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ORIGINAL RESEARCH

Integrative eQTL and Multi-Omics Analysis Reveals the Role of N6-Methyladenosine Modification in Polycystic Ovary Syndrome and Predictive Model Construction

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Objective: Polycystic ovary syndrome (PCOS) is a heterogeneous disorder with incompletely understood epigenetic regulation. We investigated the role of N6-methyladenosine (m6A)-related single nucleotide polymorphisms (m6A-SNPs) in PCOS.

Methods: Bulk RNA sequencing (RNA-seq) data from the GSE277906 dataset, comprising 23 PCOS patients and 17 healthy controls, was analyzed for differential expression. m6A-SNPs with significant expression quantitative trait locus (eQTL) signals were obtained by integrating eQTLGen and RMVar. Gene Ontology, KEGG, and Reactome supported enrichment analyses. Immune infiltration was estimated with CIBERSORT. Logistic-regression models were built based on the entire cohort without data splitting and evaluated using receiver operating characteristic (ROC) curves.

Results: A total of 362 differentially expressed genes and 45 PCOS-related candidate m6A-SNPs were identified. Enrichment analysis revealed that these genes were mainly involved in cell cycle dysregulation and interferon-α/β signaling pathways. Immune infiltration analysis showed no extensive remodeling of the overall immune landscape in PCOS, but correlation analysis identified significant associations between key genes and specific immune subsets, including monocytes, dendritic cells, and M2 macrophages. The predictive model integrating gene expression and immune cell infiltration achieved the highest diagnostic value (AUC = 0.836), outperforming models based on single features. Moreover, genomic annotation of the core gene SECTM1 indicated an open chromatin state and potential regulation by a complex transcriptional network.

Conclusion: m6A-SNPs likely contribute to PCOS pathogenesis via gene-regulatory effects. The integrative model shows high diagnostic promise, and SECTM1 emerges as a potential candidate for further functional validation and diagnostic exploration.

Keywords: polycystic ovary syndrome, m6A-SNPs, immune microenvironment

Introduction

Polycystic ovary syndrome (PCOS) is one of the most common endocrine and metabolic disorders in women of reproductive age, with a global prevalence of 6–20%.[1] Clinically, it is highly heterogeneous, featuring hyperandrogenism, chronic anovulation, and polycystic ovarian morphology, and is frequently associated with insulin resistance, obesity, dyslipidemia, and long-term cardiovascular risks.[2–4] The etiology of PCOS is complex, involving interactions between genetic, environmental, and lifestyle factors, while its precise molecular mechanisms remain unclear, posing significant challenges for diagnosis and treatment.[5]

Genome-wide association studies (GWAS) have identified multiple single nucleotide polymorphisms (SNPs) significantly associated with PCOS susceptibility, implicating immune, metabolic, and steroidogenic pathways in disease development.[6] However, most of these loci are located in non-coding regions, with poorly defined functions. Some SNPs may influence PCOS risk by regulating gene expression rather than altering protein structure, acting as expression quantitative trait loci (eQTLs).[7] Thus, integrating genetic variation with transcriptional regulation is essential to deepen

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our understanding of PCOS pathogenesis. While PCOS is fundamentally an ovarian and endocrine disorder, it is also characterized by chronic systemic inflammation and immune dysregulation. Therefore, utilizing blood-derived eQTL data provides a valuable and non-invasive window into systemic immune-related genetic regulation. However, it is important to acknowledge that gene regulation in circulating immune cells may not fully reflect the specific transcriptional dynamics in local ovarian tissues, such as granulosa or theca cells. This tissue specificity must be considered when interpreting the downstream regulatory mechanisms.

Epigenetic modifications add another critical layer of regulation. Among these, N6-methyladenosine (m6A) is the most abundant internal modification of eukaryotic mRNA. m6A is dynamically and reversibly regulated by methyltransferases, demethylases, and binding proteins, affecting mRNA splicing, export, stability, degradation, and translation.[8] Through these processes, m6A plays essential roles in cell differentiation, immune response, and metabolic homeostasis.[9] Dysregulated m6A has been linked to various metabolic and inflammatory diseases. Particularly in the context of ovarian physiology, emerging evidence suggests a potential role of m6A modification in follicular development, oocyte maturation, and granulosa cell function.[10] The dynamic balance of m6A appears to be involved in maintaining maternal mRNA stability and proper meiotic initiation during oogenesis. Furthermore, aberrant m6A landscapes have been increasingly implicated in PCOS. For instance, altered expression of m6A regulators, such as the demethylase FTO, and changes in overall m6A RNA methylation levels within ovarian granulosa cells have been associated with hyperandrogenism, altered gene expression profiles, and impaired follicle selection in PCOS patients.[11,12] These functional insights provide crucial biological context, indicating that m6A dysregulation may contribute to the ovarian pathophysiology of PCOS. Importantly, genetic variants located within m6A consensus motifs may alter m6A modification levels, thereby affecting mRNA stability and translation efficiency, leading to transcriptional variation and phenotypic heterogeneity.[13] These findings suggest that m6A-SNPs may represent a crucial bridge linking genetic susceptibility to epitranscriptomic regulation in PCOS.

This study therefore aims to systematically investigate the regulatory roles of m6A-SNPs in PCOS by integrating multi-omics and bioinformatics analyses. Particular emphasis is placed on their influence on gene expression and immune microenvironmental changes, as well as the construction of a predictive model based on key molecular and cellular features, providing novel insights and potential targets for mechanistic research and precision diagnosis of PCOS.

Materials and Methods

Data Preprocessing

The transcriptome dataset, GSE277906, used in this study were obtained from the Gene Expression Omnibus (GEO, https://www.ncbi.nlm.nih.gov/geo/), which includes 23 PCOS and 17 normal samples. Raw expression files were normalized and preprocessed. Empty probes were first removed to avoid invalid signals. Probes mapping to multiple genes were discarded to prevent bias from ambiguous mapping. For genes mapped by multiple probes, the median expression value was used as the final expression level to ensure matrix stability. The preprocessed expression matrix was applied to differential expression and subsequent analyses. As all samples in the GSE277906 dataset were from a single platform, batch effect correction was unnecessary. Standard normalization was applied to ensure data comparability. The present study was approved by the Institutional Review Board of the Seventh Affiliated Hospital, Sun Yat-sen University (Approval No. KY-2024-258-02).

eQTL Dataset

Blood eQTL data were derived from the eQTLGen database (https://eQTLgen.org/), a large multi-center consortium integrating 31,684 blood samples from European ancestry individuals, covering cis-eQTL information for 16,989 genes. Significant cis-eQTLs (false discovery rate, FDR < 0.05) and allele frequency information were extracted to explore the functional links between SNPs and gene expression.[14]

Disease-Related m6A-SNPs

RNA modification-associated variant data were obtained from the RMVar database (https://www.rmvar.renlab.org/), which integrates multiple RNA modification types and related genetic variation. In this study, m6A-SNPs from RMVar were intersected with eQTLGen data to identify m6A-SNPs with significant eQTL signals and their corresponding genes.[15]

Differential Expression and Functional Enrichment Analysis

Differentially expressed genes (DEGs) were identified using the limma R package, with thresholds $|\log_2 FC| > 0.25$ and $p < 0.05$.[16] DEGs were subjected to functional enrichment analysis. Gene Ontology (GO) analysis included three categories: cellular component (CC), molecular function (MF), and biological process (BP), revealing potential localization and functional roles. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis was performed to identify metabolic, signaling, and disease-related pathways. Analyses were conducted with the clusterProfiler package using enrichGO and enrichKEGG functions.[17] Reactome pathway enrichment was conducted using the ReactomePA package to assess overall biological networks. Results were visualized with bubble and bar plots.

Immune Microenvironment Analysis

To assess immune characteristics of PCOS, immune cell infiltration was analyzed using the CIBERSORT algorithm, quantifying proportions of 22 immune cell types per sample.[18] The IOBR R package was used to implement CIBERSORT, providing infiltration levels in PCOS and normal samples.[19] Spearman correlation analysis was performed to evaluate associations between immune cells. Additionally, the psych R package was applied to assess correlations between infiltration levels and candidate m6A-related genes,[20] with visualization using corrplot R package.

Disease Prediction Model

To evaluate the clinical predictive value of key features, predictive models were built using the rms R package.[21] Given the limited sample size (n = 40), the entire dataset was utilized for model construction to maximize statistical power, without splitting into training and testing sets or performing internal cross-validation. Furthermore, predictor variables were strictly predefined based on our upstream bioinformatic screening rather than algorithmic feature selection, mitigating the risk of data dredging. Separate models were constructed for gene features and immune cell features, and compared with a combined model. Receiver operating characteristic (ROC) curves were generated to calculate area under the curve (AUC) values for predictive efficacy. A nomogram was then constructed to translate regression results into a scoring system. Each variable corresponded to a score, which was summed to estimate total risk, mapped to a probability of PCOS.

m6A-SNPs and RNA-Binding Protein Interactions

To further investigate epigenetic regulation of m6A-related genes, the UCSC Genome Browser (GRCh37/hg19 version; https://genome.ucsc.edu/) was used for visualization and annotation. Integrated genomic data included RefSeq annotations, transcriptome profiles, common SNPs, DNase I hypersensitivity regions, and transcription factor binding peaks. Candidate m6A gene loci and overlapping regulatory elements were examined to infer potential transcriptional regulation mechanisms.

Statistical Analysis

All data processing and statistical analyses were performed in R (version 4.1.2). For continuous variables with normal distribution and homogeneity of variance, independent *t*-tests were applied. For non-normally distributed data, Wilcoxon rank-sum tests were used. Comparisons among three or more groups employed one-way ANOVA if assumptions were met, or the Kruskal–Wallis test otherwise. Correlation analyses were performed using Spearman correlation. All tests were two-sided, and $p < 0.05$ was considered statistically significant unless otherwise specified.

Results

Identification and Clustering Characteristics of DEGs

To systematically elucidate the role of m6A modification in PCOS, we established an overall research workflow (Figure 1). Specifically, we obtained m6A-SNP information from the RMVar database, significant eQTL sites (FDR < 0.05) from the eQTLGen database, and DEGs from the PCOS dataset. The intersection of these three datasets yielded m6A-SNPs and candidate genes associated with the disease. We further conducted immune cell infiltration analysis, correlation analysis between m6A genes and immune cells, construction of a predictive nomogram, and chromatin state profiling of key genes, aiming to comprehensively reveal the potential roles of m6A modification in the pathogenesis of PCOS.

To investigate transcriptional differences between PCOS and normal samples, we performed differential expression analysis. A total of 362 DEGs were identified, including 300 upregulated and 62 downregulated genes. The volcano plot clearly displayed the distribution of DEGs, among which RIOK1, MRPL46, DET1, TSG10, GUCA1B, RXYLT1, AKAP7, CTBS, ZNF559, and FCF1 were significantly upregulated in PCOS, while ADGRE5, C15orf48, and ENG were the most significantly downregulated (Figure 2A). These findings provide candidate targets for further exploration of the molecular mechanisms underlying PCOS. Moreover, the heatmap based on DEG expression profiles further revealed distinct expression differences between PCOS and normal samples. Clustering analysis showed clear separation between the two groups at the transcriptional level (Figure 2B).

Functional Enrichment Analysis of DEGs

To further explore the potential roles of DEGs in the pathogenesis of PCOS, KEGG and GO enrichment analyses were performed. KEGG pathway analysis revealed that DEGs were significantly enriched in multiple signaling pathways, including the basal transcription factor pathway and cellular senescence (Figure 3A). In the GO BP category, DEGs were mainly enriched in fatty acid synthesis and metabolic regulation, peptide hormone response, and protein mannosylation (Figure 3B), suggesting that metabolic imbalance and immune regulation play important roles in PCOS. In the GO CC category, DEGs were enriched in the transcription initiation complex, respiratory chain complex, RNA polymerase II holoenzyme, and phagocytic vesicles (Figure 3C), indicating possible transcriptional dysregulation and energy metabolism abnormalities in PCOS. At the GO MF level, DEGs were significantly enriched in Toll-like receptor binding, TGF-β receptor binding and activity, transcription initiation factor activity, and transmembrane transporter activity (Figure 3D), suggesting that inflammatory responses and signal transduction may be involved in the occurrence and development of PCOS.

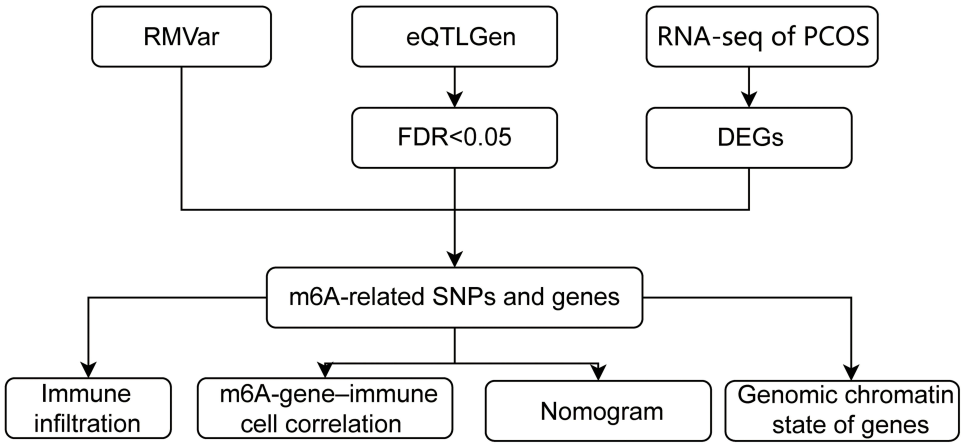

Figure 1 Flowchart of the study design. This workflow details the integration of transcriptomic, eQTL, and m6A-SNP data to identify PCOS-related genes, followed by downstream functional, immune, and predictive model analyses.

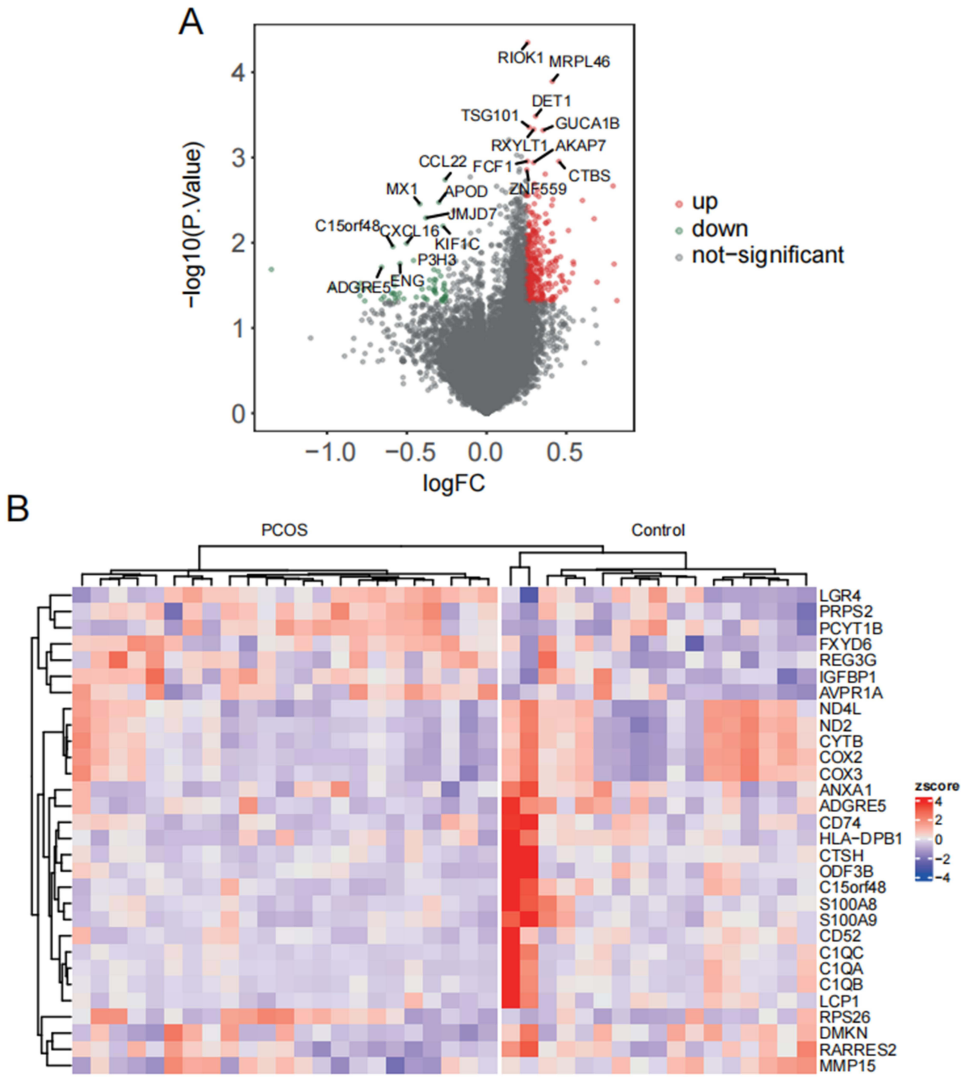

Figure 2 Differential expression analysis of PCOS and normal samples. (**A**) The volcano plot shows the distribution of DEGs. The x-axis represents log_2FC, and the y-axis represents -log(*p*value). Red dots indicate upregulated genes, green dots indicate downregulated genes, and gray dots indicate non-significant genes. Several representative DEGs are labeled in the plot. (**B**) Hierarchical clustering heatmap based on DEGs. The x-axis represents samples, and the y-axis represents genes. Colors indicate standardized expression values (Z-scores), with red representing high expression and blue representing low expression.

Functional Enrichment Analysis of PCOS-Related m6A-SNP Genes

To identify m6A-SNPs associated with PCOS, we applied a two-step intersection strategy. First, at the SNP level, SNPs with significant eQTL signals (FDR < 0.05) from the eQTLGen database were intersected with m6A-SNPs from the RMVar database to obtain eQTL-supported m6A-SNPs. Second, at the gene level, these m6A-SNPs were mapped to their cis eQTL target genes and further intersected with DEGs from the GSE277906 dataset, ultimately yielding 45 PCOS-related candidate genes (Supplementary Box 1). These genes represent critical nodes closely associated with m6A modification, transcriptional regulation, and the molecular pathology of PCOS (Figure 4A).

Functional enrichment analysis of the intersected genes revealed significant enrichment in biological processes such as aberrant regulation of the mitotic cell cycle due to RB1 defects, diseases of the mitotic cell cycle, and interferon α/β signaling (Figure 4B). These enriched pathways suggest that the development and progression of PCOS may be closely linked to dysregulation of the cell cycle, antiviral immune responses, and interferon signaling regulation.

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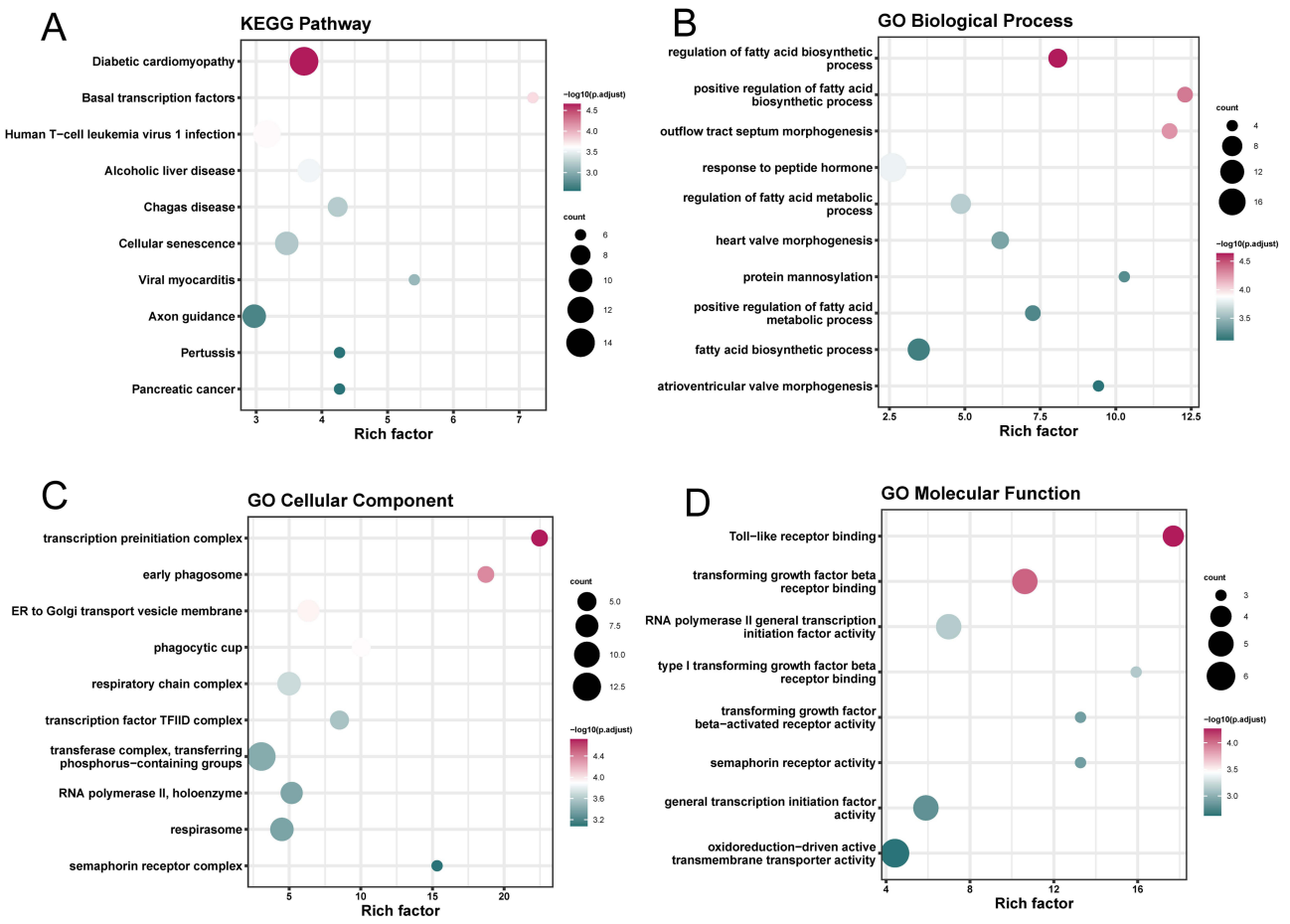

Figure 3 Functional enrichment analysis of differentially expressed genes in PCOS. (**A–D**) KEGG pathway (**A**) and GO enrichment analysis, including BP (B), CC (C), and MF (**D**) enrichment analysis. In the bubble plot, the x-axis represents the Rich factor, the y-axis represents pathway names, bubble size indicates the number of genes, and color intensity reflects the level of significance ($-\log_{10}$adjusted *p*value).

Correlation Between Immune Cell Infiltration Patterns and PCOS-Related m6A-SNP Genes

By systematically analyzing the immune cell composition of PCOS patients, we further uncovered their immunological characteristics and potential pathological mechanisms, which are critical for understanding the onset and pathophysiological changes of PCOS. Using the CIBERSORT algorithm to perform immune infiltration analysis on transcriptome data, we obtained the proportional distribution of 22 immune cell types across all samples (Figure 5A). Overall, PCOS patients and controls exhibited a high degree of consistency in immune cell infiltration patterns, suggesting that PCOS is not accompanied by large-scale remodeling of immune cell proportions. To more intuitively compare differences between the two groups, boxplots of immune cell proportions were generated. The results showed that most immune cell types displayed no significant differences between PCOS and controls, indicating that the immune microenvironment of PCOS patients remains relatively stable (Figure 5B).

On this basis, we calculated the correlations between immune cell proportions and PCOS-related m6A-SNP genes in both groups, and selected the top five immune cell types most significantly correlated with multiple genes, as well as the top five genes most significantly correlated with multiple immune cell types (Figure S1). The results revealed that monocytes, activated dendritic cells, resting dendritic cells, eosinophils, and M2 macrophages showed the strongest correlations with candidate genes, while at the gene level, AKT3, CCDC88B, FSTL1, C21orf91, and SECTM1 exhibited strong correlations with multiple immune cell types. Further analysis demonstrated that in the PCOS group, monocytes

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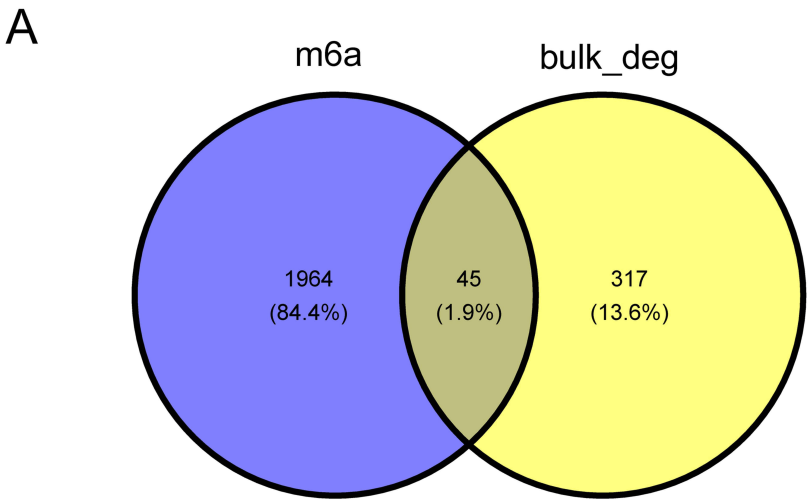

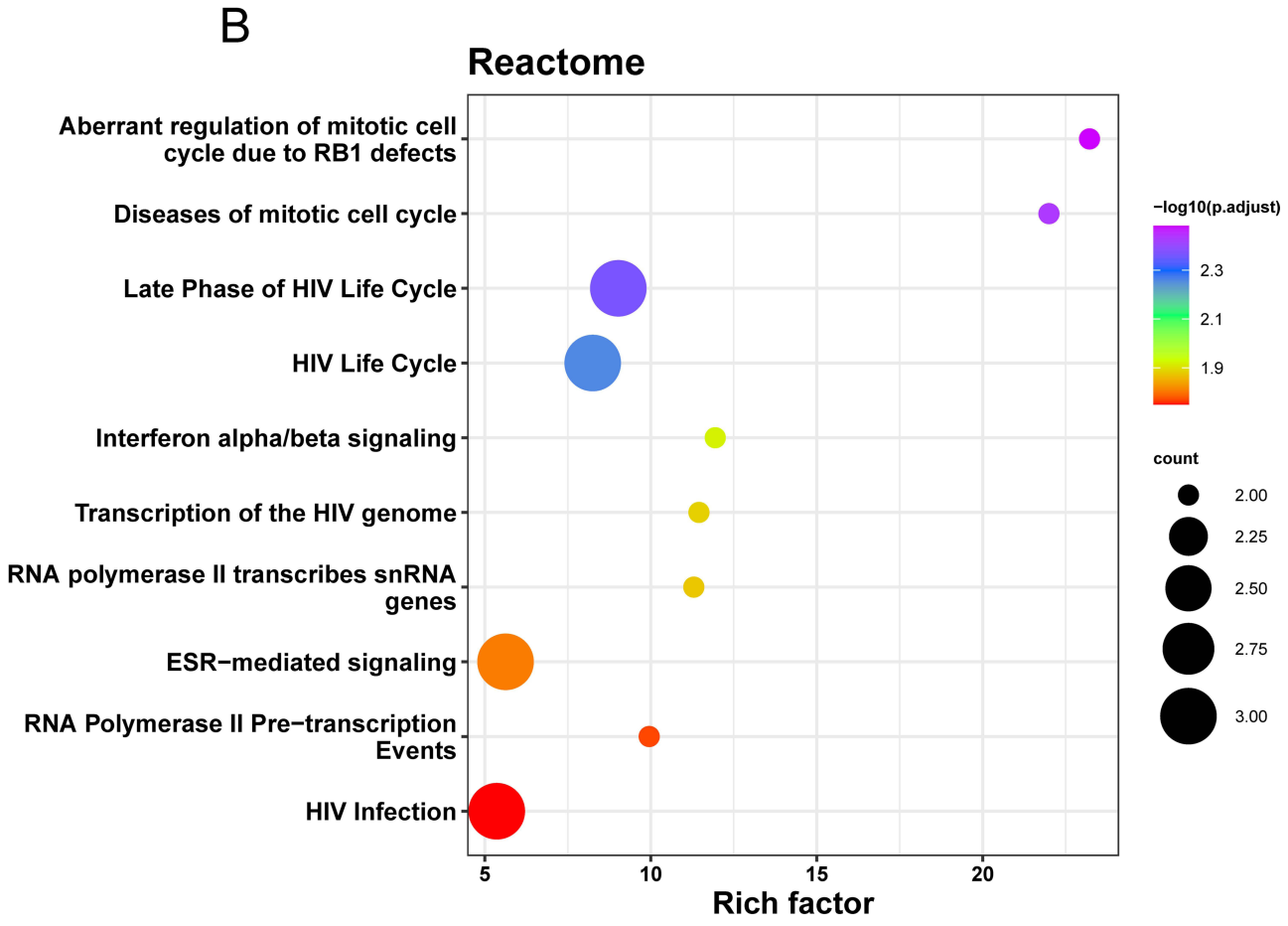

Figure 4 Identification and functional enrichment of PCOS-related m6A-SNP genes. Venn diagram illustrating the overlap between eQTL-supported m6A-SNP genes (n = 1964) and DEGs (n = 317). A total of 45 overlapping genes were identified as PCOS-related candidates. (**B**) Reactome pathway enrichment analysis. The x-axis represents the Rich factor, the y-axis represents pathway names, bubble size indicates the number of enriched genes, and color intensity reflects the significance level ($-\log_{10}$adjusted *p*value).

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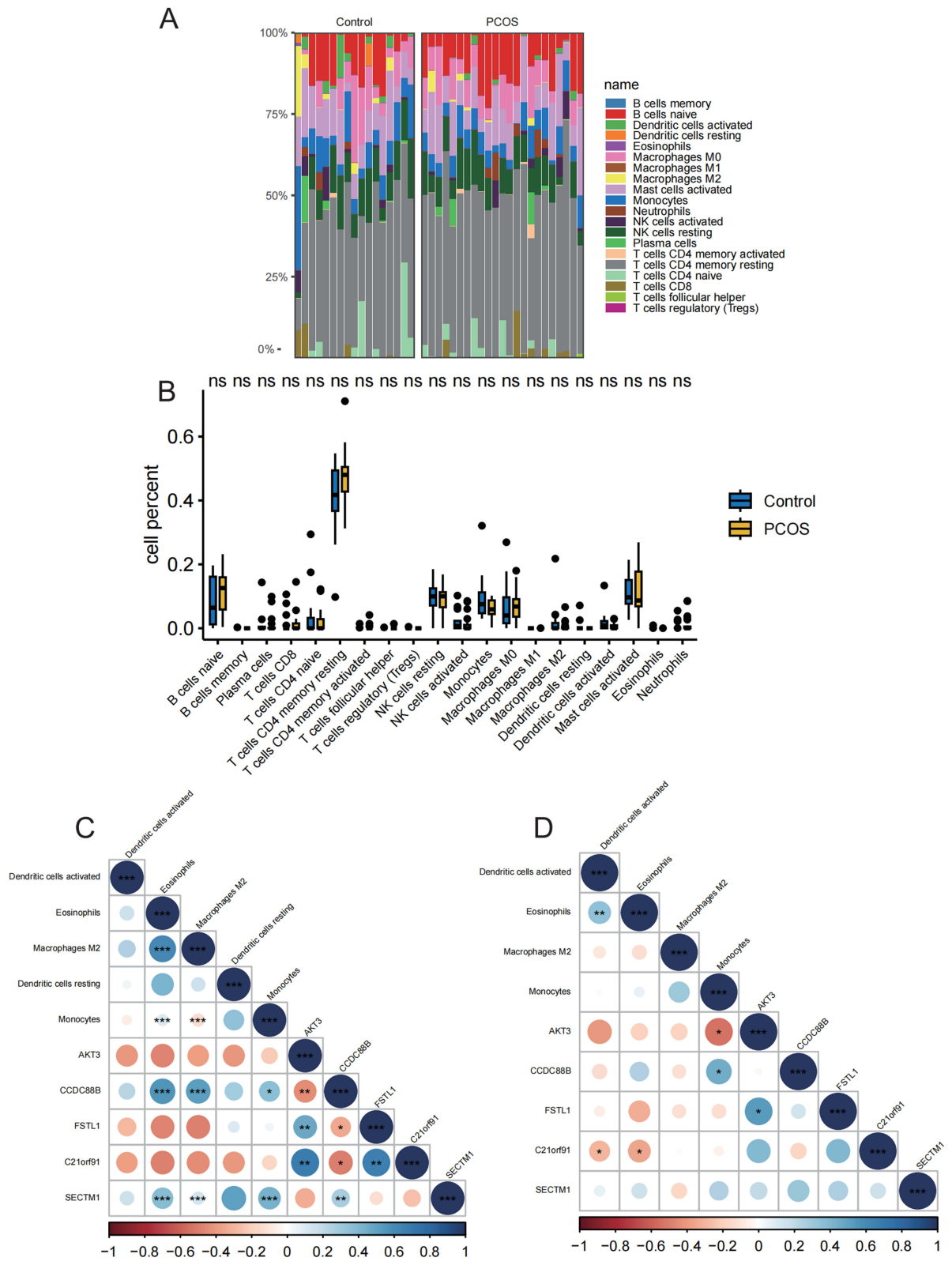

Figure 5 Immune cell infiltration and correlation with PCOS-related m6A-SNP genes. (**A**) Stacked bar plot showing the proportional distribution of 22 immune cell types in PCOS and control samples. (**B**) Boxplots comparing differences in immune cell proportions between the two groups. (**C and D**) Correlation heatmaps showing the associations between five key PCOS-related m6A-SNP genes (AKT3, CCDC88B, FSTL1, C21orf91, SECTM1) and immune cell proportions in PCOS patients. Circle size represents the strength of correlation, and color indicates positive or negative correlations. (***$p < 0.001$, **$p < 0.01$, *$p < 0.05$), ns = not significant).

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were positively correlated with AKT3 and CCDC88B, whereas FSTL1 and SECTM1 showed varying degrees of correlation with dendritic cells and M2 macrophages (Figure 5C and D).

Risk Prediction Model Based on Genes and Immune Cells

To further evaluate the role of PCOS-related m6A-SNP genes and immune cells in predicting PCOS risk, we constructed three types of predictive models based on the PCOS-related m6A-SNP genes and immune cell infiltration results, and compared their performance using ROC curves. The results showed that the combined gene and immune cell model achieved an AUC of 0.836, which was higher than that of the gene-only model (AUC = 0.818) and the immune cell-only model (AUC = 0.731), indicating that integrating both features provides a more comprehensive reflection of the molecular and immunological characteristics of PCOS (Figure 6A).

Based on the optimal model, we further developed a risk prediction nomogram, incorporating the key genes, including AKT3, CCDC88B, FSTL1, C21orf91 and SECTM1, and five immune cell types, including activated dendritic cells, eosinophils, M2 macrophages, resting dendritic cells, and monocytes. In the nomogram, each variable was assigned a score, and the cumulative score was converted into a total risk probability, enabling individualized prediction of PCOS risk. This model not only provides quantitative risk assessment but also facilitates intuitive interpretation of inter-individual differences in clinical applications (Figure 6B).

In addition, we validated the predictive stability of the model using calibration curves. The results demonstrated good agreement between predicted risk and observed outcomes, particularly in medium- and high-risk groups, where predicted values closely overlapped with actual observations. These findings indicate that the model has strong robustness and potential for clinical application (Figure 6C).

Chromatin Accessibility and Regulatory Features of SECTM1

DNase I hypersensitivity reflects regions of chromatin with increased sensitivity to DNase I digestion, typically indicating an open chromatin state closely associated with transcription factor binding sites and gene regulatory activity. SECTM1 was identified as the top gene contributing to outcomes in the constructed predictive model. To further investigate the transcriptional regulatory characteristics of this key candidate gene, we performed chromatin accessibility analysis of SECTM1. The results revealed multiple DNase I hypersensitive peaks within the chromatin region of SECTM1, suggesting that this gene resides in an open chromatin state across various cell types, conferring high transcriptional potential. Moreover, integration of GTEx, ENCODE, and ReMap datasets demonstrated that this region not only overlapped with common SNP loci but also contained multiple transcription factor binding sites, indicating that SECTM1 expression is likely influenced by a complex transcriptional regulatory network. These findings provide important clues for further elucidating the role of SECTM1 in the pathogenesis of PCOS (Figure 7).

Discussion

PCOS is a highly heterogeneous and complex disorder, and its etiological mechanisms have long been a major focus and challenge in reproductive medicine. Given the extreme clinical heterogeneity of PCOS, m6A modifications act as critical convergence points integrating diverse genetic signals. Targeting upstream m6A-SNPs helps uncover shared molecular pathways underlying varied phenotypes, providing a biological basis for universal diagnostic biomarkers. Although GWAS have identified numerous susceptibility loci, the functional interpretation and downstream mechanisms remain poorly understood. In this study, we focused on m6A, a core epitranscriptomic modification, and systematically investigated the regulatory roles of m6A-SNPs in PCOS. Specifically, we intersected SNPs with significant eQTL signals, m6A-SNPs from the RMVar database, and DEGs of PCOS samples to identify PCOS-related m6A-SNP genes. We then examined their association with immune cell infiltration and constructed a predictive model integrating gene expression and immune features, highlighting SECTM1 as a key regulator with clinical relevance.

We first identified 362 DEGs at the transcriptome level, which showed clear distinctions between PCOS patients and normal samples. Functional enrichment analysis demonstrated that these genes were significantly enriched in pathways, including basal transcription factors and cellular senescence, suggesting that global transcriptional dysregulation and cell cycle disturbances may contribute to PCOS pathogenesis. GO enrichment further revealed roles in fatty acid metabolism,

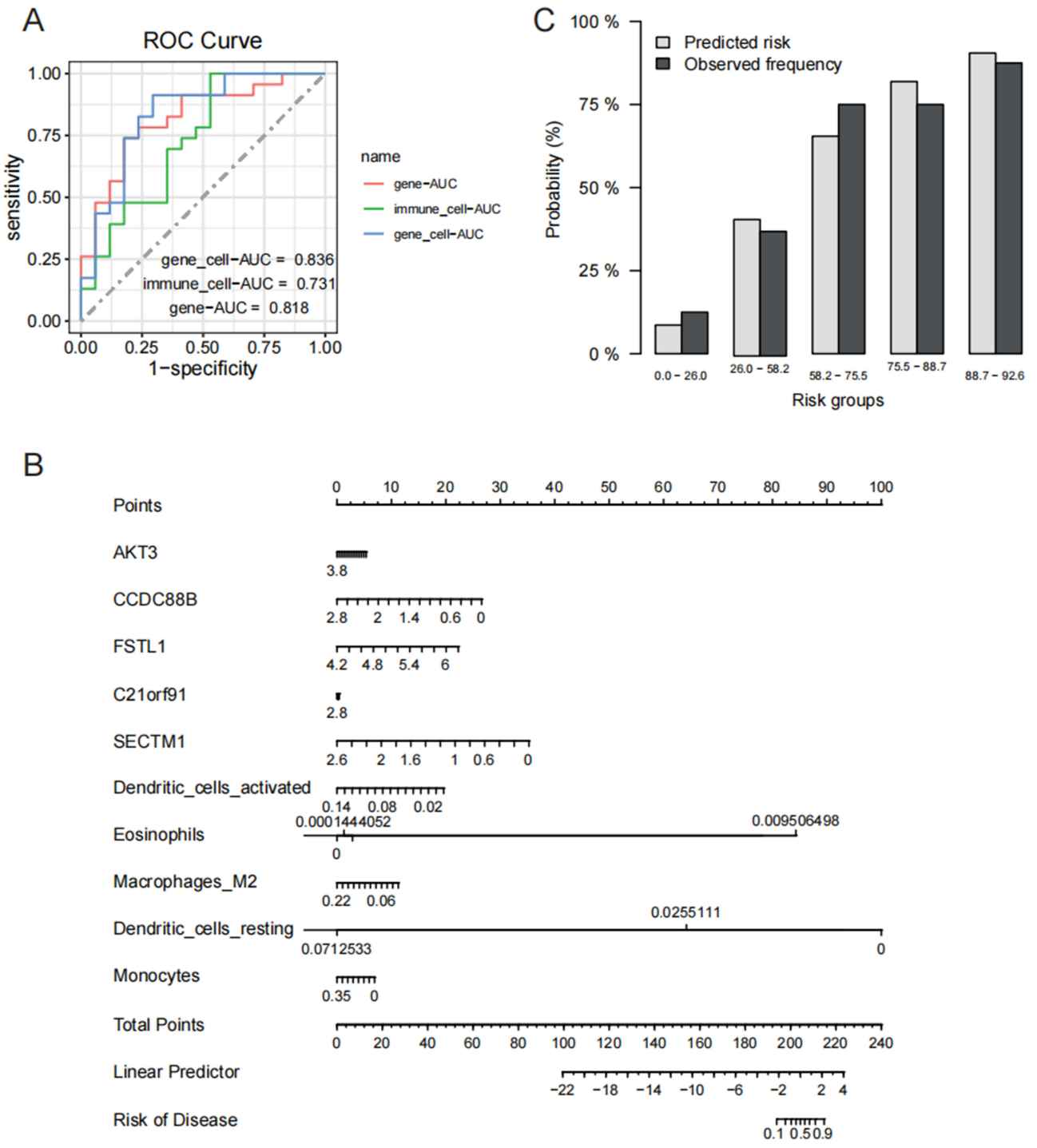

Figure 6 Construction and validation of a predictive model based on PCOS-related m6A-SNP genes and immune cells. (**A**) ROC curves comparing the predictive performance of three models. (**B**) Risk prediction nomogram incorporating the key genes and immune cell types. (**C**) Calibration bar chart of the predictive model. The light gray columns represent the predicted risk of PCOS, and the dark gray columns represent the actual observed frequency across different risk groups.

peptide hormone response, and protein modification, consistent with the metabolic imbalance and abnormal hormonal responses commonly observed in PCOS.[22,23] At the cellular component and molecular function levels, DEGs were linked to transcription initiation complexes, respiratory chain complexes, and Toll-like receptor binding, suggesting combined defects in energy metabolism and immune signaling.

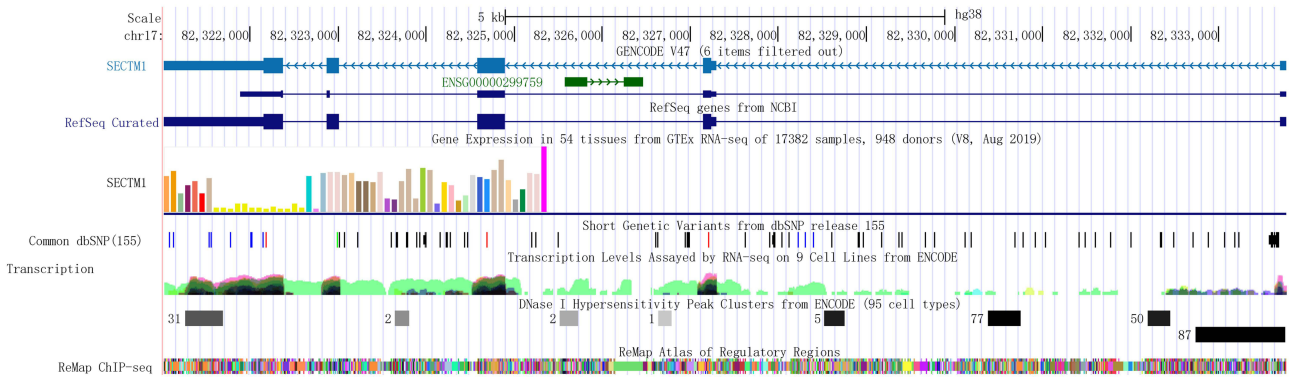

Figure 7 Chromatin accessibility and regulatory features of SECTM1. Genomic location and transcript structure of the SECTM1 gene, along with its expression levels across multiple tissues. The figure indicates the distribution of dbSNP sites, RNA transcription levels, DNase I hypersensitive regions, shown as green peaks, and transcription factor binding sites in ReMap database.

Previous studies on PCOS have primarily focused on the differential expression of core m6A regulators like FTO or METTL3.[11,12] In contrast, our study bridges genetic susceptibility with epitranscriptomics by systematically integrating m6A-SNPs, eQTL signals, and immune microenvironmental profiling. This multi-omics approach identifies specific m6A-altering variants linked to immune dysregulation. By constructing a genetic-immune predictive nomogram, we provide a novel systems-level framework for understanding PCOS pathogenesis, moving beyond single-gene functional analyses.

Through the integration of eQTL signals, RMVar-derived m6A-SNPs, and DEGs, we identified 45 candidate m6A-SNP genes associated with PCOS. This strategy underscored the dual importance of gene expression regulation and genetic variation. These intersecting genes likely reside at the crossroads of genetic variation, transcriptional regulation, and epigenetic modification, offering greater mechanistic insight than single-level markers. Enrichment analysis revealed strong involvement in aberrant mitotic cell cycle regulation and cell cycle-related diseases, highlighting the link between PCOS and impaired cell cycle control. As oocyte maturation and follicular development rely on precise cell cycle regulation, its dysregulation may contribute to follicular arrest, anovulation, and other key phenotypic features of PCOS.[24] Moreover, enrichment in interferon α/β signaling implicates immune homeostasis, consistent with the chronic low-grade inflammation frequently observed in PCOS.[25–27]

Immune infiltration analysis using CIBERSORT showed that PCOS and normal samples shared a broadly consistent immune cell landscape, suggesting that global immune cell redistribution is not a hallmark of PCOS. Instead, immune abnormalities may manifest in specific immune subpopulations or at the gene-regulatory level.[28] Although broad compositional shifts in immune cell proportions were absent, correlation analysis revealed strong associations between key candidate genes and specific immune subsets. It is plausible that these genetic variations (eg., in *AKT3* and *SECTM1*) influence the functional state, polarization, or activation status of resident immune cells—such as monocytes and M2 macrophages—rather than altering their absolute abundance. For instance, *AKT3*, a key member of the AKT signaling pathway, may exacerbate chronic inflammation in PCOS through regulation of monocyte responses.[29] CCDC88B, which has been implicated in immune cell migration and inflammatory regulation, further supports the notion of overactive immune signaling in PCOS patients.[30] Additionally, FSTL1 was associated with dendritic cells, potentially influencing antigen presentation and immune activation, while SECTM1 correlated with M2 macrophages, suggesting a role in immune suppression and inflammatory balance. Given the reported importance of M2 macrophages in ovarian tissue repair and resolution of local inflammation, aberrant regulation of SECTM1 may disrupt follicular development and ovulation.[31,32] However, we must exercise caution in interpreting these subtle correlations. Without direct functional assays to assess immune cell activity, the biological and clinical relevance of these specific gene-immune associations remains speculative. Therefore, these findings should be viewed primarily as hypothesis-generating, pointing towards potential gene-regulatory mechanisms that require future validation through single-cell RNA sequencing or functional immunology assays.

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We then constructed predictive models integrating key PCOS-related m6A-SNP genes and immune infiltration features. The combined model achieved superior predictive performance (AUC = 0.836) compared with gene-only (AUC = 0.818) or immune-only (AUC = 0.731) models, with calibration curves confirming its robustness. These results indicate that neither molecular nor immunological markers alone sufficiently capture the complexity of PCOS, while their integration provides a more comprehensive representation of disease mechanisms. This highlights the advantages of multimodal data integration for disease prediction and clinical translation.

Among these genes, SECTM1 emerged as the top contributor to the predictive model. Chromatin accessibility analysis revealed multiple DNase I hypersensitive peaks in the SECTM1 locus, indicating an open chromatin state and high transcriptional potential across diverse cell types. Furthermore, overlap with common SNP loci and transcription factor binding sites, supported by GTEx, ENCODE, and ReMap datasets, suggested complex transcriptional regulation of SECTM1 expression. Taken together, these findings indicate that SECTM1 is influenced by both genetic variation and epigenetic regulation, while also playing a key role in immune modulation. Previous studies have shown that SECTM1 interacts with immune cells and participates in inflammatory and immunoregulatory processes.[33,34] In the ovary, a healthy balance of M1/M2 macrophages is vital for normal follicular development, ovulation, and corpus luteum regression.[35] Our data show that *SECTM1* expression strongly correlates with M2 macrophages. Therefore, we hypothesize that variant-driven changes in *SECTM1* (via m6A-SNPs) might disrupt this local immune balance, potentially worsening the chronic inflammation seen in PCOS.[36] However, while bioinformatics and current literature support this mechanism, *SECTM1*'s exact role in ovarian physiology requires direct proof. Future in vitro and in vivo studies are needed to confirm these functional mechanisms.

Limitations

Several limitations of this study should be noted. First, we relied on blood-derived eQTL data. Although systemic immune profiles are highly relevant to the chronic inflammation of PCOS, circulating blood cells may not perfectly mirror the transcriptional dynamics of local ovarian tissues (eg., granulosa cells). Thus, our findings warrant cautious interpretation and direct validation in ovarian cells. Second, the public GSE277906 dataset lacked detailed clinical metadata, such as exact age, body mass index (BMI), and metabolic status. This prevented us from adjusting for these important clinical confounders in our models. Furthermore, we used nominal p-values ($p < 0.05$) rather than multiple-testing corrections for downstream DEG and immune correlation analyses to preserve potentially meaningful biological signals in this small cohort. We acknowledge that this approach increases the risk of false positives, which must be addressed in future large-scale studies. Fourth, regarding our predictive model, the modest sample size precluded the use of data splitting or internal cross-validation, raising the possibility of overfitting. The absence of an independent external validation cohort further restricts the immediate generalizability of the model.Finally, as an entirely in silico study, large-scale independent clinical cohorts and targeted molecular experiments are essential to fully validate our findings.

Conclusion

In conclusion, this study demonstrates the potential role of m6A-SNPs in PCOS, emphasizing the interplay between genetic variation, epigenetic regulation, and the immune microenvironment. SECTM1, as a key candidate gene, exhibits unique epigenetic and immunoregulatory features, highlighting it as an intriguing candidate that warrants further experimental investigation to determine its functional mechanisms and clinical utility in PCOS.

Data Sharing Statement

The datasets analyzed during the current study are available from the corresponding author (Lin Ma, malin8@mail.sysu.edu.cn) upon reasonable request. The raw data are accessible in the GEO database under accession number GSE277906.

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Disclosure

The authors report no conflicts of interest in this work.

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