., Z.L.S., P.T.

DISCLOSURES

Conflict of Interest: No conflict of interest was declared by the authors.

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