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# The Role of Chromatin Regulator-Related Genes GADD45A and TAF5 in Polycystic Ovary Syndrome: A Bioinformatics Analysis

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**Background: Polycystic ovary syndrome (PCOS) is a common disease that often results in miscarriage among females. The study aimed to elucidate the relationship between chromatin regulators and PCOS to explore potential biomarkers for PCOS.**
**Methods: Differential genes between PCOS patients and healthy controls were screened based on GSE10946 dataset. Pivotal genes were screened using LASSO (Least Absolute Shrinkage and Selection Operator), XGBoost (eXtreme Gradient Boosting) and random forest, and crossover genes of these three machine learning methods were further screened using a Venn diagram. The predictive potential of these key genes was evaluated using receiver operating characteristic (ROC) curve analysis, and functional enrichment analysis was conducted to elucidate the associated signaling pathways. To investigate their roles in PCOS and their effects on the immune microenvironment, immunity profiles between PCOS patients and healthy controls were compared; the correlations between key genes and immune cell populations were also examined, and then the associations of these genes with potential drug candidates were explored.**
**Results: *GADD45A* and *TAF5* were identified as critical genes associated with PCOS, exhibiting significantly decreased expression levels in PCOS patients compared to healthy controls (both $p < 0.05$). Both genes showed strong predictive performance. Functional enrichment revealed their involvement in pathways such as cholesterol homeostasis and E2F targets. Immune infiltration analysis indicated distinct differences in immune cell composition between the PCOS and control groups, with gene expression levels closely correlating with specific immune cell subsets (all $p < 0.05$).**
**Conclusion: *GADD45A* and *TAF5* have high predictive ability for PCOS and have the potential to be new biomarkers and therapeutic targets for PCOS.**

**Keywords:** polycystic ovary syndrome; *GADD45A*; *TAF5*; predictive biomarker; machine learning

## Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder among women of reproductive age, affecting an estimated 8–13% of women worldwide based on diagnostic criteria applied [1,2]. A comprehensive meta-analysis published in 2024, encompassing over 12 million women, reported a global PCOS prevalence of 9.2% (95% CI: 6.8–12.5%), with estimates varying by diagnostic approach—5.5% under NIH (National Institutes of Health) criteria, 11.5% under Rotterdam, and 7.1% under AES (American Epilepsy Society) [3]. Data from the Global Burden of Disease (GBD) 2021 report an age-standardized PCOS prevalence of 867.7 per 100,000, with an incidence of 30.7 and 7.6 DALYs per 100,000, reflecting an increase of approximately 28%, 27%, and 28%, respectively, since 1990 [4]. This upward trend is particularly pronounced among younger women, especially adolescents and those aged 20–29 years, with reported prevalence rates reaching 6–18% [5]. PCOS exerts a profound burden on reproductive, metabolic, and psychological health, and is associated with increased risks of infertility, type 2 diabetes, cardiovascular disease, and mood disorders [6–9]. These compelling epidemiological trends highlight an urgent need to elucidate the molecular mechanisms underlying PCOS and to identify robust, clinically relevant biomarkers for early diagnosis and personalized management.

In recent years, increasing attention has been directed toward the role of chromatin regulators in PCOS pathophysiology. These factors modulate gene expression through nucleosome repositioning, histone modification, and higher-order chromatin organization, thereby shaping transcriptional outcomes in reproductive and metabolic tissues [10,11]. Key regulators include chromatin-remodeling complexes, histone acetyltransferases (HATs), histone deacetylases (HDACs), and DNA methylation-associated

enzymes, many of which are dynamically expressed in ovarian, endometrial, and adipose tissues [12–14]. Recent studies have reported altered expression of multiple chromatin regulators -related genes in ovarian tissues of women with PCOS. For example, Sagvekar *et al.* [15] found that granulosa cells from PCOS patients exhibit dysregulated expression levels of AKR1C3, CASR, and MAMLD1, suggesting a disturbed chromatin state that may impair follicular development and steroidogenesis. More recently, Weng *et al.* [16] demonstrated that METTL3, an $m^6A$ RNA methyltransferase, enhances the stability of CD36 mRNA via $m^6A$ modification, thereby exacerbating glucose metabolic dysfunction and promoting inflammatory cytokine release in the PCOS follicular microenvironment. Suppression of CD36, both *in vitro* and *in vivo*, reversed these pathologies, highlighting the pathogenic role of RNA- and chromatin-modifying enzymes in PCOS. Despite these emerging insights, the transcriptomic landscape of chromatin regulator-related genes in PCOS remains uncharacterized, and few studies have prioritized them as diagnostic or mechanistic biomarkers. Given their dual involvement in gene activation and repression, chromatin regulators represent an underexplored but potentially crucial node in the pathophysiology of PCOS.

Based on these findings, we conducted a comprehensive multi-database analysis to identify the chromatin regulator-related genes associated with PCOS. Leveraging multiple machine learning approaches, we systematically screened and validated these genes to ensure their robustness and reliability. Furthermore, we explored their role within the immune microenvironment through comprehensive immune infiltration analysis, elucidating their impact on immune cell dynamics. Additionally, we investigated the involvement of these key genes in critical signaling pathways, shedding light on the intricate molecular mechanisms underlying PCOS. Finally, drug sensitivity analysis was performed to identify potential therapeutic agents targeting these genes. By deepening the understanding of the pathophysiology of PCOS, our study aims to uncover novel biomarkers and provide a theoretical framework for advancing diagnostic and therapeutic strategies.

## Materials and Methods

### *Data Sources*

The chromatin regulator gene set including 870 genes was retrived from the previous study published by Lu *et al.* [17]. RNA-Seq datasets about immue and PCOS were acquired from the GEO repository (https://www.ncbi.nlm.nih.gov/geo/). Transcriptomic profiles associated with PCOS were extracted from the GSE10946 dataset, comprising 12 PCOS patients and 11 healthy controls [18]. Similarly, gene expression signatures of PCOS were validated in the GSE34526 dataset, which consisted of the gene expression profiles of seven PCOS patients and three normal controls [19]. Differential gene expression analysis between PCOS patients and controls was conducted utilizing the 'limma' package in R, with stringent thresholds set at $|\log2FC| > 1$ and adjusted $p$-value (adj.$p$) $< 0.05$ for identifying significant genes. The DEGs were subsequently visualized through volcano plots and hierarchical clustering heatmaps using the R package "ggplot2" and "pheatmap". Venn diagram analysis was performed to identify overlapping genes in the DEG profiles of PCOS and chromatin regulators. Principal Component Analysis (PCA) [20] was employed to validate the distinguishing ability of crucial genes by reducing the dimensionality.

### *Functional Enrichment Analysis*

To decipher the molecular underpinnings of PCOS pathogenesis, we conducted a systematic functional enrichment analysis based on DEGs between PCOS patients and controls using Gene Ontology (GO) enrichment analysis, Kyoto Encyclopedia of Genes and Genomes (KEGG), and Gene Set Enrichment Analysis (GSEA). GO enrichment analysis was systematically performed by the R package "clusterProfiler" to categorize genes into three principal domains: biological processes (BP), cellular components (CC), and molecular functions (MF). Concurrently, pathway-centric analysis was executed using the KEGG database to delineate enriched signaling cascades. Furthermore, GSEA was employed with the Molecular Signatures Database (MSigDB) to uncover critical biological pathways and regulatory networks associated with key molecular determinants [21]. Enrichment significance was defined as an adj. $p < 0.05$ and a false discovery rate (FDR, q value) $<0.25$ using the Benjamini-Hochberg (BH) method.

### *Machine Learning*

To elucidate potential predictive biomarkers for PCOS, we employed a comprehensive machine learning framework encompassing three distinct algorithms: LASSO regression, Random Forest, and XGBoost. Through rigorous crossover analysis utilizing Venn diagrams, we delineated consensus pivotal genes that demonstrated consistent significance across all three computational approaches. Subsequently, the overall predictive accuracy of these genes was evaluated using the receiver operating characteristic curve (ROC) curves generated with the R package pROC. For each gene, we calculated the area under the curve (AUC) with optimal cutoff, specificity and sensitivity to comprehensively assess the biomarkers' discriminatory power.

### *Immune Infiltration Analysis*

To comprehensively characterize the immune microenvironment in patients with PCOS, an integrated, multi-dimensional analytical framework was implemented. Initially, the CIBERSORT algorithm was utilized to quantitatively determine the relative proportions of 22 distinct im-

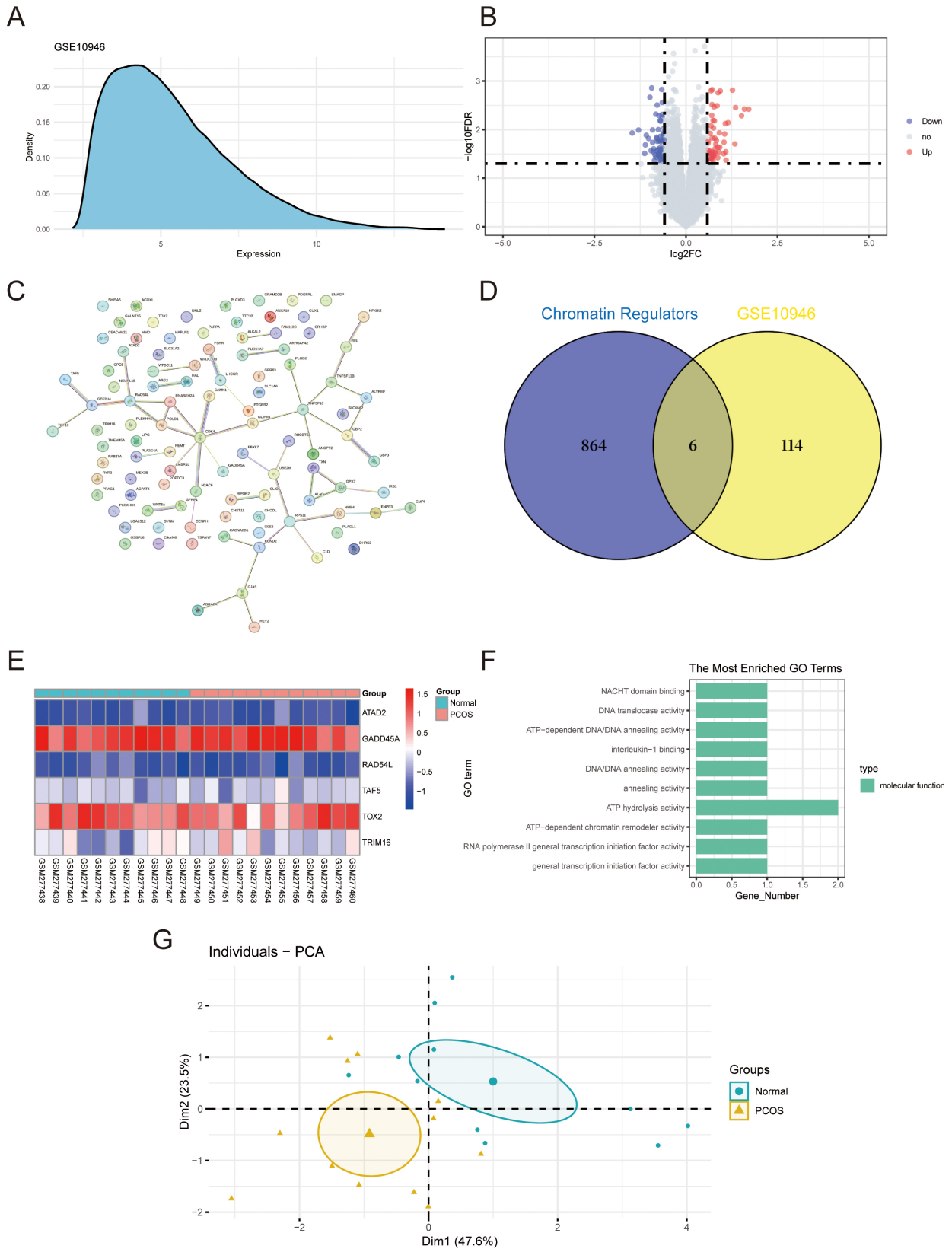


**Fig. 1. Screening for differential genes.** (A) Distribution of gene expression in GSE10946. (B) Volcano map of GSE10946 differential genes. (C) PPI network of differential genes. (D) Screening of differential genes for crossover genes with chromatin regulators using a Venn diagram. (E) Expression of crossover genes in GSE10946. (F) GO enrichment analysis of crossover genes. (G) Distinguishing ability of crossover genes between PCOS and controls. PPI, protein-protein interaction; GO, Gene Ontology; PCOS, Polycystic ovary syndrome.

mune cell subtypes, providing a high-resolution profile of immune cell composition within the affected tissues [22]. Subsequently, single-sample gene set enrichment analysis (ssGSEA) was employed to evaluate the immune functional activity across different clinical subgroups, offering a nuanced understanding of the immune responses associated with PCOS. Furthermore, correlation analyses were conducted to explore potential relationships between the expression patterns of key molecular regulators and specific immune cell populations [23].

### *Construction of Regulatory Networks*

To map the interactome landscape and identify hub genes, a protein-protein interaction (PPI) network using the STRING database (https://cn.string-db.org/) was constructed to pinpoint central molecular components within the network architecture. Secondly, the 'GENIE3' package was used to construct and visualize the regulatory network of hub genes, thereby delineating the intricate interactions between transcription factors and their target genes [24].

### *Drug Sensitivity Analysis*

Drug candidates associated with hub genes were predicted based on the Drug Signature Database (DSigDB, http://dsigdb.tanlab.org/DSigDBv1.0/), accessed via the EnrichR platform [25]. Potential therapeutic compounds were systematically identified and ranked based on their adjusted p-values, ensuring statistical robustness in prioritizing drug-gene interactions.

### *Statistical Analysis*

All analyses were conducted using R (version 4.3.0; R Foundation for Statistical Computing, Vienna, Austria). For two-group comparisons, Student's *t*-tests was used for normally distributed variables, and Wilcoxon test were used for non-normally distributed variables. Spearman correlation analysis was used to calculate correlation coefficients between different groups. Univariate and multivariate logistic regression were used to explore the association between the genes expression and PCOS. A threshold of $p < 0.05$ with two-sided statistical tests was considered indicative of statistical significance. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

## Results

### *Screening and Characterization of Differentially Expressed Genes (DEGs)*

RNA-seq data from GSE10946 were analyzed for DEGs (Fig. 1A), revealing 120 significant alterations in PCOS individuals, including 59 up-regulated genes and 61 down-regulated genes (Fig. 1B). The construction of the PPI network highlights the complex regulatory and interaction relationships among the proteins encoded by the differentially expressed genes (Fig. 1C). A cross-comparative analysis using Venn diagrams identified 6 chromatin regulator-associated genes implicated in PCOS pathogenesis (Fig. 1D). The heatmap illustrates the expression patterns of 6 differential genes related to chromatin regulatory factors in polycystic ovary syndrome (PCOS) patients and the normal control group (Fig. 1E). GO-MF enrichment analysis indicated that these genes were predominantly associated with energy metabolism, gene expression regulation, DNA stability and immune inflammation (Fig. 1F). Principal component analysis (PCA) demonstrated that these genes effectively discriminated between PCOS patients and healthy controls (Fig. 1G).

### *Identification of Hub Genes*

To further elucidate chromatin regulator-associated DEGs in PCOS patients compared to controls, we utilized three distinct machine learning algorithms: LASSO, Random Forest, and XGBoost (Fig. 2A–F). There were five genes in LASSO that were related to PCOS. Besides, GADD45A, TRIM16, and TAF5 were considered important in Random Forest and XGBoost.

### *Expression Characterization and Predictive Ability of Hub Genes*

Univariate regression analysis revealed that *GADD45A*, *TRIM16*, and *TAF5* were all correlated with PCOS; however, multifactor logistic regression analysis only confirmed the statistical relationship between *GADD45A* and *TAF5* with PCOS (Fig. 3A). We therefore included *GADD45A* and *TAF5* for subsequent analysis. In the independent validation cohort GSE10946, the expression levels of both *GADD45A* and *TAF5* in PCOS patients were significantly lower compared to healthy controls ($p < 0.05$). But in GSE34526, only the *GADD45A* level in PCOS patients was significantly lower compared to healthy controls ($p < 0.05$) (Fig. 3B). ROC analysis showed high predictivity of *GADD45A*, *TAF5*, and especially the combination of *GADD45A* and *TAF5* for PCOS (Fig. 3C).

### *Functional Analysis of Hub Genes*

To explore the functions of the hub genes, we first mapped their regulatory networks and found a complex association between *GADD45A* and *TAF5* (Fig. 4A). To investigate the involvement of the identified key genes in the pathogenesis of PCOS, GSEA analysis was conducted. Our analysis revealed that *GADD45A* was predominantly enriched in the cholesterol homeostasis pathway, the E2F targets pathway, and other pathways (Fig. 4B). Notably, *TAF5* displayed a similar enrichment profile, aligning closely with that of *GADD45A* (Fig. 4C). GSEA enrichment plots of hub genes *GADD45A* and *FZD5* further demonstrate the pathways they are enriched in, highlighting their potential roles in PCOS pathogenesis (Fig. 4D,E).

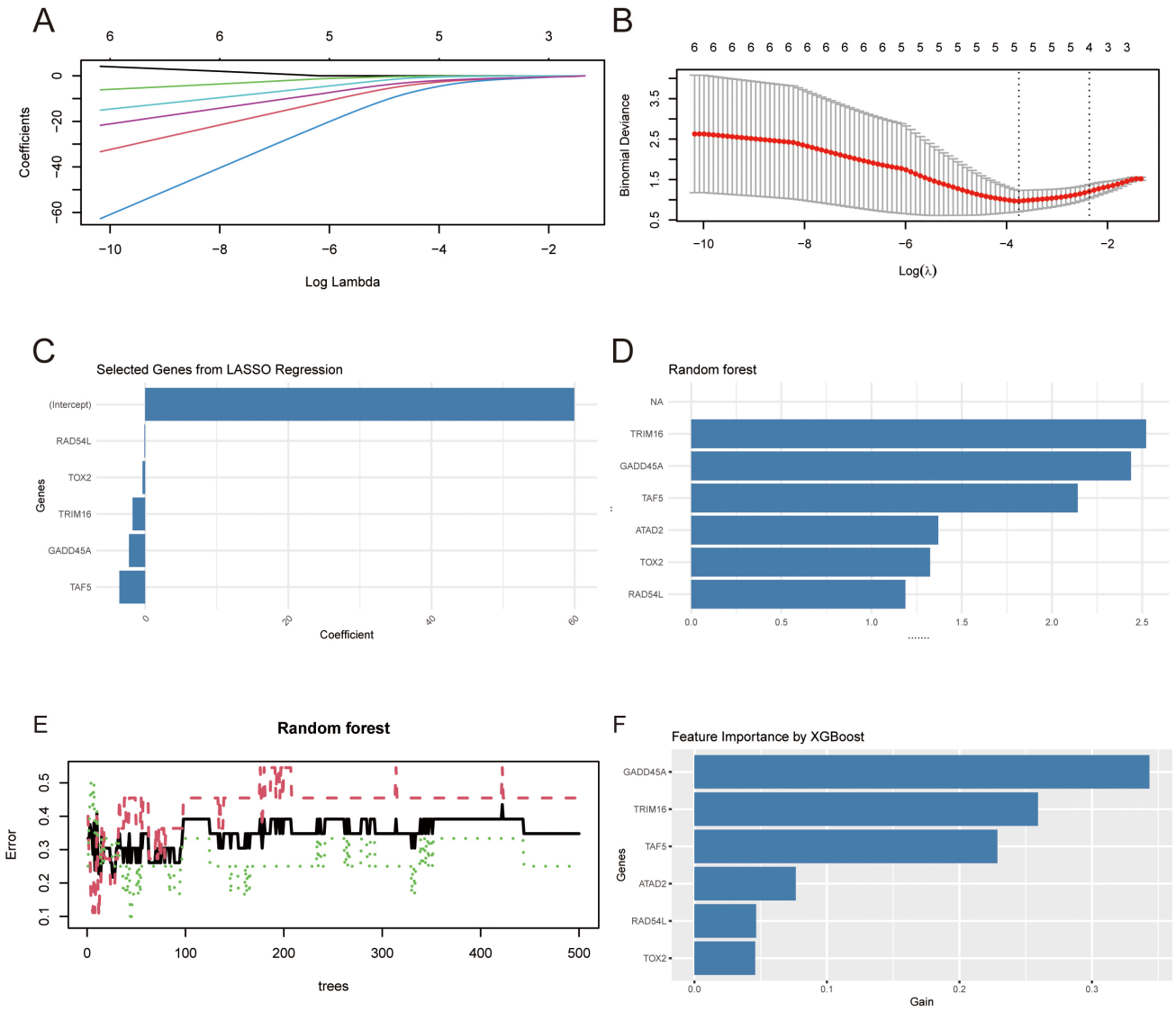


**Fig. 2. Screening of hub genes.** (A) The change of LASSO coefficients with the increasing lambda. Each line represents a gene. (B) Binormal deviance from changing lambda values. (C) The coefficients for hub genes in LASSO regression. (D) The importance of genes ranked in Random Forest. (E) The error of Random Forest. (F) The importance of genes ranked in XGBoost. LASSO, Least Absolute Shrinkage and Selection Operator; XGBoost, eXtreme Gradient Boosting.

### *Microenvironmental Characterization of Key Genes*

To further explore the interplay between PCOS and the immune system, an immunogenomic analysis was conducted. Utilizing the CIBERSORT algorithm, we quantified the immune cell composition in all samples, which were stratified into two groups based on the cohort-specific median value, revealing significant heterogeneity in immune profiles across the cohorts (Fig. 5A). To investigate the relationship between key genes and immune microenvironment alterations, the ssGSEA algorithm was applied, and high and low groups were divided by the median of gene expression. Notably, eosinophil population was significantly decreased in *GADD45A* in the low group ($p < 0.05$) (Fig. 5B), while a significant difference was observed in neutrophil, natural killer T cell, type 1 T helper cell, central memory CD4 T cell, plasmacytoid dendritic cell, activated B cell, and activated dendritic cell between the *TAF5* high and low groups (all $p < 0.05$) (Fig. 5C). Furthermore, between PCOS patients and normal controls, only neutrophil was significantly different ($p < 0.05$) (Fig. 5D). Moreover, we identified a significant correlation between the expression levels of *GADD45A* and *TAF5* and the infiltration of specific immune cell populations (Fig. 5E).

### *Drug Sensitivity Analysis*

To assess the potential of *GADD45A* and *TAF5* as novel therapeutic targets, a drug sensitivity analysis was conducted. Utilizing the DSigDB database via the EnrichR platform, a strong association between these two genes and several therapeutic agents was identified, including

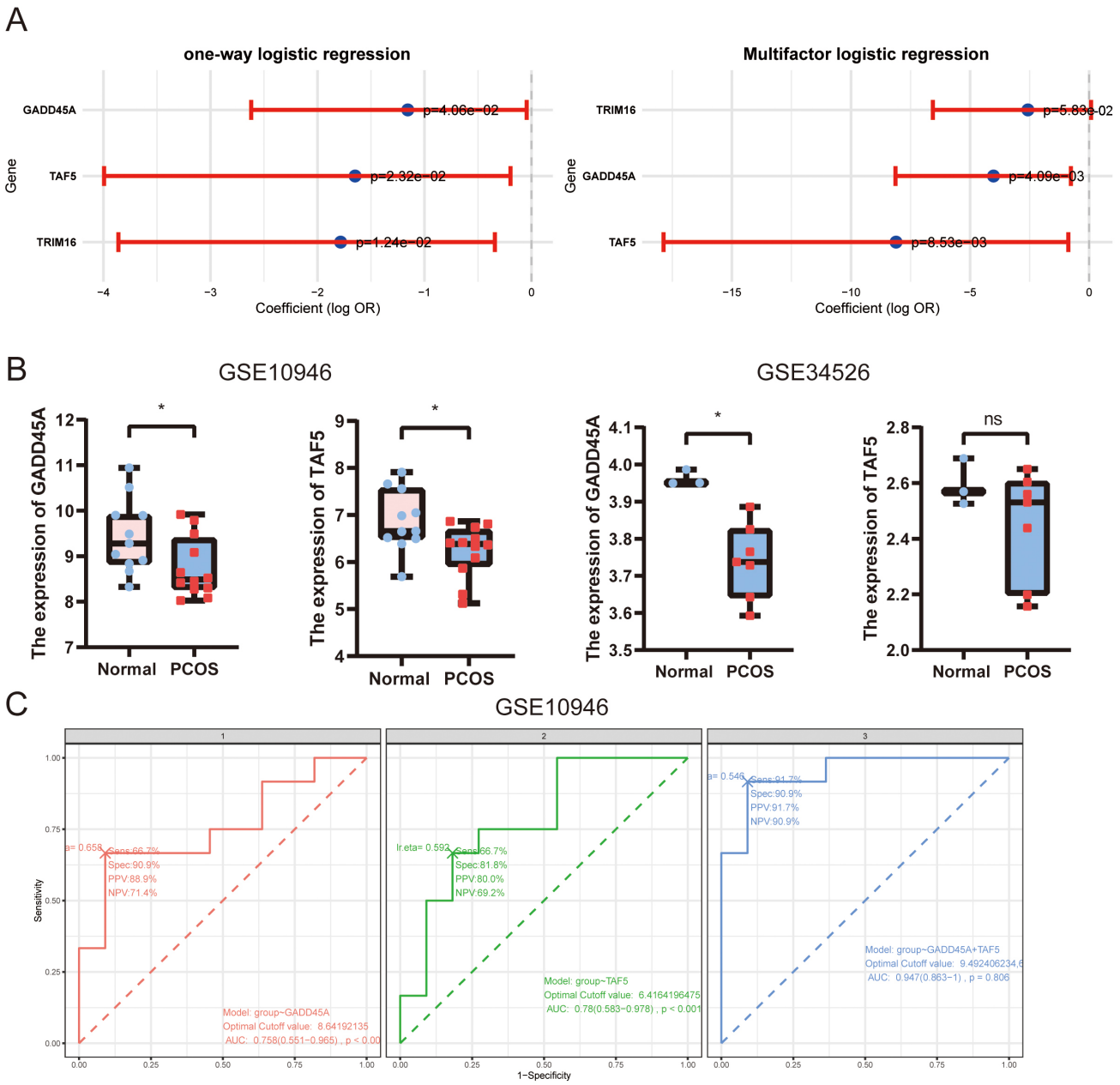


**Fig. 3. Expression characterization and predictive ability of hub genes.** (A) Association analysis between hub genes and diseases. (B) Expression characterization of hub genes in GSE10946 and GSE34526. (C) Disease prediction ability of hub genes in test set GSE10946 and validation set GSE34526. *, $p < 0.05$; ns, $p \geq 0.05$.

10'(Z),13'(E),15'(E)-Heptadecatrienylhydroquinon, benzyl isothiocyanate, allyl isothiocyanate, Dimethyl-IQX, and others (Fig. 6).

## Discussion

PCOS arises from a multifactorial interplay involving hormonal imbalance, metabolic dysregulation, and immune disturbances. It is characterized by ovulatory dysfunction, insulin resistance, and chronic low-grade inflammation. Current diagnostic and therapeutic strategies for PCOS rely primarily on clinical symptoms and hormone assessments, lacking reliable molecular markers [26,27]. Chromatin regulators, which modulate gene expression by altering chromatin structure and accessibility, have been increasingly recognized for their roles in immune regulation and metabolic homeostasis [28,29]. However, their specific mechanisms in PCOS pathophysiology remain poorly understood. Identifying key chromatin regulator-related genes may provide novel insights into disease mechanisms and explore the potential diagnostic biomarkers and targeted therapeutic strategies for PCOS.

In this study, we used integrated bioinformatics approaches to identify chromatin regulator-related genes in-

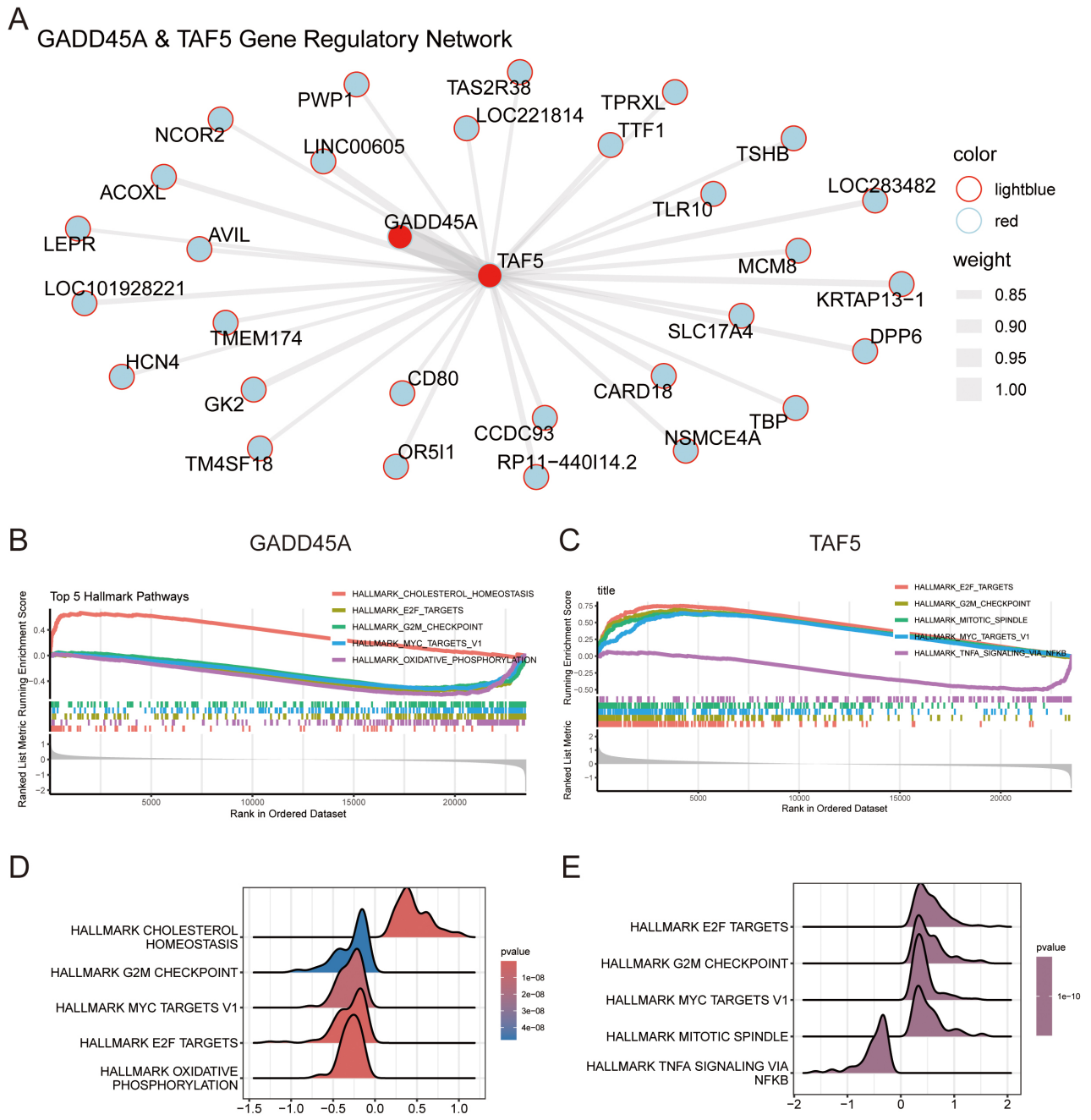


**Fig. 4. Functional analysis of hub genes.** (A) Regulatory network analysis of the hub genes. (B,C) GSEA analysis of the hub genes GADD45A and FZD5. (D,E) Mountain range maps of GSEA analysis of hub genes GADD45A and FZD5. GSEA, Gene Set Enrichment Analysis.

volved in the pathogenesis of PCOS. Through the application of three independent machine learning algorithms—LASSO, Random Forest, and XGBoost—we identified *GADD45A* and *TAF5* as two hub genes. These genes showed significantly decreased expression in PCOS patients and exhibited strong predictive power in both discovery and validation datasets. Functional enrichment, immune infiltration analysis, and drug sensitivity prediction further highlighted their potential mechanistic and clinical utility.

Our findings indicate that *GADD45A* and *TAF5* are involved in key biological pathways contributing to the pathophysiology of PCOS. GSEA revealed that both genes are enriched in cholesterol homeostasis and E2F targets pathways. These pathways regulate lipid metabolism, cell proliferation, and inflammatory responses, which are central to the metabolic and reproductive dysregulation in PCOS [30–34]. *GADD45A*, a key member of the *GADD45* family, plays crucial roles in cell cycle regulation, DNA damage response, and immunomodulation. These functions

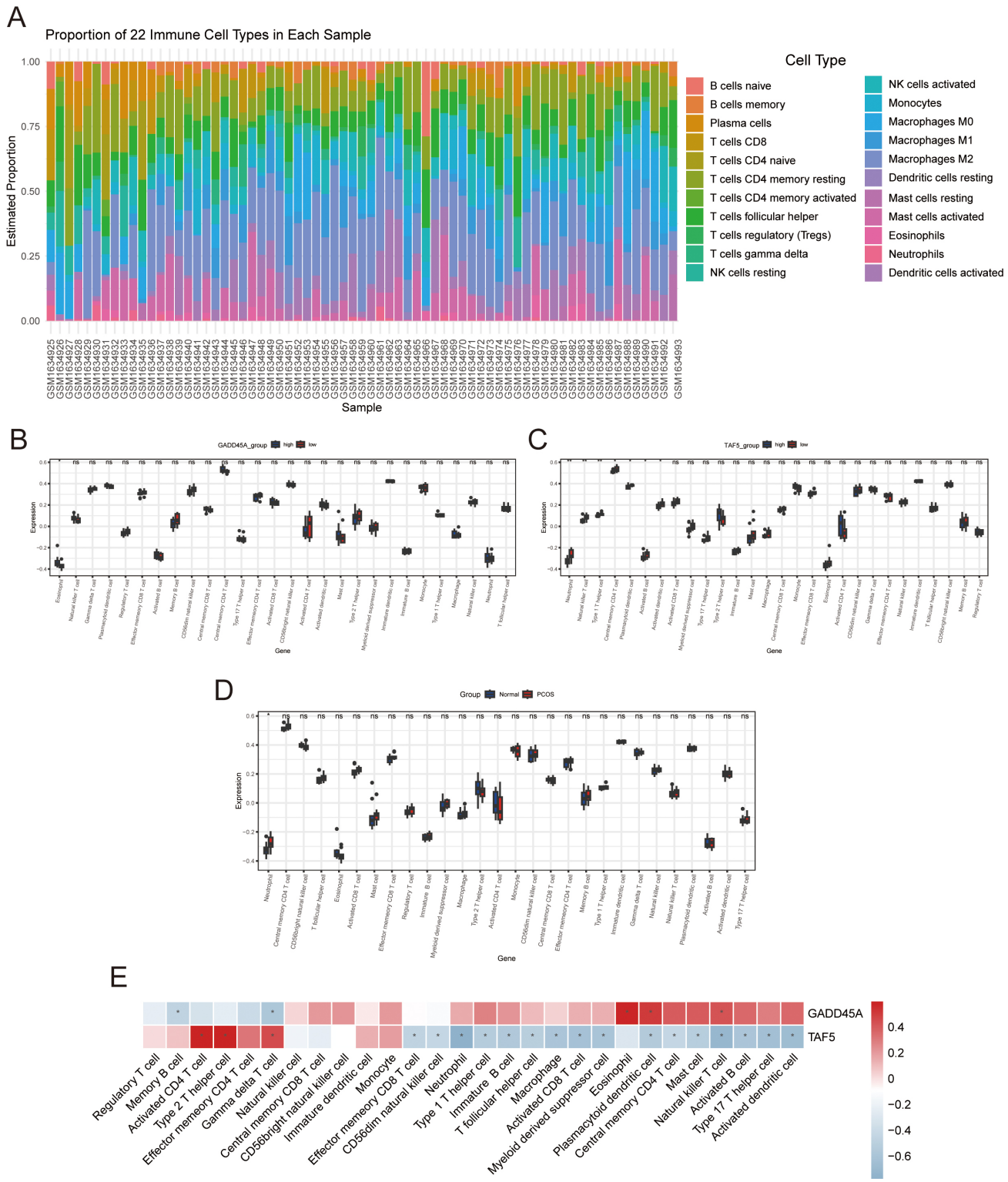


**Fig. 5. Immune microenvironmental analysis of hub genes.** (A) Calculation of the composition of the immune signature of individual patients with PCOS and healthy controls based on the CIBERSORT algorithm. Differences in levels of (B) GADD45A high and low expression, (C) FZD5 high and low expression, and (D) immune cells between PCOS patients were calculated based on ssGSEA analysis. (E) Association analysis between the expression levels of hub genes and immune cell expression.*, $p < 0.05$; **, $p < 0.01$; ns, $p \geq 0.05$.

align with its enrichment in inflammatory pathways and its association with altered immune cell profiles. For example, Xie *et al.* [35] identified a close association between *GADD45A* and plasmacytoid dendritic cell infiltration in renal ischemia-reperfusion injury through bioinformatics analysis. Another study demonstrated that *GADD45A* in-

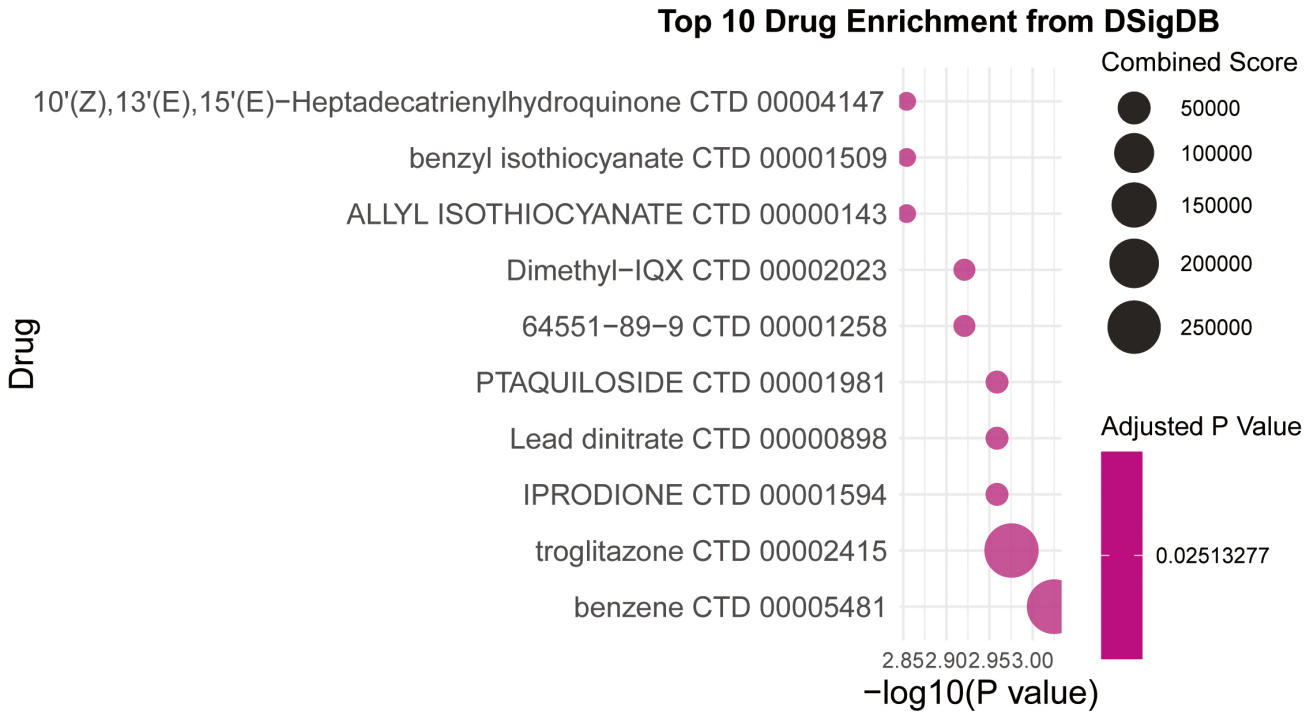


**Fig. 6. Drug sensitivity analysis of pivotal genes based on DSigDB database.**

hibits liver cancer cell proliferation by arresting G2/M phase cell cycle [36]. In addition, Carrier *et al*. [37] showed that *GADD45* promotes DNA repair by enhancing topoisomerase activity and modulating the accessibility of DNA repair enzymes to damaged chromatin. Similarly, *TAF5* is a core component of the transcription factor IID (TFIID) complex, positioning it to influence broad transcriptional programs associated with hormonal regulation and ovarian function. Freiman and colleagues reported that *TAFII105*, a tissue-specific TAF variant, is expressed in mouse ovarian granulosa cells, and its loss results in defective folliculogenesis and infertility [38]. Further studies have highlighted that distinct expression patterns of TAF subunits within TFIID can decisively influence ovary-specific transcriptional programs, including hormone responsiveness, granulosa cell function, and follicular maturation [39,40]. Taken together, these findings suggest that *GADD45A* and *TAF5* may contribute to PCOS by bridging transcriptional regulation with immune and endocrine dysfunction. Their involvement in cell cycle control, inflammation, and ovarian development highlights their potential as mechanistic biomarkers and therapeutic targets in PCOS.

In addition to their transcriptional and ovarian regulatory roles, a notable aspect of this study is the immune infiltration analysis of *GADD45A* and *TAF5*. By comparing the high- and low-expression groups, we observed significant differences in eosinophils, neutrophils, natural killer T cells, and T helper cell subsets. These results suggest that both genes may be involved in modulating immune cell composition in PCOS. This finding is particularly relevant, as chronic low-grade inflammation and immune imbalance are known to contribute to insulin resistance and anovulatory dysfunction in PCOS [34,41–43]. The close relationship between gene expression levels and immune cell abundance suggests that *GADD45A* and *TAF5* may serve as immune-interacting nodes within the chromatin–inflammation axis. Furthermore, drug sensitivity analysis identified multiple bioactive compounds associated with the modulation of *GADD45A* and *TAF5*, including isothiocyanate derivatives with known anti-inflammatory and antiproliferative effects [44–46]. These findings may offer new therapeutic directions for PCOS, especially for patients who are unresponsive to conventional hormonal treatments.

Despite these strengths, several limitations should be acknowledged. First, our study relied solely on transcriptomic data from public datasets with relatively small sample sizes, which could increase the risk of overfitting in machine learning algorithms and undermine the robustness of the ROC curves and logistic regression analyses. Second, while *in silico* validation was performed across independent datasets, the findings lack confirmation through experimental validations of gene expression or biological effects. Third, the cross-sectional design of transcriptomic profiling limits causal interpretation. Lastly, the regulatory role of *TAF5* in ovarian physiology remains underexplored and warrants further mechanistic investigation. Future studies should integrate multi-omics approaches, including proteomics and epigenomics, to validate the role of these genes and their downstream targets [47,48]. Additionally, *in vitro* and *in vivo* experiments (e.g., qPCR, West-

ern blot, and functional assays), along with validation in large, prospective clinical cohorts, are necessary to establish their diagnostic and therapeutic utility [49–51]. Understanding how chromatin regulators interact with hormonal and metabolic networks may pave the way for precision medicine approaches in PCOS [52].

## Conclusion

In conclusion, this study identifies *GADD45A* and *TAF5* as key chromatin regulator-related genes with strong relevance to PCOS. Integrated analyses reveals their potential roles in immune-related pathways and highlights their value as candidate biomarkers and therapeutic targets. These findings could provide a theoretical foundation and a clue for advancing early diagnosis and precision treatment strategies in PCOS.

## Availability of Data and Materials

The datasets used or analyzed during the current study are available from the corresponding author via email upon request.

## Author Contributions

Conceptualization: LFG and YD; Data curation: YLX, HYR, XXW and XYK; Formal Analysis: QX, XWHF, DL and YD; Writing-original draft: LFG; Writing-critical revision & editing: all authors. All authors gave final approval of the version to be published. All authors have participated sufficiently in the work to take public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to its accuracy or integrity.

## Ethics Approval and Consent to Participate

Not applicable.

## Acknowledgment

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## Funding

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## Conflict of Interest

The authors declare no conflict of interest.

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