

Identification of Autophagy-Related Biomarkers for Subtyping and Immune Microenvironment Remodeling in Ulcerative Colitis

Meng Li

The First Affiliated Hospital of Guilin Medical University

Ying Xu

The First Affiliated Hospital of Guilin Medical University

Songnan Zhang

The First Affiliated Hospital of Guilin Medical University

Nanfang Qu

Nanxishan Hospital of Guangxi Zhuang Autonomous Region

Junfeng Zhu

zhu.junfeng@lnu.edu.cn

Guilin Medical University

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Abstract

Background

Ulcerative colitis (UC) is an autoimmune inflammatory disease of the digestive tract. While autophagy plays a crucial role in inflammation and immunity, its precise mechanism in UC remains unclear. This study aimed to investigate the molecular characteristics and immune landscape associated with autophagy-related differentially expressed genes (AR-DEGs) in UC.

Methods

Differentially expressed genes (DEGs) were identified through analysis of the UC dataset (GSE87466). Subsequently, AR-DEGs were identified by intersecting these DEGs with established autophagy-related genes (ARGs) sets. A UC risk diagnostic model was constructed using logistic regression, and its diagnostic value was evaluated via the receiver operating characteristic (ROC) curve analysis. Utilizing AR-DEGs, consensus clustering analysis was performed to stratify UC patients into two distinct subtypes, whose differential characteristics were then thoroughly analyzed and compared. Single-cell sequencing (scRNA-seq) analysis was employed to investigate alterations in cell populations and intercellular communication within UC patients. The expression levels of the identified AR-DEGs were further validated by quantitative real-time PCR (qRT-PCR).

Results

Our study identified three AR-DEGs: *APOL1*, *CCL2*, and *IL-24*. A robust risk diagnostic model was successfully constructed based on these genes, demonstrating strong diagnostic performance (AUC = 0.99). UC patients were successfully stratified into two distinct subtypes. Subtype 1 exhibited heightened immune cell infiltration and significantly elevated expressions of these AR-DEGs. At the single-cell level, these three AR-DEGs mainly showed high expression in endothelial cells and fibroblasts, with concomitant significant alterations in cell proportions and intercellular communication. Validation via qRT-PCR confirmed significantly increased expression of *APOL1*, *CCL2*, and *IL-24* in intestinal tissues from UC mouse models and UC patients.

Conclusion

This study identifies *APOL1*, *CCL2*, and *IL-24* as critical autophagy-related biomarkers in the pathogenesis of UC. The diagnostic model constructed based on these genes demonstrates robust clinical predictive value. Furthermore, our findings reveal the significance of these signatures in stratifying UC patients into distinct molecular subtypes with unique immune infiltration patterns and highlight their specific enrichment in endothelial cells and fibroblasts, suggesting a role in remodeling

the immune microenvironment via altered intercellular communication. Collectively, these insights not only deepen the understanding of autophagy-mediated immune regulation in UC but also provide potential biomarkers and novel strategies for diagnosis and targeted therapy.

1. Introduction

Ulcerative colitis (UC), a principal form of inflammatory bowel disease (IBD), exhibits an increasing global prevalence and imposes a significant burden on healthcare systems[1, 2]. The etiology of UC is multifactorial, involving complex interactions among genetic susceptibility, immune dysregulation, and intestinal microbiota[3, 4]. Despite advances in understanding its pathogenesis, the precise molecular mechanisms remain elusive. Clinically, current diagnostic markers often lack the requisite sensitivity and specificity for early risk stratification and precise differentiation of UC from other intestinal disorders[5, 6]. Furthermore, while biological agents have revolutionized UC management, a substantial proportion of patients exhibit primary non-response or develop secondary resistance[7, 8]. Consequently, there is an urgent need to identify novel molecular biomarkers and therapeutic targets to improve diagnostic accuracy and treatment outcomes.

Autophagy is an evolutionarily conserved catabolic process essential for maintaining cellular homeostasis by degrading damaged organelles and misfolded proteins via the lysosomal pathway[9, 10]. In the context of intestinal health, autophagy plays a pivotal role in regulating innate immunity, maintaining the intestinal epithelial barrier, and modulating inflammatory responses[11, 12]. Dysregulation of autophagy has been implicated in the pathogenesis of UC, contributing to aberrant immune activation and tissue damage[13, 14]. However, the specific molecular landscape of ARGs in UC remains incompletely characterized. Moreover, how these genes influence the immune microenvironment and intercellular communication across different cell types—particularly at the single-cell resolution—is not yet fully understood. Bridging this knowledge gap is crucial for uncovering new pathogenic mechanisms.

In this study, we integrated bulk RNA sequencing and scRNA-seq data to systematically investigate the molecular characteristics and immune landscape associated with autophagy in UC. We identified key AR-DEGs and constructed a robust risk diagnostic model. Furthermore, we explored the expression patterns of these genes in specific cell populations, such as endothelial cells and fibroblasts, and analyzed their role in intercellular communication. Finally, the expression of identified key genes was validated in clinical samples and mouse models. This study aims to provide a novel theoretical basis for the molecular diagnosis and targeted therapy of UC.

2. Materials and method

2.1 Data Sources

We queried the gene expression omnibus (GEO) database (<http://www.ncbi.nlm.nih.gov/geo/>) for datasets using the keyword "ulcerative colitis". Six datasets pertaining to intestinal mucosal expression in UC patients were selected. Detailed information regarding these datasets is provided in in Table 1. The GSE87466 served as the training set, while GSE231993 was utilized for single-cell analysis. For external validation, the GSE36807, GSE75214, and GSE38713 datasets were merged, and batch effects were corrected using the "combat" package. Additionally, GSE73661 was employed to analyze correlations between AR-DEGs and clinical scores. A total of 222 ARGs were retrieved from the Human Autophagy Database (HADb).

Table 1
The Information Corresponding to All Datasets in the Study.

Datasets	Platform	UC samples	Control samples	Tissues
GSE84766	GPL13158	87	21	Colon mucosa
GSE36807	GPL570	15	7	Colon mucosa
GSE75214	GPL6244	97	11	Colon mucosa
GSE38713	GPL570	30	13	Colon mucosa
GSE73661	GPL6244	166	12	Colon mucosa
GSE231993	GPL18573	8	4	Colon mucosa

2.2 Identified DEGs and Functional Enrichment Analysis

To identify DEGs associated with UC pathogenesis, we used the "Limma" package in R software (version 4.3.1) to analyze the GSE87466 dataset, applying screening criteria of $|\log_2FC| > 1.5$ and adjusted P-value < 0.05 . Volcano plots and heatmaps of the identified DEGs were visualized using the "ggplot2" and "pheatmap" packages. Gene ontology (GO) and kyoto encyclopedia of genes and genomes (KEGG) analyses were conducted using the "ClusterProfiler" package. Overlapping genes between DEGs and ARGs were determined using Venn diagrams. These intersecting genes were termed AR-DEGs.

2.3 Establishment and Evaluation of Risk Score Diagnostic Model

We constructed a logistic regression diagnostic model using the "glmnet" R package, incorporating AR-DEGs as input features. To assess the model's performance, we generated ROC curves and calculated the AUC using the "ROCR" package. To enhance clinical utility, we developed a nomogram to visualize the diagnostic model. The predictive accuracy and clinical net benefit of the nomogram were evaluated via confusion matrices, decision curve analysis (DCA), and clinical impact curves using the "rms" and "rmda" packages. Finally, the diagnostic capability of individual feature genes was assessed by generating ROC curves for both the training and validation sets using the "pROC" package.

2.4 Autophagy-related Subtype Identification based on AR-DEGs

Consensus clustering was performed on the GSE87466 dataset using the "ConsensusClusterPlus" R package to identify distinct molecular subtypes. The optimal number of clusters was determined based on consensus matrix plots, consensus cumulative distribution function (CDF) plots, and the relative change in area under the CDF curve. Principal component analysis (PCA) was subsequently performed to visualize the distribution and variance between the identified clusters.

2.5 Immune Cell Infiltration Analysis

The "CIBERSORT" algorithm was utilized to quantify immune infiltration levels and identify significant differences in immune cell composition between UC subtypes. Spearman correlation analysis was performed to assess the relationship between feature genes and immune cell infiltration. Differential expression analysis was conducted using the "Limma" package, the expression of AR-DEGs across subtypes was visualized via boxplots using "ggplot2". Additionally, GO and KEGG enrichment analyses were performed on the identified subtype-specific DEGs.

2.6 Drug Prediction

We utilized the CellMiner database (<https://discover.nci.nih.gov/cellminer>) to predict potential therapeutic agents (FDA-approved and in clinical trials) targeting the AR-DEGs. The association between risk scores and drug sensitivity was evaluated using "impute" and "Limma" R packages.

2.7 Single-Cell Sequencing Analysis

Single-cell data processing and quality control were conducted using the "Seurat" R package. Cells were filtered based on the following exclusion criteria: (1) expression of fewer than 200 genes; (2) expression of more than 4,000 genes; and (3) mitochondrial gene content exceeding 20%. Dimensionality reduction was performed via PCA, selecting the top 30 principal components. Cell clustering was achieved using the FindNeighbors function followed by the FindClusters function with a resolution of 0.7. Clusters were visualized using uniform manifold approximation and projection (UMAP). Cell type annotation was performed using the "SingleR" package in conjunction with reference markers from the CellMarker database (<https://biocc.hrbmu.edu.cn/CellMarker/>). Cell proportions and gene expression features were visualized using the "ggplot2" package.

2.8 Cell Communication Analysis

Intercellular communication networks were inferred and quantified using the "CellChat" package, utilizing its database of known ligand-receptor interactions. Signaling networks were analyzed separately for normal and UC groups and subsequently integrated to compare communication patterns between conditions.

2.9 Animal Experiments

Male C57BL/6 mice were housed under specific pathogen-free conditions. Mice were randomly assigned to two groups (n = 9 per group). To induce acute colitis, the experimental group was administered 2.5% dextran sulfate sodium (DSS; MP Biomedicals) in drinking water for 7 days while the control group received normal drinking water. After the experiment, we collected the entire colon tissue. A 1-cm segment of colonic tissue was fixed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin (HE) for histological evaluation. An additional colonic segment was collected for total RNA extraction. All animal procedures were approved by the Ethics Committee of the Affiliated Hospital of Guilin Medical University (No:GLMC202303104).

2.10 Sample Collection

Inflamed intestinal tissues were collected from six UC patients recruited at the Digestive Endoscopy Department of the Affiliated Hospital of Guilin Medical University between January 2024 and September 2025. Adjacent non-inflamed tissues from the same patients served as the control group. Inclusion criteria were: (1) age > 18 years; (2) confirmed diagnosis of ulcerative colitis via colonoscopy and pathological examination, in accordance with the Chinese Guidelines for the Diagnosis and Treatment of Inflammatory Bowel Disease (2023); and (3) provision of informed consent. Exclusion criteria included: (1) presence of systemic lupus erythematosus, Sjögren's syndrome, or other autoimmune diseases; (2) malignancy or active infection; (3) use of corticosteroids or immunosuppressive drugs within the preceding month; and (4) family history of autoimmune diseases. This study was approved by the Ethics Committee of the Affiliated Hospital of Guilin Medical University (Approval No.: 2024YJSLL-74).

2.11 Quantitative Real-Time PCR.

Total RNA was isolated from colonic tissues using RNAiso™ Plus kit (Takara, Dalian, China) following the manufacturer's instructions. cDNA was synthesized from the total RNA using the PrimeScript™ RT Reagent Kit (Takara, Dalian, China). Gene expression levels were quantified via qRT-PCR using SYBR Premix Ex Taq™ Kit (Takara, Dalian, China).

2.12 Statistical Analysis

Relative gene expression was calculated using the $2^{-\Delta\Delta CT}$ method. Statistical analyses were performed using GraphPad Prism 8.0 software. Data are presented as mean $\pm$ standard error of the mean (SEM). Differences between two groups were evaluated using the independent Student's t-test for normally distributed data or the Mann-Whitney U test for non-normally distributed data. A P-value < 0.05 was considered statistically significant.

3. Result

3.1 Identification of AR-DEGs and Functional Enrichment Analysis in UC

Differential expression analysis was performed on colonic mucosa samples from 87 UC patients and 21 healthy controls retrieved from the GSE87466 dataset. A total of 327 DEGs were identified based on the criteria of $|\log_2FC| > 1.5$ and adjusted P-value < 0.05 . The expression profiles of these genes were visualized using a volcano plot (Fig. 1a) and a heatmap (Fig. 1b). A venn diagram (Fig. 1c) was used to intersect the identified DEGs with a set of ARGs obtained from the HADb database, resulting in three AR-DEGs: *APOL1*, *CCL2*, and *IL-24*. The results of GO (Fig. 1d) and KEGG (Fig. 1e) enrichment analyses showed that these DEGs were significantly enriched in biological processes and pathways such as granulocyte chemotaxis, G protein-coupled receptor binding, cytokine activity, cytokine receptor binding, chemokine signaling pathway, and *IL17* signaling pathway.

3.2 Model Efficacy Verification and External Data Validation

A diagnostic model was constructed using multivariate logistic regression to predict UC risk based on the three identified AR-DEGs. A nomogram was subsequently established to visualize the prediction model (Fig. 2a). In this model, the total score was calculated by summing the scores of each feature gene, indicating the UC risk. Subsequently, the confusion matrix (Fig. 2b), DCA curve (Fig. 2c), and impact curve (Fig. 2d) demonstrated that the model possessed robust predictive performance and potential clinical utility.

In the training set, the AUC of the constructed model was 0.99, and the AUC values of AR-DEGs were all greater than 0.9 (Fig. 2e-h). Furthermore, the genes *APOL1*, *CCL2*, and *IL-24* were significantly upregulated in UC samples (Fig. 2m). In the validation set, the expression trends of these genes remained consistent, showing significant upregulation in UC samples, as confirmed by the boxplots (Fig. 2i-l and 2n).

3.3 Correlation analysis of clinical scores

To investigate the association between AR-DEGs and clinical disease severity, we utilized an independent external dataset, GSE73661. This dataset contains clinical disease activity data of UC patients, allowing us to assess the expression levels of these genes across different disease severities. Significant differential expression was observed among UC patients with varying mayo scores (Fig. 3a-c). Specifically, the expression levels of AR-DEGs increased progressively with the elevation of the mayo score. To further explore this relationship, we analyzed the correlation between gene expression and the mayo score. Notably, spearman correlation analysis revealed a significant positive correlation between the expression levels of these genes and UC-related clinical activity scores (*APOL1*: $R = 0.586$, $P < 0.001$; *CCL2*: $R = 0.377$, $P < 0.001$; *IL-24*: $R = 0.468$, $P < 0.001$; Fig. 3d-f). These results suggest that AR-DEGs may contribute to UC pathogenesis and hold potential as biomarkers for assessing disease severity in UC patients.

3.4 Identification of Autophagy-Related Clusters in UC

To elucidate the the heterogeneity of autophagy-related patterns in UC, unsupervised consensus clustering was performed on 87 UC samples from the GSE87466 dataset based on the expression profiles of the three AR-DEGs using the ConsensusClusterPlus R package. The clustering stability was optimal at $k = 2$, identifying two distinct molecular subtypes (Fig. 4a). This classification was supported by the significant relative change in the area under the CDF curve from $k = 2$ to $k = 6$ (Fig. 4b and 4c). PCA further confirmed a clear segregation between the two clusters (Fig. 4.d). Consequently, the cohort was stratified into Cluster 1 ($n = 44$) and Cluster 2 ($n = 43$).

To characterize the underlying molecular differences between the two subtypes, we compared the expression levels of the AR-DEGs. As expected, cluster 1 exhibited significantly higher expression levels of all three AR-DEGs compared to cluster 2, designating it as the "autophagy-high" subtype (Fig. 4e). We further investigated the immune landscape differences between the two subtypes. The "autophagy-high" cluster 1 demonstrated a significantly higher abundance of infiltrating immune cells compared to cluster 2, suggesting a more active immune microenvironment (Fig. 4f). Notably, correlation analysis of *IL-24* revealed a robust positive correlation with various adaptive immune cells, including T cells and B cells, across the subtypes (Fig. 4g). This indicates a potential role for IL-24 in bridging autophagy regulation and immune cell recruitment in UC.

3.5 Identification of Potential Small Molecule Drugs for AR-DEGs

To explore potential therapeutic strategies targeting the identified AR-DEGs, we utilized the CellMiner database to analyze the correlation between gene expression and drug sensitivity. This analysis identified several small molecule compounds, including Staurosporine, AEG-40730, lenvatinib, and motesanib, as potential autophagy-modulating agents linked to the key genes. Specifically, high expression of *APOL1* was associated with increased sensitivity to Staurosporine ($\text{Cor} = 0.38$, $P = 0.002$; Fig. 5a and 5e). Regarding *IL-24*, a significant positive correlation was observed with sensitivity to AEG-40730 ($\text{Cor} = 0.34$, $P = 0.009$; Fig. 5b and 5f). Furthermore, elevated *CCL2* expression indicated potential sensitivity to VEGFR inhibitors such as lenvatinib ($\text{Cor} = 0.41$, $P = 0.001$; Fig. 5c and 5g) and motesanib ($\text{Cor} = 0.36$, $P = 0.004$; Fig. 5d and 5h). Based on these findings, the expression profiles of these three AR-DEGs could serve as potential biomarkers for predicting drug response. These results provide a theoretical basis for exploring targeted pharmacological interventions in UC patients exhibiting specific autophagy-related molecular patterns, warranting further experimental validation.

3.6 Single-Cell Transcriptomic Landscape of UC

To elucidate the cellular heterogeneity in UC, we analyzed scRNA-seq data from the GSE231993 dataset. After rigorous quality control to filter out low-quality cells and doublets, a total of 27298 cells were retained. Unsupervised dimensional reduction and clustering identified 23 distinct clusters (Fig. 6a-c). Based on canonical marker genes and SingleR annotation, these clusters were characterized into 8 major cell lineages: B cells, T cells, monocytes, epithelial cells, endothelial cells, smooth muscle cells,

fibroblasts, and mast cells (Fig. 6d), constructing a comprehensive cellular atlas of the UC microenvironment. Furthermore, differential expression analysis highlighted widespread transcriptomic alterations between UC and normal tissues across all identified cell types (Fig. 6e), emphasizing the multicellular nature of UC pathogenesis.

We next investigated the cell-type-specific expression patterns of the identified AR-DEGs. *APOL1* was predominantly enriched in endothelial cells and fibroblasts, while *CCL2* was showed broad expression in fibroblasts, smooth muscle cells, and endothelial cells. In contrast, *IL-24* exhibited relatively sparse expression in this dataset (Fig. 6f). Consistent with our bulk RNA-seq findings, violin plots confirmed that *APOL1* and *CCL2* were significantly upregulated in UC samples compared to the healthy controls particularly within their specific cell populations.

Analysis of cellular composition revealed significant remodeling of the immune and stromal compartments in UC. Specifically, we observed a marked expansion of B cells, indicative of an active adaptive immune response, alongside a significant reduction in epithelial cells and fibroblasts, reflecting mucosal damage and stromal dysregulation (Fig. 6g and 6h).

3.7 Altered Intercellular Communication Networks in UC

Comparative analysis of cell-cell interactions revealed a global expansion in both the number and interaction strength of cellular communications in the UC group compared to healthy controls (Fig. 7a). Notably, the crosstalk among stromal components—specifically fibroblasts, endothelial cells, and smooth muscle cells—was significantly potentiated in the disease state (Fig. 7b). Analysis of information flow patterns highlighted the hyperactivation of specific pro-inflammatory signaling pathways in UC (Fig. 7c), characterized by the enrichment of protease-activated receptors (PARs), C-C motif chemokine ligand (CCL) chemokines, and macrophage migration inhibitory factor (MIF) signaling, suggesting a coordinated stromal-immune response.

Given the identification of *CCL2* as a key AR-DEGs, we specifically focused on the CCL signaling network. Circle plot visualization identified T cells and fibroblasts as the primary "Senders" (sources), exhibiting strong ligand-receptor interaction probabilities with endothelial cells acting as the "Receivers" (targets) (Fig. 7d). Furthermore, comparative pathway analysis confirmed that the CCL signaling axis was markedly upregulated in UC compared to normal tissue (Fig. 8e). This suggests that fibroblasts and T cells may drive endothelial activation and immune cell recruitment through the CCL-CCR axis, thereby exacerbating the inflammatory microenvironment in UC.

3.8 Experimental Validation in Clinical Samples and in Vivo Models

To corroborate the in silico findings and validate the diagnostic potential of the three identified AR-DEGs, we quantified their mRNA expression levels using qRT-PCR. This was performed on intestinal mucosal biopsies from UC patients and colon tissues from a DSS-induced murine colitis model. Following DSS

induction, a murine model of acute colitis was successfully established. Mice in the DSS group developed typical clinical signs. At sacrifice, the colons were markedly shortened and displayed gross signs of congestion and edema (Fig. 8a and 8e). Histopathological analysis further revealed severe mucosal inflammation, characterized by crypt distortion and ulceration (Fig. 8b and 8f). A progressive body weight loss from day 4 onward (Fig. 8c), accompanied by diarrhea and hematochezia, leading to significantly increased disease activity index (DAI) scores (Fig. 8d). Consistent with the transcriptomic analysis, *APOL1*, *CCL2*, and *IL-24* were all found to be significantly upregulated in the intestinal mucosa of UC patients compared to healthy controls (Fig. 8i-k; $P < 0.05$). Furthermore, parallel validation in the murine model confirmed that *CCL2* and *IL-24* maintained significantly elevated expression trends (Fig. 8g-h), thereby supporting the conservation of these autophagy-related signatures across species.

4. Discussion

Autophagy is pivotal in maintaining intestinal epithelial homeostasis by facilitating the clearance of intracellular pathogens and damaged organelles[15, 16]. Dysregulated autophagy compromises the intestinal barrier and amplifies inflammatory cascades, thereby driving the pathogenesis of IBD[17, 18]. Additionally, autophagy is involved in regulating the immune response of the intestinal mucosa, aiding in the clearance of invading pathogens and preventing excessive inflammatory responses[19, 20]. In IBD patients, variations in ARGs are associated with disease susceptibility. Moreover, reduced or dysregulated autophagy activity may contribute to the persistence of intestinal inflammation[21]. Despite these associations, the specific autophagy-related molecular signatures and their diagnostic implications in UC remain to be fully elucidated. In this study, we systematically screened for AR-DEGs and identified three key genes (*APOL1*, *CCL2*, and *IL-24*). Functional enrichment analysis highlighted that the coordinated dysregulation of granulocyte chemotaxis and cytokine activity underlies the inflammatory milieu of UC. Based on these AR-DEGs, we constructed a diagnostic nomogram (rather than a "risk model") to distinguish UC patients from healthy controls. The model demonstrated robust predictive value and stability, suggesting that these autophagy-related signatures could serve as reliable biomarkers for clinical diagnosis and disease assessment. *APOL1*, a primate-specific innate immune gene involved in pathogen defense, functions as a BH3-only lipid-binding protein capable of inducing autophagic cell death[22]. While primarily studied in kidney disease where variants disrupt autophagic flux[23]. Its role in the gut is emerging. Our findings of elevated *APOL1* in UC suggest a similar pathogenic mechanism: *APOL1* overexpression may trigger excessive autophagy-dependent cell death or pyroptosis in intestinal epithelial cells. This hypothesis is supported by evidence that autophagy defects can activate the NLRP3 inflammasome[24, 25], leading to barrier loss and persistent inflammation characteristic of UC. *CCL2* acts as a critical chemoattractant for monocytes and macrophages. Although some studies suggest that autophagy is required for *CCL2* induction during wound healing[26, 27], our data and others observe significantly upregulated *CCL2* in the inflamed UC mucosa. This elevation likely reflects a compensatory response to autophagy dysfunction or direct activation by NF- κ B signaling in the inflammatory microenvironment. In the context of UC, high *CCL2* levels perpetuate inflammation by recruiting innate immune cells, thereby creating a feedback loop that

hinders mucosal healing[28, 29]. *IL-24*, a member of the IL-10 family, exhibits pleiotropic effects in tissue remodeling and immunity. While *IL-24* can induce protective mucin production and SOCS3 expression[30], its chronic upregulation in UC—as observed in our study—may tip the balance towards pathogenicity. Elevated *IL-24* has been linked to cytotoxic autophagy (via Beclin-1 activation) and pro-fibrotic processes[31, 32]. Thus, *IL-24* may act as a double-edged sword: initially attempting to restore barrier integrity but ultimately driving tissue damage and fibrosis under conditions of chronic inflammation[33–35]. Our consensus clustering identified two distinct molecular patterns. Cluster 1, characterized by high expression of the three AR-DEGs, exhibited a "hot" immune microenvironment with extensive infiltration of immune cells. This aligns with the notion that autophagy dysregulation exacerbates inflammation by recruiting immune effectors via mediators like *CCL2*[36]. Consequently, patients in cluster 1 may require more aggressive anti-inflammatory strategies. Finally, our drug sensitivity analysis identified potential therapeutic agents, including Staurosporine and CUDC-305. These compounds, known to modulate autophagy and kinase signaling[37], may offer novel targeted interventions for UC patients exhibiting this specific autophagy-defective molecular profile. Our scRNA-seq analysis mapped the cellular localization of the identified AR-DEGs. *APOL1* and *CCL2* were predominantly enriched in endothelial cells and fibroblasts, implicating the stromal compartment as a key driver of autophagy-mediated inflammation in UC. Interestingly, while *IL-24* showed sparse expression in the single-cell dataset (likely due to the "dropout" effect common in scRNA-seq for low-abundance transcripts), our bulk tissue qRT-PCR validation confirmed its robust upregulation in UC patients. This discrepancy highlights the necessity of multi-platform validation. Biologically, the observed expansion of B cells in the UC microenvironment suggests a shift towards humoral immunity, which may disrupt epithelial-stromal crosstalk and impede mucosal healing, a process heavily reliant on functional extracellular matrix remodeling[38–40]. Cell-cell communication analysis revealed a hyperactive stromal signaling network in UC, particularly involving fibroblasts and endothelial cells. We identified enriched MIF signaling, where MIF likely acts via CD74 and CXCR2/4 to promote inflammatory cell recruitment and survival[41–43]. Crucially, our study highlights a specific fibroblast-endothelial axis mediated by *CCL2-ACKR1* interactions. Since *ACKR1* (DARC) on endothelial cells facilitates chemokine transcytosis, the high *CCL2* secretion by fibroblasts may serve as a "homing beacon," efficiently recruiting circulating leukocytes into the inflamed tissue[44, 45]. This suggests that targeting fibroblast-derived chemokines could be a strategy to block immune infiltration at its source. Importantly, our bioinformatics predictions were corroborated by experimental validation. qRT-PCR analysis in both clinical UC biopsies and a murine colitis model confirmed the significant upregulation of *APOL1*, *CCL2*, and *IL-24*. This cross-species consistency reinforces the reliability of these autophagy-related signatures and supports their potential utility as robust biomarkers for UC diagnosis. Several limitations should be acknowledged. First, while we validated expression levels, the precise molecular mechanisms by which these genes modulate autophagy flux in specific cell types require further in vitro and in vivo functional assays. Second, our single-cell analysis did not delve into finer subtypes (e.g., fibroblast subsets), which may mask subtle heterogeneity. Third, the diagnostic model requires validation in larger, multi-center cohorts to ensure its clinical generalizability. Future studies will focus on elucidating the specific signaling pathways downstream of these AR-DEGs using spatial transcriptomics and knockout mouse models.

5. Conclusion

In conclusion, this study comprehensively characterizes the autophagy-related transcriptomic landscape of Ulcerative Colitis. We identified a novel three-gene signature (*APOL1*, *CCL2*, *IL-24*) and elucidated its multicellular expression patterns, highlighting the critical role of stromal-immune crosstalk in driving mucosal inflammation. These findings not only provide a robust diagnostic tool but also uncover potential therapeutic targets—specifically within the fibroblast-endothelial signaling axis—offering new avenues for precision medicine in IBD management.

Abbreviations

AR

DEG (autophagy-related differentially expressed genes)

ARGs (autophagy
related genes)

AUC (area under the curve)

CD (Crohn's disease)

CDF (consensus cumulative distribution function)

DAI (disease activity index)

DEGs (differentially expressed genes)

DSS (dextran sulfate sodium salt)

GAPDH (glyceraldehyde

3-phosphate dehydrogenase)

GEO (gene expression omnibus)

GO (gene ontology)

GWAS (genome

wide association study)

HADb (human autophagy database)

HE (hematoxylin eosin)

IBD (inflammatory bowel disease)

KEGG (Kyoto Encyclopedia of Gene and Genomes)

PCA (principal component analysis)

qRT

PCR (quantitative real-time PCR)

ROC (receiver operating characteristic)

scRNA

seq (single-cell RNA sequencing)

UC (ulcerative colitis)

UMAP (uniform manifold approximation and projection)

PARs (protease

activated receptors)

CCL (C
C motif chemokine ligand)
MIF (macrophage migration inhibitory factor).

Declarations

Ethics approval and consent to participate

The animal experiment protocol was approved by the Ethics Committee of Guilin Medical University (No:GLMC202303104). This study was approved by the Ethics Committee of the Affiliated Hospital of Guilin Medical University (Approval No.: 2024YJSLL-74).

Consent for publication

Not applicable.

Availability of data and materials

The datasets presented in this study can be accessed via the relevant online repositories.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

All authors read and approved the final manuscript.

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Figures

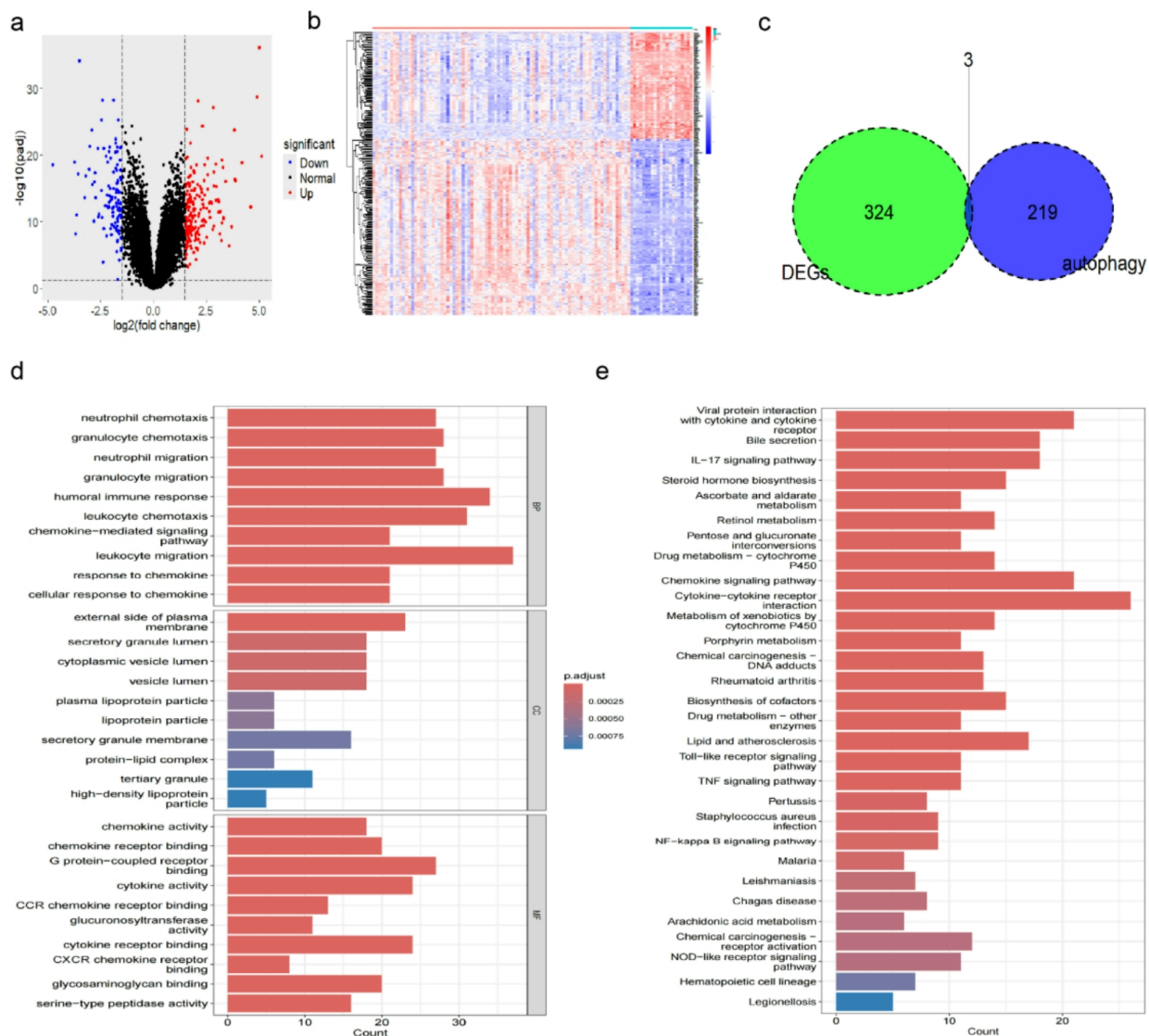


Figure 1

Identification of AR-DEGs. (a) Volcano plot of DEGs. Blue dots represent downregulated genes, red dots represent upregulated genes, and black dots represent genes with no significant difference. (b) Heatmap of DEGs. (c) Venn diagram showing the intersection between DEGs and ARGs. (d) GO enrichment analysis of DEGs. (e) KEGG enrichment analysis of DEGs.

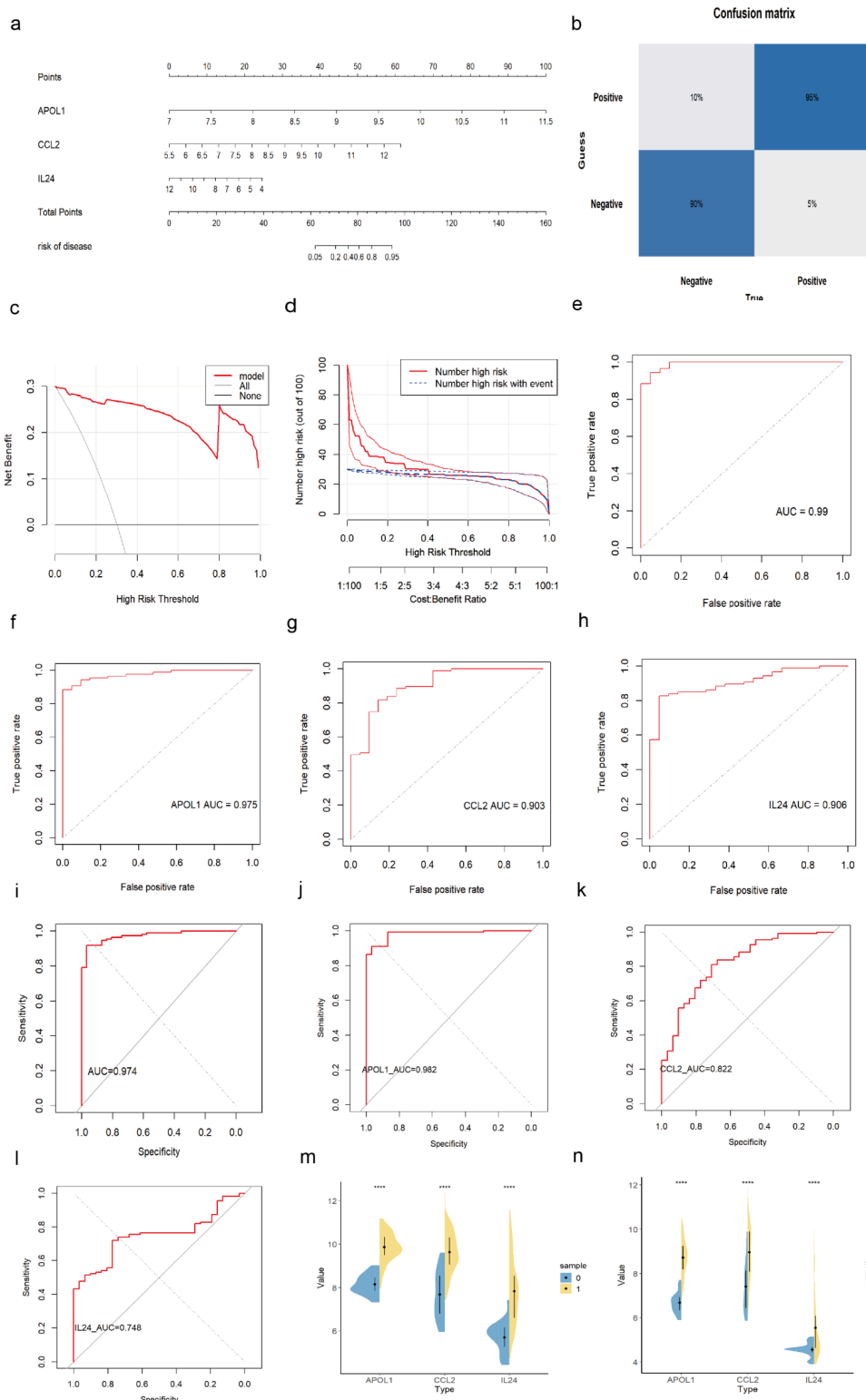


Figure 2

Construction and Evaluation of the Diagnostic Model. (a) Nomogram for predicting UC diagnosis based on AR-DEGs. (b) Confusion matrix of model. (c) DCA based on the training set. (d) Clinical impact curve based on the training set. (e-h) ROC curve of the UC diagnostic model, *APOL1*, *CCL2*, and *IL-24* in the training set. (i-l) ROC curve of the UC diagnostic model, *APOL1*, *CCL2*, and *IL-24* in the validation set. (m) Expression of AR-DEGs in the training set (0=healthy controls, 1=UC patients). (n) Expression of AR-DEGs

in the validation set (0=healthy controls, 1=UC patients). *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001.

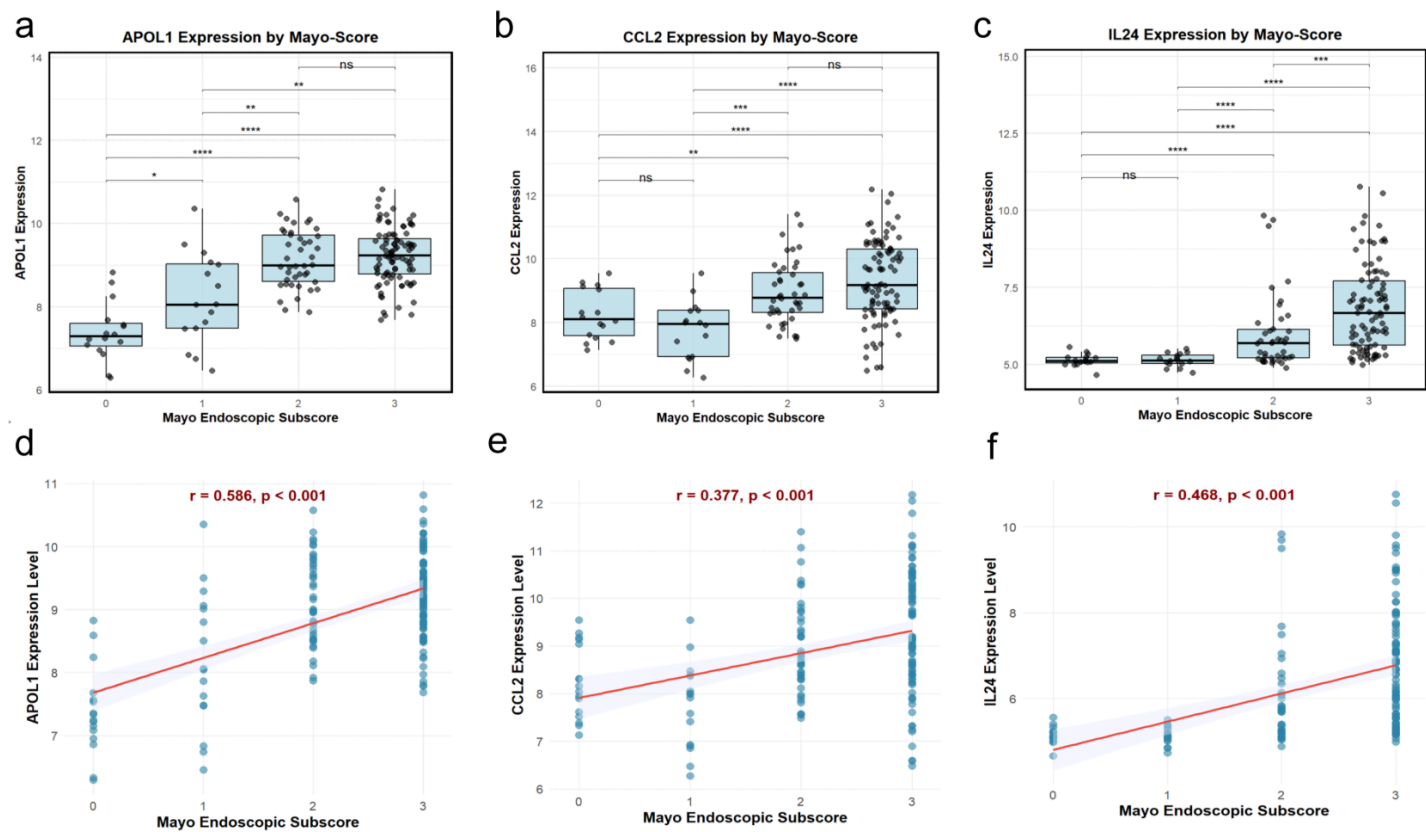


Figure 3

AR-DEGs Expression and Correlation with Clinical Scores. In the GSE73661 dataset, the levels of *APOL1*(a, d), *CCL2*(b, e), and *IL-24*(c, f) vary with UC severity, and correlation analysis between their expression and UC-related clinical scores was performed. *p < 0.05; ** p < 0.01; *** p < 0.001; **** p < 0.001.

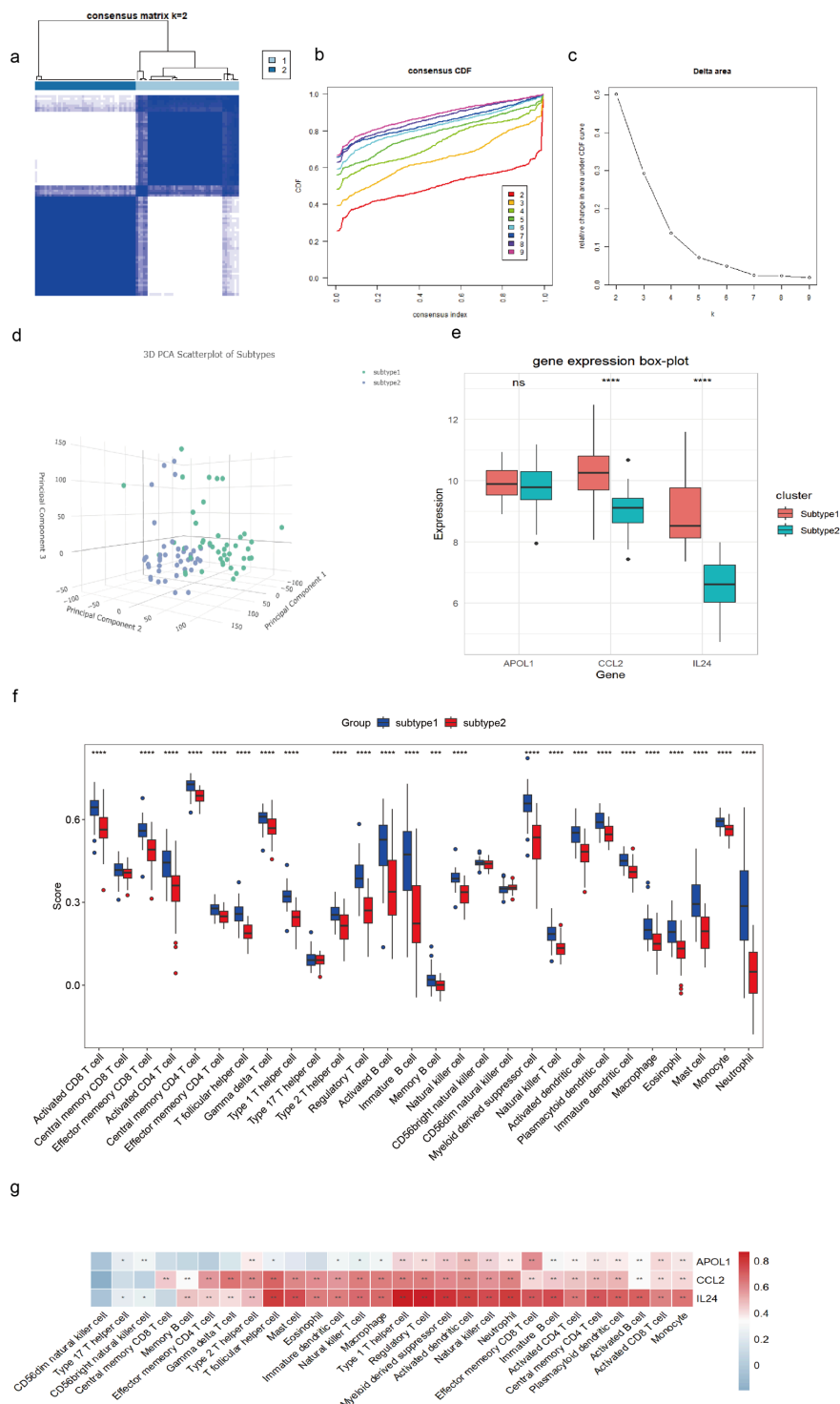


Figure 4

Consensus Clustering Analysis and Landscape of Immune Cell Infiltration. (a) Consensus matrix plot. (b) Consensus cumulative distribution function. (c) Relative change in the area under the CDF curve. (d) PCA visualization of the two subtypes, showing good clustering performance. (e) Expression of AT-DEGs in subtype 1 and subtype 2. ns=no significance. (f) Differences in the abundance of 22 immune-infiltrating

cells between subtype 1 and subtype 2. (g) Correlation heatmap between AR-DEGs and immune-infiltrating cells. *p < 0.05; ** p < 0.01; *** p < 0.001; **** p < 0.001.

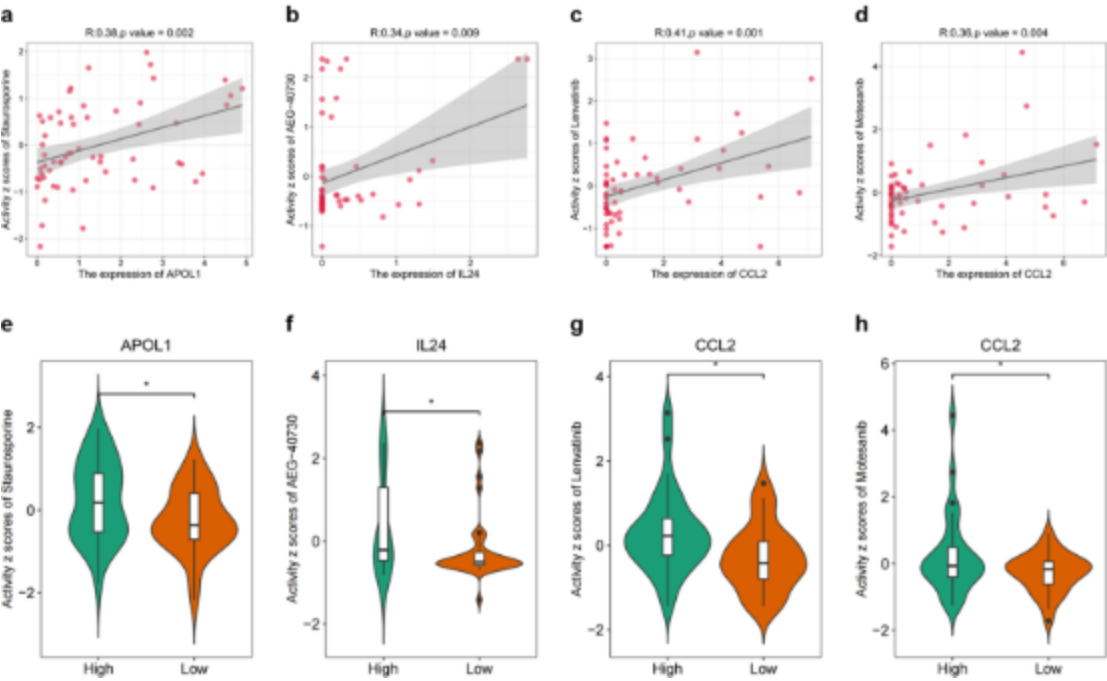


Figure 5

Drug Prediction and Correlation Analysis. Drug sensitivity analysis of *APOL1*with Staurosporine (a) and the relationship between gene expression and drug activity (e). Drug sensitivity analysis of *IL-24* with AEG-40730 (b) and the relationship between gene expression and drug activity (f). Drug sensitivity analysis of *CCL2* with lenvatinib (c) and the relationship between gene expression and drug activity (g). Drug sensitivity analysis of *CCL2*with motesanib (d) and the relationship between gene expression and drug activity (h). *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001.

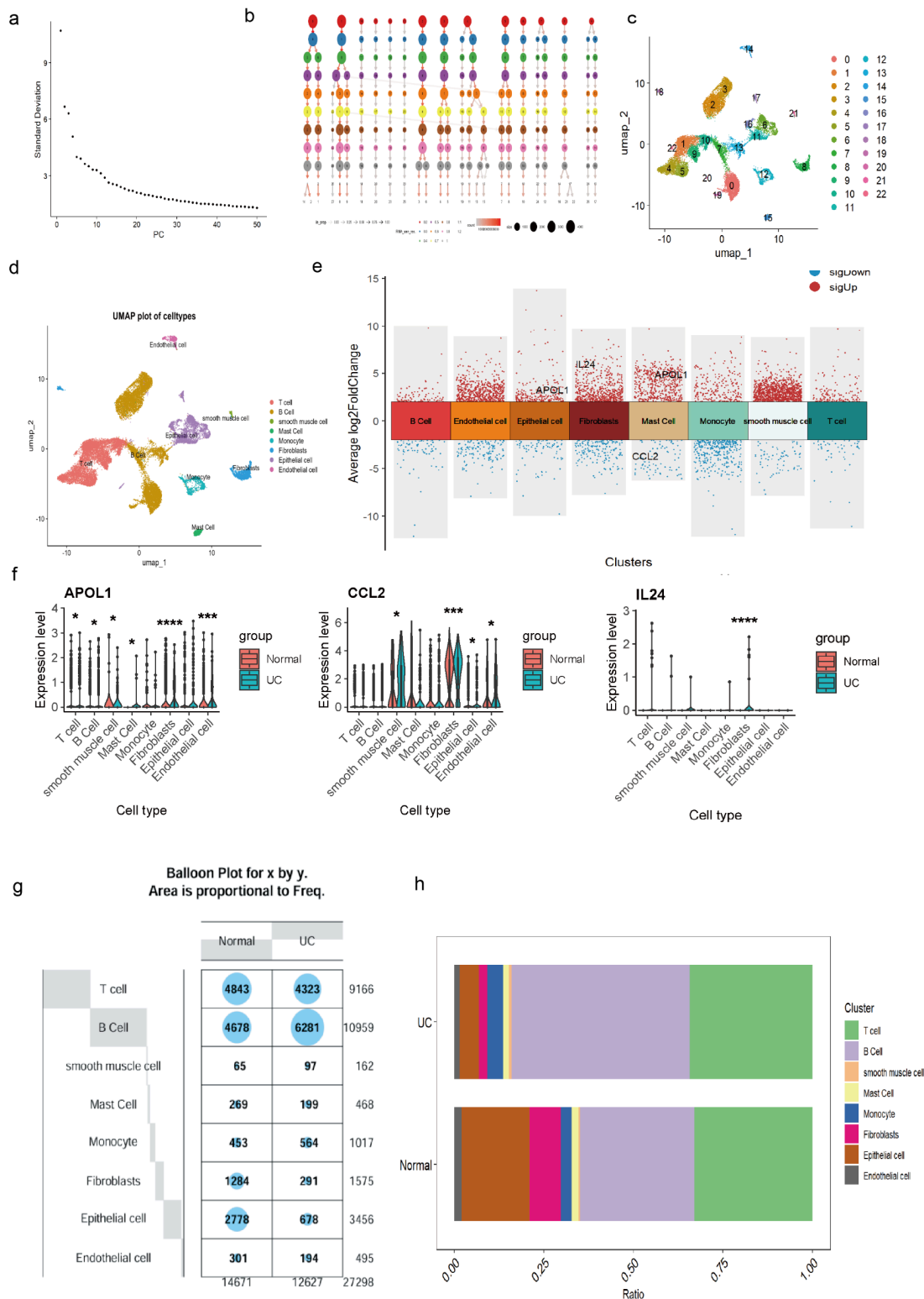


Figure 6

Single-Cell Sequencing Data Analysis of the GSE231993 dataset. (a) Elbow plot showing the standard deviation contributed by each principal component (PC), aiming to identify the inflection point where the standard deviation begins to stabilize. (b) Seurat clustering results with resolutions from 0 to 1. The color of each point represents the resolution. Each row shows clustering results at a specific resolution. Point size corresponds to cluster cell count (larger points indicate more cells). Clustering was stable at a

resolution of 0.7 without the emergence of new clusters. (c) UMAP showing cells grouped into 23 clusters. (d) UMAP plot after cell annotation. (e) Volcano plots of AR-DEGs in cell types. Blue indicates down regulated genes, and red indicates up regulated genes. (f) Violin plot of AR-DEGs expression in ulcerative colitis and normal groups from the single-cell dataset. (g) Comparison of cell counts between UC patients and healthy controls in the single-cell dataset. (h) Proportion plot of cell counts. *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001.

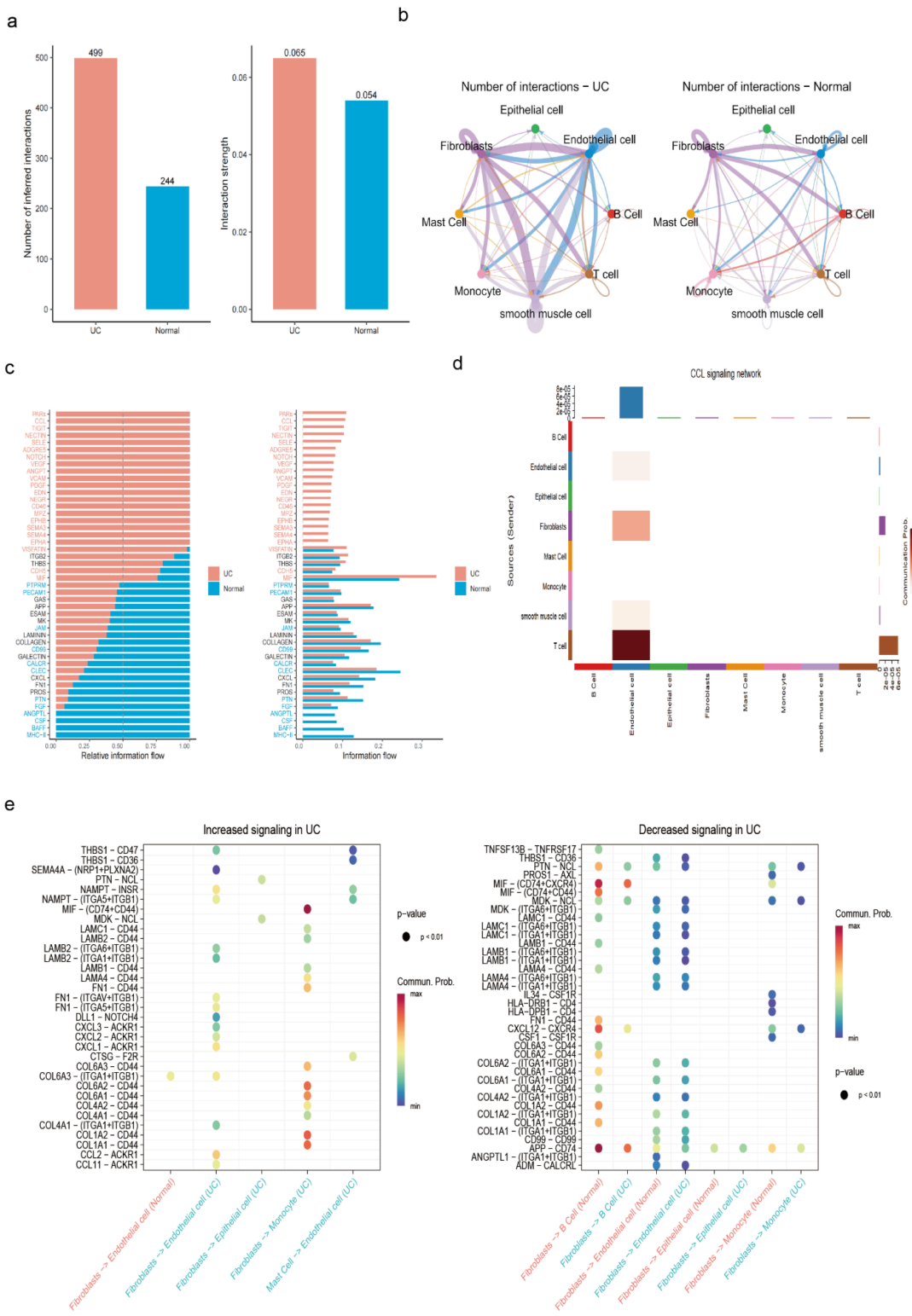


Figure 7

Analysis of Cell Signaling Pathways. (a) and (b) Comparison of cell communication between UC patients and healthy controls in the single-cell dataset. (c) Information flow of specific pathways in cell communication.(d) Identification of major signal sender and receiver cell types in the specific CCL signaling pathway. (e) Communication networks under different conditions.

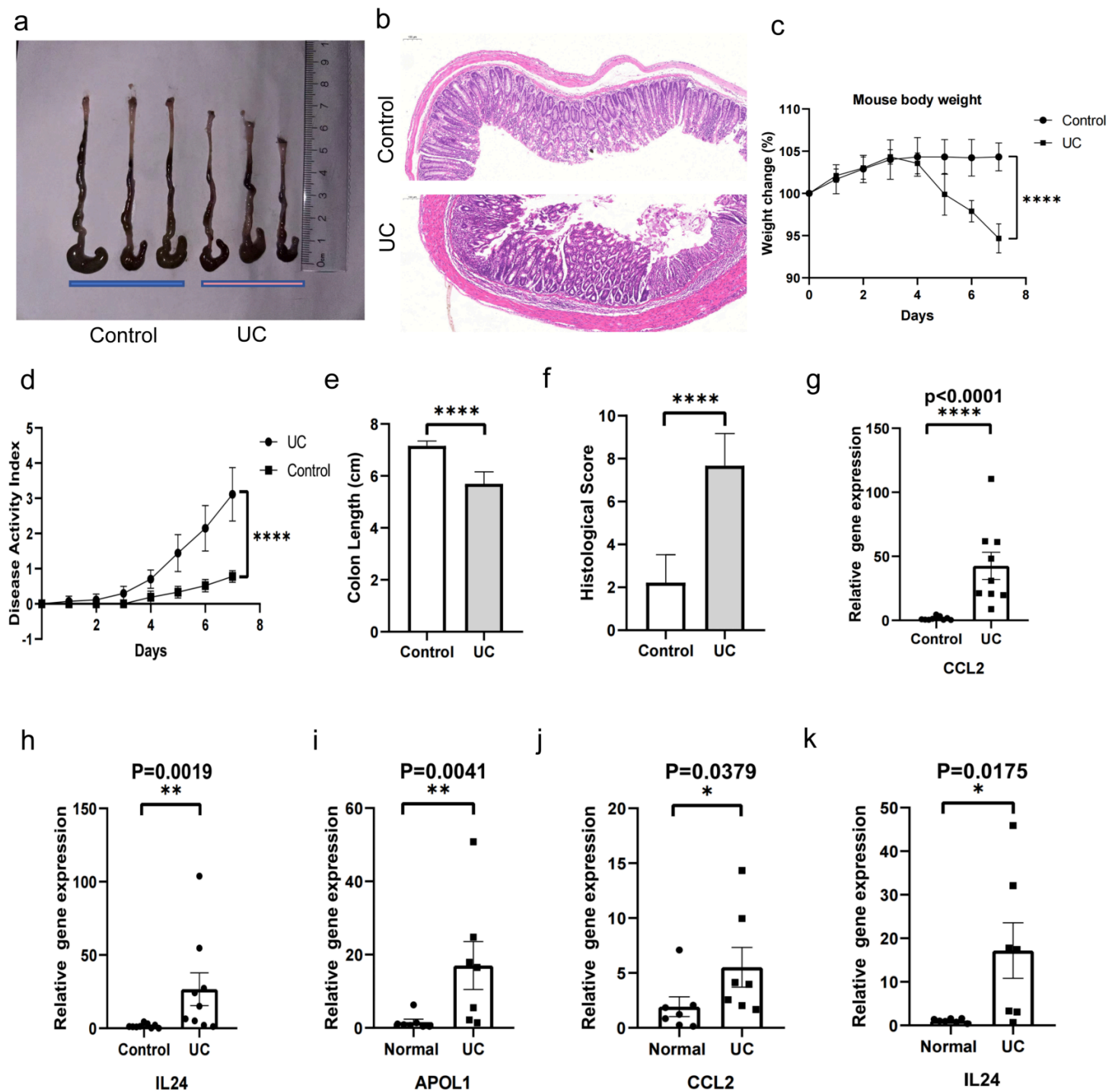


Figure 8

qRT-PCR experiments. (a) Comparison of intestinal measurements in mice. (b) Representative H&E staining images of distal colon sections from mice in the water and DSS groups. (c-f) Body weight curve, DAI, colon length and histological score of mice. (g-h) Relative mRNA expression levels of *CCL2* and *IL-24* in colon tissues of mice in the water group (n=9) and DSS group (n=9). (i-k) Relative mRNA expression levels of *APOL1*, *CCL2*, and *IL-24* in tissues from inflamed intestinal areas (n=7) and adjacent non-inflamed areas (n=7) from UC patients. Data are presented as mean±SEM. * p < 0.05; ** p < 0.01; *** p < 0.001; **** p < 0.0001.