Transcriptomic Analysis Reveals the Regulatory Effects of EGCG on Ovarian Function in PCOS Mice

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Abstract. Polycystic ovary syndrome (PCOS) is a common endocrine and metabolic disorder that causes reproductive dysfunction and is often accompanied by insulin resistance and metabolic abnormalities, thereby increasing long-term cardiovascular risk. Current therapeutic strategies mainly focus on symptomatic management and present limitations with prolonged use. Epigallocatechin gallate (EGCG), the main bioactive polyphenol in green tea, has anti-inflammatory, antioxidant, and metabolic regulatory properties. However, its molecular mechanisms in promoting ovarian health and quality in PCOS remain incompletely understood. In this research, we established a PCOS mice model via DHEA induction and subsequently treated the animals with EGCG. Ovarian transcriptomic profiling was performed using RNA sequencing (RNA-seq) to investigate EGCG-mediated gene expression changes. Comparative gene expression analysis revealed 278 genes with differential expression between the EGCG intervention group and the PCOS model group, with 98 genes displaying increased expression and 180 showing decreased expression, indicative of profound transcriptional remodeling induced by EGCG. Functional analysis revealed significant upregulation of several metabolism- and cellular function–related genes, such as *Vpreb1b*, *Pifo*, *Ldlrad1*, *Tmem196*, and *Cyp2b10*, while genes including *Ces2a*, *Wnt10b*, *Sfrp4*, *S100g*, and *Odaph* were markedly downregulated. Collectively, these findings demonstrate that EGCG exerts broad regulatory effects on ovarian biological processes by modulating key metabolic and functional gene pathways, providing novel molecular insights into the therapeutic potential of EGCG as a targeted intervention for PCOS.

1. Introduction

As a common endocrine and metabolic condition, polycystic ovary syndrome (PCOS) affects an estimated 6–20% of females in their childbearing years[1]. It is clinically characterized by the presence of ovulatory dysfunction[1], hyperandrogenism, and polycystic changes in the ovaries, and is often associated with menstrual irregularities, infertility, hirsutism, and acne. Apart from compromised reproductive function, PCOS is tightly linked to various metabolic abnormalities, among which are insulin resistance, obesity, dyslipidemia, and a heightened risk of type 2 diabetes mellitus. These metabolic disturbances not only aggravate reproductive dysfunction but also markedly elevate long-term cardiovascular risk, making PCOS a chronic disorder with both reproductive and metabolic consequences[1].

Presently, clinical strategies for PCOS focus predominantly on managing its manifestations, including the administration of oral contraceptives for menstrual regulation, insulin sensitizers (e.g., metformin) to improve metabolic dysfunction, and ovulation-inducing agents for fertility treatment[2]. Although oral contraceptives can regulate the menstrual cycle, long-term use increases the risk of thrombosis[3]. The use of metformin may affect patient compliance due to its gastrointestinal side effects[3]. Furthermore, the administration of ovulation-inducing agents carries the risk of ovarian hyperstimulation. However, long-term pharmacological therapy is often accompanied by undesirable effects, including gastrointestinal side effects, weight changes, and endocrine imbalance[4. Moreover, these treatments do not offer a cure and typically require prolonged or even lifelong administration, raising concerns about cumulative side effects and long-term safety[5]. Given these limitations, it is critical to develop novel therapeutic approaches that are safer, more effective, and associated with fewer adverse reactions[5]. Such strategies might include targeting inflammatory pathways, modulating the gut microbiome, utilizing natural products with multi-target effects, or developing selective androgen receptor modulators, ultimately aiming for a more holistic and patient-friendly management of PCOS[6]. Accordingly, it is critical to develop novel therapeutic approaches that are safer, more effective, and associated with fewer adverse reactions.

Epigallocatechin gallate (EGCG)[7], a predominant and biologically active polyphenol derived from green tea, has gained growing interest owing to its strong oxidative stress-alleviating effects, inflammation-suppressive effects, and metabolic-modulating activities REF _Ref226460820 \r \h [7]. Accumulating evidence indicates that EGCG can stimulate the AMP-activated protein kinase

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(AMPK) signaling pathway, suppress nuclear factor-kappa B(NF-κB)[8] -mediated inflammatory responses, and alleviate oxidative stress. In experimental models of obesity, type 2 diabetes, endocrine disorders and cardiovascular diseases, EGCG has demonstrated significant protective effects. Recently, several studies have reported that EGCG supplementation can improve hormonal profiles, restore estrous cyclicity, and alleviate ovarian pathological damage in PCOS models, suggesting its potential therapeutic value.

Despite these promising findings, most existing studies have focused primarily on phenotypic and biochemical changes, while the molecular mechanisms underlying EGCG-mediated regulation of ovarian function remain poorly understood. In particular, the comprehensive transcriptional regulatory network through which EGCG exerts its effects in PCOS has not been systematically elucidated. Transcriptomic analysis based on RNA sequencing (RNA-seq) provides a powerful tool to globally characterize gene expression changes and identify key regulatory pathways involved in disease progression and therapeutic intervention.

Therefore, in the present research, we developed a DHEA-induced PCOS mouse model and investigated the effects of EGCG on ovarian gene expression profiles using RNA-seq. By combining differential expression profiling, functional enrichment assessment, and the construction of protein–protein interaction (PPI) networks. This study intended to identify core regulatory genes and pathways mediated by EGCG, so as to provide new molecular evidence for the therapeutic effects of EGCG on PCOS and lay a foundation for its potential application as a targeted intervention.

2. Methods

2.1. Establishment of animal models and design of experimental protocols

3-weeks healthy female C57BL/6J mice were used in this study and acclimated for one week under standard laboratory conditions. Mice were randomly assigned to experimental groups. Intraperitoneal injection of dehydroepiandrosterone was performed to generate a mouse model of PCOS . The control group received equal volumes of vehicle solution, while the model group was continuously injected with DHEA to induce PCOS-like phenotypes[10].

After successful model establishment, PCOS mice were further randomly allocated to two groups: a model-only group and an EGCG intervention group. Mice in the EGCG group were administered EGCG through oral administration at 200 mg/kg. This dose was selected based on pilot dose-screening experiments (100 and 200 mg/kg), in which reduction of serum testosterone (T) in PCOS mice was used as an indicator of biological responsiveness. The control and PCOS groups received equal volumes of vehicle solution by oral gavage.

After the intervention period, mice were sacrificed, and bilateral ovarian tissues were rapidly collected, rapidly frozen in liquid nitrogen and preserved at -80 °C until further analysis.

2.2. RNA extraction and library preparation

Total RNA was isolated from ovarian tissue specimens with TRIzol reagent following the manufacturer's instructions. The purity, concentration, and integrity of RNA were evaluated using a NanoDrop spectrophotometer and an Agilent 2100 Bioanalyzer. Library preparation was only performed using qualified high-quality RNA samples with appropriate RNA integrity number (RIN) scores.

Magnetic bead-based enrichment was carried out to isolate messenger RNA (mRNA), which was then fragmented into short segments. We synthesized the first and second strands of complementary DNA (cDNA), followed by end repair, 3′-end adenylation, adapter ligation, and PCR amplification to generate the sequencing libraries. Prior to sequencing, library quality was assessed via Qubit quantitation and size distribution analysis.

2.3. RNA sequencing and data quality control

Sequencing was performed on an Illumina instrument using a paired-end, high-throughput strategy. First, quality assessment of the raw sequencing data was performed using FastQC software. Low-quality reads and residual adapter sequences were eliminated to generate high-quality clean data. The qualified reads were subsequently mapped to the mouse reference genome, and mapping rates and genome coverage were calculated to evaluate sequencing reliability.

Differential expression profiling and subsequent functional enrichment analyses were employed to identify genes and biological pathways potentially modulated by EGCG intervention. These analyses were intended to provide pathway-level insights rather than definitive mechanistic validation.

2.4. Identification of gene expression changes

After normalization of gene expression levels, comparisons of differential expression between the EGCG intervention group and the PCOS model group were conducted using the DESeq2 package. Differentially expressed genes (DEGs) were defined under the thresholds of $p < 0.05$ and $|\log_2 FC| > 1$. Volcano maps and hierarchical clustering heatmaps were constructed to intuitively display the expression patterns of DEGs..

2.5. Protein interaction network analysis

PPI network analysis was conducted using the STRING database. Protein interaction relationships were obtained either by direct association using species-specific annotations provided by STRING or, when direct annotation was unavailable, by aligning gene sequences to the corresponding species protein sequences in the STRING database using BLAST (E-value < 1×10^{-10}).

Interaction information for differentially expressed genes was extracted from the resulting dataset, and interaction pairs were ranked according to their confidence scores. The top 300 interactions were selected to construct the PPI network, which was visualized as a three-dimensional interaction network.

2.6. Data statistical assessment

DESeq2 was utilized to carry out comparisons of gene expression differences. Genes with adjusted *p*-values below 0.05 were regarded as statistically significant. R programming language and GraphPad Prism software were employed for data visualization and the generation of statistical figures.

3. Results

3.1. EGCG treatment alters the ovarian transcriptomic profile in PCOS mice

Differential expression analysis was performed to compare ovarian transcriptomes between the EGCG-treated group (G-treatment) and the PCOS group (G-PCOS). Under the criteria of $p < 0.05$ and $|\log_2 FC| > 1$, we identified 278 differentially expressed genes (DEGs) in total. Relative to the G-PCOS group, the G-treatment group exhibited 98 genes with elevated expression and 180 genes with reduced expression, as illustrated in Figure 1.

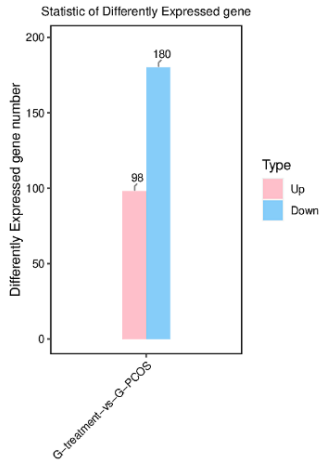

Figure 1. Summary of differentially expressed genes between the G-treatment and G-PCOS groups

3.2. Detection of genes with differential expression following EGCG intervention

As shown in the volcano plot (Figure 2), significantly increased genes are labeled in red, while significantly reduced genes are annotated in blue. The distribution of DEGs demonstrated a clear transcriptional shift induced by EGCG intervention. Notably, several genes associated with metabolic regulation and cellular function, such as *Vpreb1b, Pifo, Ldlrad1, Tmem196, and Cyp2b10,* were markedly upregulated. In contrast, genes including *Ces2a, Wnt10b, Sfrp4, S100g, and Odaph* were significantly downregulated following EGCG intervention.

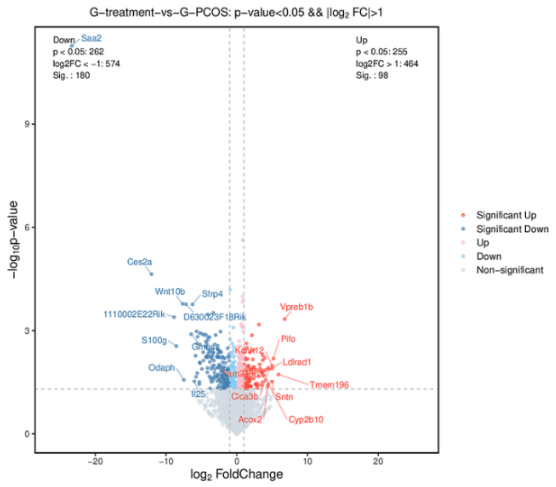

Figure 2. Volcano map illustrating gene expression differences between the G-treatment and G-PCOS groups

These results indicate that EGCG intervention induces substantial transcriptional reprogramming in the ovaries of PCOS mice, suggesting its regulatory effects on multiple biological processes.

3.3. Hierarchical clustering reveals distinct gene expression patterns between EGCG-treated and PCOS ovaries

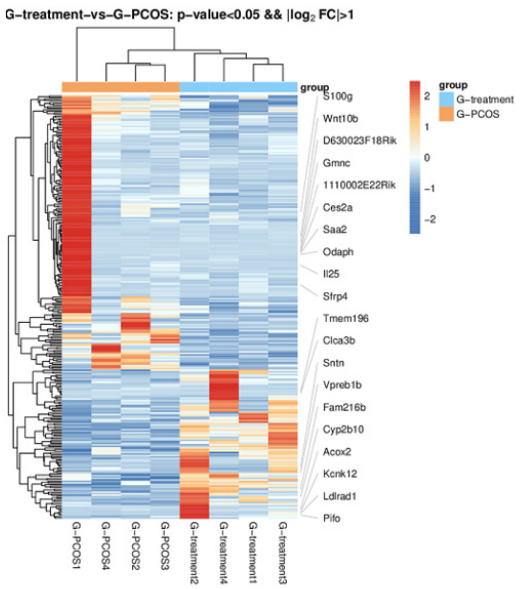

Figure 3. Cluster heatmap of differentially expressed genes between the G-treatment and G-PCOS groups

Differential expression analysis was performed on ovarian transcriptomes from the EGCG-treated group (G–treatment) and the PCOS model group (G–PCOS), selecting genes with *p*-value < 0.05 and $|\log_2 FC| > 1$. A heatmap with hierarchical clustering was generated to visualize these differentially expressed genes (Figure 3). The findings demonstrated a distinct separation between

the two treatment groups: G–PCOS samples (orange) exhibit high expression levels for multiple genes (red regions), whereas the corresponding genes in the G–treatment samples (blue) are significantly downregulated (blue regions), indicating that EGCG markedly modulates their expression.

For the 20 most significantly dysregulated genes displayed on the right side of the heatmap, including *S100g*, *Wnt10b*, *Saa2*, *Cyp2b10*, and *Acox2*, most show downregulation following EGCG treatment, suggesting potential involvement in PCOS-related metabolic and inflammatory pathways. Hierarchical clustering further reveals that samples within the same group have highly similar expression profiles, whereas distinct differences are observed between groups, further confirming the modulatory role of EGCG on the ovarian transcriptome in PCOS mice.

3.4. Protein–protein interaction network analysis identifies key regulatory nodes affected by EGCG

Based on the protein–protein interaction (PPI) analysis, a subnetwork was extracted from the top-ranking interactions to visualize key interaction patterns among differentially expressed genes (Figure 4). The network revealed a heterogeneous interaction structure, consisting of both upregulated and downregulated genes with varying degrees of connectivity.

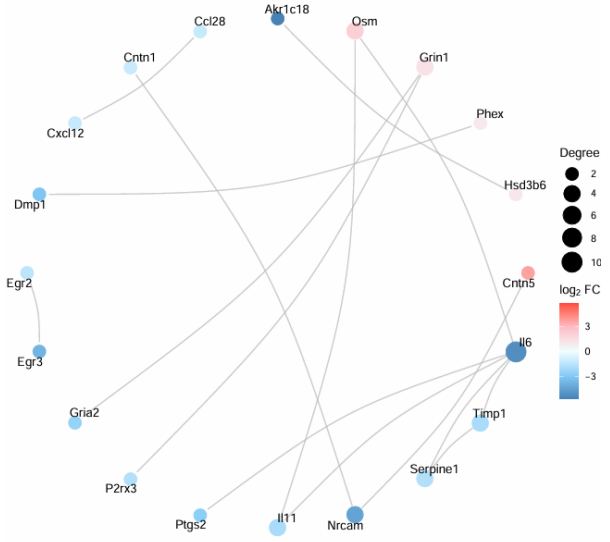

Figure 4. Interaction network of differentially expressed genes.

Several nodes exhibited relatively higher degrees within the network, indicating increased connectivity at the protein interaction level. Among them, *Il 6* showed a comparatively high degree and was connected with multiple interacting partners, including *Serpine1*, *Timp1*, *Il11*, and *Nrcam*. In contrast, other genes such as *Cxcl12*, *Cntn1*, and *Egr family members* formed smaller interaction clusters.

Notably, the network comprised genes displaying distinct expression patterns, as reflected by the $\log_2$ fold change–based color scale, suggesting coordinated but heterogeneous transcriptional alterations at the protein interaction level following EGCG treatment.

4. Conclusion

Employing a murine PCOS model induced by DHEA, this work comprehensively analyzed the impacts of EGCG on transcriptomic features in ovarian tissue. EGCG treatment significantly altered several pathways associated with hormone regulation and ovarian function in PCOS mice, suggesting a potential role of EGCG in modulating PCOS-related molecular processes. Although dose selection was guided by pilot observations, dose-dependent effects were not systematically evaluated in the present study. Therefore, the current findings should not be interpreted as defining an optimal therapeutic dose.

Notably, a total of 278 differentially expressed genes were identified between the EGCG-treated group and the PCOS group. Functional annotation indicated that these genes were primarily involved in hormone metabolism and cellular function–related biological processes, supporting a potential role of EGCG in regulating ovarian function in PCOS mice. Although dose selection was guided by pilot observations, dose-dependent effects were not systematically evaluated, and therefore the present findings should not be interpreted as defining an optimal therapeutic dose.

Overall, this study provides transcriptomic and network-level evidence that contributes to the understanding of the potential biological effects of EGCG in PCOS. Nevertheless, given the limitations of the current study, further investigations incorporating dose-dependent analyses and experimental validation will be required to elucidate the underlying molecular pathways and to confirm the functional roles of key genes identified herein.

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