Heliyon 10 (2024) e29163

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Single-cell transcriptomics reveals activation of endothelial cell and identifies LHPP as a potential target in ulcerative colitis

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ARTICLE INFO

Keywords:
Ulcerative colitis
Single-cell RNA sequencing
LHPP
Inflammation regulation

ABSTRACT

This study delves into Ulcerative colitis (UC), a persistent gastrointestinal disorder marked by inflammation and ulcers, significantly elevating colorectal cancer risk. The emergence of single-cell RNA sequencing (scRNA-seq) technology has opened new avenues for dissecting the intricate cellular dynamics and molecular mechanisms at play in UC pathology. By analyzing scRNA-seq data from individuals with UC, our study has revealed a consistent enhancement of inflammatory response pathways throughout the course of the disease, alongside detailing the characteristics of endothelial cell damage within colitis environments. A noteworthy finding is the downregulation of Phospholysine Phosphohistidine Inorganic Pyrophosphate Phosphatase (LHPP), which exhibited a inversely correlate with STAT3 expression levels. The markedly reduced expression of LHPP in both the tissues and plasma of UC patients positions LHPP as a compelling target for therapeutic intervention. Our findings highlight the pivotal role LHPP could play in moderating inflammation, spotlighting its potential as a crucial molecular target in the quest to understand and treat UC.

1. Introduction

Ulcerative colitis, a chronic inflammatory condition of the intestinal tract, primarily targets the colon, manifesting through extensive mucosal inflammation and ulceration [1,2]. This condition is identified as a pivotal risk factor for the onset of colorectal cancer. The etiology of UC is multifaceted, weaving a complex interplay of genetic predispositions, environmental exposures, and aberrant immune responses. These factors collectively orchestrate a disrupted immune equilibrium within the gastrointestinal milieu, culminating in the pathological landscape characteristic of UC [3,4]. Individuals afflicted with UC experience a spectrum of symptoms that significantly deteriorate quality of life. These include, but are not limited to, severe abdominal discomfort, persistent diarrhea often accompanied by blood, and rectal bleeding. The unpredictable nature of symptom flare-ups further compounds the psychological burden on patients [5]. Recent study efforts have unveiled critical insights on the UC pathogenesis, underscoring the pivotal role of

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https://doi.org/10.1016/j.heliyon.2024.e29163
Received 11 October 2023; Received in revised form 30 March 2024; Accepted 2 April 2024
Available online 3 April 2024

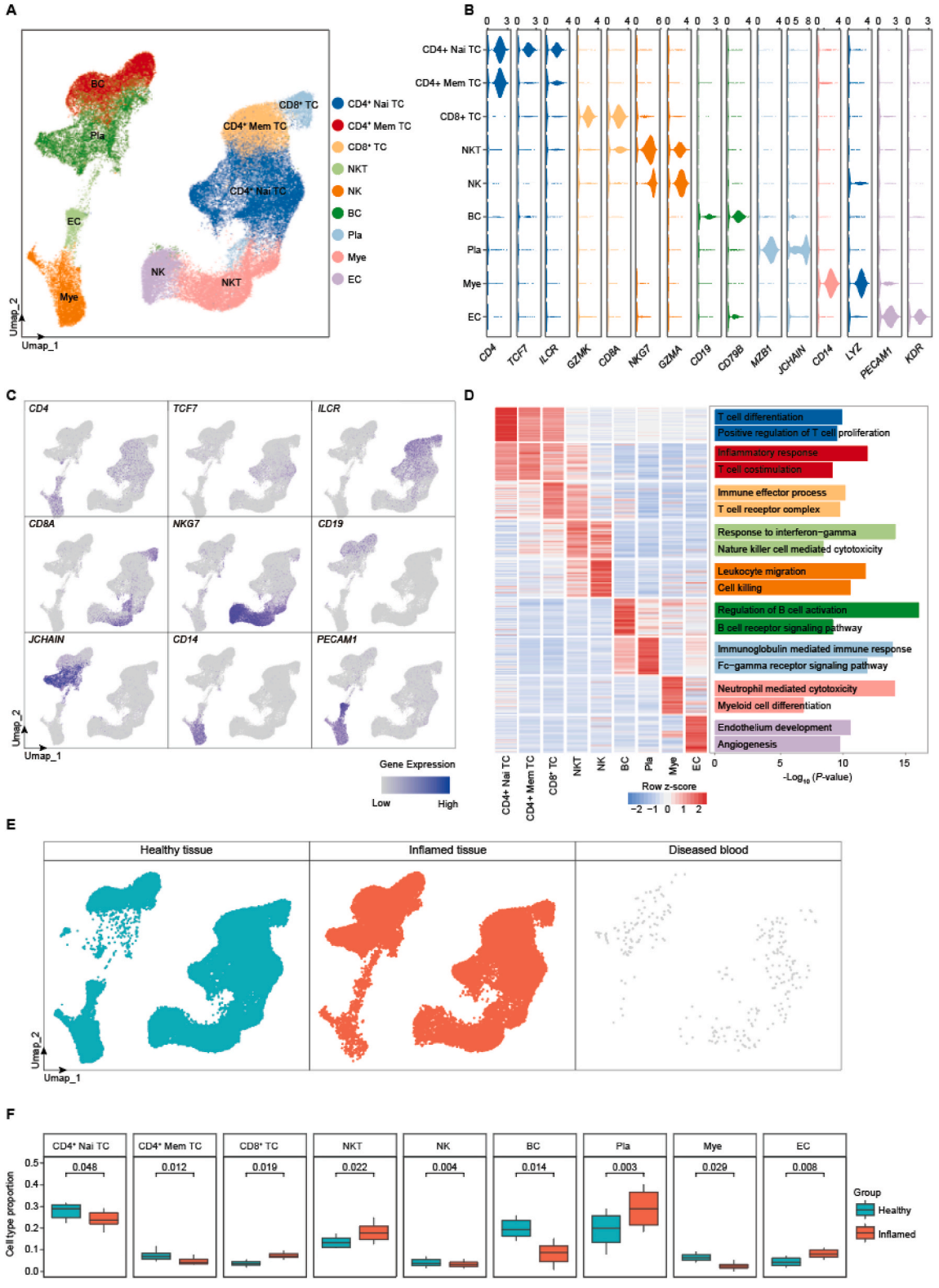

Fig. 1. Diversity of Cell Types Identified by scRNA-seq Analysis. A. UMAP visualization showcasing the distribution of various cell types within human colon tissues, annotated for clarity. B. Violin plots illustrating the distribution of expression levels for key marker genes in cell types across identified cell types, highlighting the cellular heterogeneity present in Ulcerative Colitis. C. Feature plots display the expression levels of marker genes of each cell type. The color intensity from light to dark indicates a gradient of expression levels from low to high. D. Heatmap illustrates the

gene set enrichment across cell types, highlighting the biological processes. The intensity of the colors correlates with the row z-scores of gene expression, with the significance denoted by the -log10 p-value. E. UMAP projections of cells from healthy tissue, inflamed tissue, and diseased blood demonstrate distinct cell landscapes, indicative of the progression and pathophysiology of Ulcerative Colitis. F. Boxplots depict the relative proportions of various cell types in healthy versus inflamed tissue.

dysregulated immune response and intestinal microbiota in disease's evolution [6,7]. Despite these advances, the cellular and molecular intricacies driving UC's onset and progression remain partially understood. This gap in knowledge underscores an urgent need for innovative diagnostic and therapeutic strategies that target the disease at its root [8].

The advent of single-cell RNA sequencing (scRNA-seq) technology have transformed our grasp of cell diversity in intricate tissues [9–11]. This transformative method enables researchers to identify unique cell populations, unravel novel cellular pathways, and scrutinize the immune responses dynamics at an unprecedented resolution [12,13]. By enabling the resolution of tissue composition at a single-cell level, scRNA-seq unveils the distinct cellular actors and the dynamic interplay of molecular pathways implicated in UC. This technology offers a promising avenue to elucidate the nuanced mechanisms of UC pathogenesis, setting the stage for the identification of novel therapeutic targets and the refinement of treatment paradigms [14–18].

Within this intricate landscape, LHPP, a histidine phosphatase protein, has been identified as a significant entity implicated in various biological functions. Its roles range from acting as a suppressor in the development of hepatocellular carcinoma [19] to modulating apoptotic pathways via the upregulation of cleaved-PARP and cleaved-Casp3 proteins, further extending to the orchestration of inflammatory and immune responses. Latest research is shedding light on a possible link between levels of LHPP expression and survival rates in patients with colorectal cancer [20]. Particularly, studies reveal that the diminution of LHPP in intestinal epithelial cells is closely linked with colitis manifestations in animal models, suggesting its integral involvement in inflammatory bowel diseases (IBD) [21,22]. Despite these insights, the precise role and influence of LHPP within UC's framework are still unclear, offering a compelling path for additional research. This research undertakes a comprehensive integration and analysis of existing single-cell RNA sequencing (scRNA-seq) datasets pertinent to UC [16,17]. Our endeavors illuminate the specific cell types, transcriptional landscapes, and immune signaling pathways that are central to UC's pathogenesis. By meticulous examination the single-cell landscape of UC-affected colonic tissues, we identified a sustained elevation of inflammatory response pathways throughout the disease's progression and characterized the features of damaged endothelial cells in colitis. An integrative approach, merging extensive UC disease databases with single-cell data, has highlighted a consistent trend of LHPP downregulation in UC, inversely related to STAT3 expression levels. Notably, our findings from UC patient samples confirm an increase in STAT3 expression alongside a decrease in LHPP levels, indicating STAT3's possible regulatory function in suppressing LHPP expression. Additionally, LHPP showed lower expression in both the intestinal tissue and plasma of UC patients, hinting at its significant role in UC pathogenesis.

2. Results

2.1. Single-cell transcriptomics landscape in ulcerative colitis microenvironment

To comprehensively decipher the microenvironmental alterations occurring during the development of UC, we conducted an analysis utilizing publicly available single-cell datasets from both UC patients and healthy individuals. Our analysis encompassed a total of 64,643 cells from UC (n = 8) and healthy (n = 8) individuals. We delineated nine predominant cell populations, including $CD4^+$ Naïve T cells ($CD4^+$ Nai TC) (24.56%), $CD4^+$ Memory T cells ($CD4^+$ Mem TC) (12.88%), $CD8^+$ T cells ($CD8^+$ TC) (4.70%), natural killer T cells (NKT) (15.00%), natural killer cells (NK) (7.98%), B cells (BC) (13.81%), myeloid cells (Mye) (8.80%), and endothelial cells (EC) (2.45%) (Fig. 1A and S1). The validity of our categorization of cell types is confirmed through the expression patterns of established cell type-specific markers (Fig. 1B and C), alongside the functional delineation of these markers for each identified cell type (Fig. 1D and E).

Regarding shifts in cellular composition during UC progression, we observed an increase in the proportions of $CD8^+$ T cells and NKT cells (Fig. 1F), suggesting a reorganization of the cellular milieu in response to chronic inflammation [23]. $CD8^+$ T cells, known for their cytotoxic prowess, and their augmented presence indicates a concerted effort by the immune system to target and eliminate inflamed UC tissues. NKT cells, bridging innate and adaptive immunity, likely play a crucial role in dampening inflammation. The bolstered presence of these cytotoxic immune operatives within the colonic milieu points to a proactive immune defense against UC, which contrasts with a observed reduction in $CD4^+$ Nai TC, $CD4^+$ Mem TC and B cells (Fig. 1F). $CD4^+$ T cells, critical in various immune functions like antigen presentation and immune regulation, have diminished in proportion, potentially signaling a reduced immune system capacity to recognize inflammatory cells [24]. Likewise, the decreased proportion of B cells, pivotal in antibody generation and antigen presentation, could impair the immune response's efficiency against UC. This decline might potentially hinder the cytotoxic ability against UC of $CD8^+$ T and NKT cells. This diminishing trend of $CD4^+$ T cells and B cells may suggest an emerging immune evasion tactic by UC pathology, rendering the colonic environment less amenable to an effective immune counteraction and possibly leading to sustained, chronic inflammation.

Simultaneously, an escalation in the proportion of endothelial cells was noted within the colon's microenvironment (Fig. 1F). This observation emphasizes angiogenesis's pivotal contribution to UC's development. A robust link between enhanced angiogenesis and inflammation has been established across a spectrum of inflammatory conditions The interplay between angiogenesis and inflammation is notably synergistic, with both processes often triggered by hypoxic conditions [25]. Furthermore, an intricate vascular network augments the mobilization and dissemination of inflammatory mediators, potentially broadening the scope of inflammation

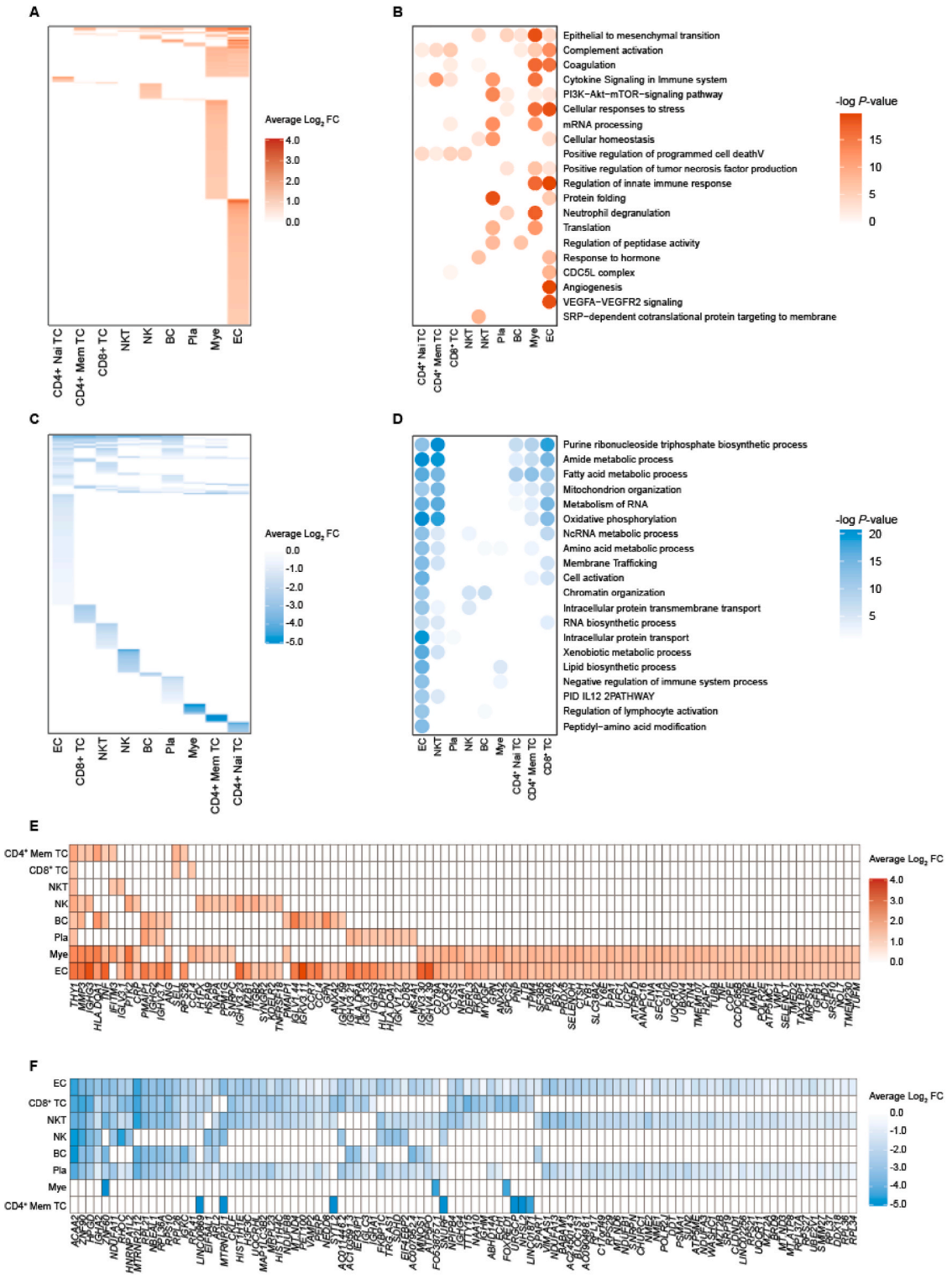

Fig. 2. Changes in the Transcriptional Profiles across Cell Types during Ulcerative Colitis. A. Heatmap displaying the distribution of upregulated DEGs across various cell types between healthy and UC groups (Diseased/healthy). B. Bubble plot illustrating the -log P-value of enriched biological processes in upregulated genes. Larger, darker bubbles represent more significant enrichment within the upregulated gene sets, with processes related to immune response, cell signaling, and metabolism highlighted. C. Heatmap displaying the distribution of downregulated DEGs across various cell types between healthy and UC groups (Diseased/healthy). D. Bubble plot for the -log P-value of enriched biological processes in downregulated genes. The size and color intensity of the bubbles correlate with the significance of the enrichment, focusing on processes such as

metabolism, cell organization, and protein transport. E. Heatmap of upregulated genes across different cell types, with color intensity representing the average log FC in gene expression, ranging from light to dark orange as expression increases. F. Heatmap of downregulated genes across different cell types, with color intensity showing the average log FC in gene expression, ranging from light to dark blue as expression decreases.

to more extensive areas.

These observations provide valuable insights into the evolving microenvironment during UC progression. The pronounced increase in angiogenesis highlights the importance of vascular development in the inflamed microenvironment, indicating a significant adaptive response to the ongoing inflammatory process.

2.2. Delineation of transcriptomic responses by cell type throughout UC progression

We conducted a comprehensive exploration of the differential gene expression across various cell types throughout the UC development process. In this exhaustive analysis, we pinpointed 764 genes exhibiting upregulation and 1625 genes showing downregulation. EC stood out, showing the most significant fluctuation, with the highest counts in both upregulated and downregulated differential expression genes (DEGs). This bidirectional alteration in gene expression profiles echoes the changing capacities of these cells throughout UC pathogenesis (Fig. 2A–D).

Delving deeper into the upregulated DEGs across different cell types, a pattern emerged where a substantial fraction of these genes was linked to the innate immune response's regulation, including pathways like "Regulation of innate immune response", "Neutrophil degranulation", and "Cytokine Signaling in the Immune system". These patterns point to a heightened immune response, with an increased deployment of immune cells to areas of inflammation, targeting the elimination of infectious agents, necrotic tissues, or other pathological entities. Noteworthy was the observation of the "Epithelial to mesenchymal transition" pathway's activation, suggesting that ongoing inflammation could lead to tissue fibrosis. Moreover, the amplification of the "Angiogenesis" pathway aligns with the previously noted expansion in EC proportions, indicating the formation of new vascular networks within inflamed regions [26]. These insights paint a picture of a multifaceted immune response to inflammation, characterized not only by the strategic recruitment of immune cells to the site of inflammation but also by concurrent tissue fibrosis and angiogenesis, marking a complex interplay of cellular processes in UC's evolution.

Turning attention to the downregulated genes, there was a pronounced decrease in expressions associated with several metabolic processes, including "Xenobiotic metabolic process", "Oxidative phosphorylation", and "Fatty acid metabolic process". This downturn suggests that inflammation might significantly impair the functional capacity of the affected tissues. Moreover, diminished expressions pertaining to "Chromatin organization", "Intracellular protein transmembrane transport" and "Cell activation" points to significant changes in cellular states and epigenetic changes within the inflamed tissue. Collectively, these patterns highlight that inflammation prompts extensive alterations in both metabolic functionality and cellular identity.

The comprehensive analysis of differential gene expression among varied cell types throughout the UC progression furnishes profound insights into the dynamic molecular alterations occurring within the inflammatory microenvironment (Fig. 2E and F). The concurrent upregulation of pathways related to inflammation, alongside the facilitation of immune cell deployment, tissue fibrosis, and angiogenesis, paints a picture of the organism's intricate response to inflammation. Simultaneously, the observed downregulation of metabolic pathways and changes in cellular condition indicate a degradation of functional integrity within the tissues involved. These distinct patterns of gene expression landscapes provide a crucial framework for identifying potential therapeutic targets in the treatment of UC.

2.3. Dynamic shifts in endothelial cell identity during UC progression

Previous studies have underscored the crucial function of endothelial cells in mediating immune responses, encompassing roles in filtration, endocytosis, antigen presentation, and the recruitment of leukocytes [27,28]. Dysfunctional interactions between endothelial cells and immune cells have been identified in injury tissues [29,30]. Additionally, endothelial cells are subject to significant changes during inflammatory conditions, directly contributing to the inflammatory process [31]. These insights not only illuminate the complex interplay between endothelial cells and inflammation milieu but also spotlight that interventions targeting specific endothelial mechanisms could hold therapeutic promise for managing UC.

To delve into the cellular evolution endothelial cells (ECs) experience in UC, we employed pseudotime trajectory analysis to reveal the dynamic shifts in EC behavior. Notably, we identified that healthy sample-derived ECs predominantly clustered in the early to middle pseudotime stages, while samples from the disease state were enriched in the middle to later stages (Fig. 3A&B). This distribution pattern furnishes robust evidence of the stepwise transformations ECs undergo throughout UC's progression.

Subsequently, we directed our focus towards the shifts in the transcriptomic landscape of ECs during UC, by meticulously tracing gene expression alterations along the pseudotime continuum. This endeavor led to the classification of genes into three patterns based on their expression dynamics: pattern 1 displayed increasing expression as pseudotime advanced, pattern 2 exhibited an initial upregulation followed by downregulation, and pattern 3 displayed a consistent downregulation. (Fig. 3C).

The pattern 1 genes revealed profound transcriptional changes in endothelial cells amidst the inflammatory backdrop of UC. These alterations were closely tied to pivotal signaling cascades, among which the PI3K-Akt pathway stood out for its integral role in mediating various aspects of the inflammatory response [32]. Equally significant was the TGF-beta pathway, implicated in cell growth, differentiation, and even oncogenesis, signaling a key regulatory mechanism in play. Additionally, the upregulation of genes in the

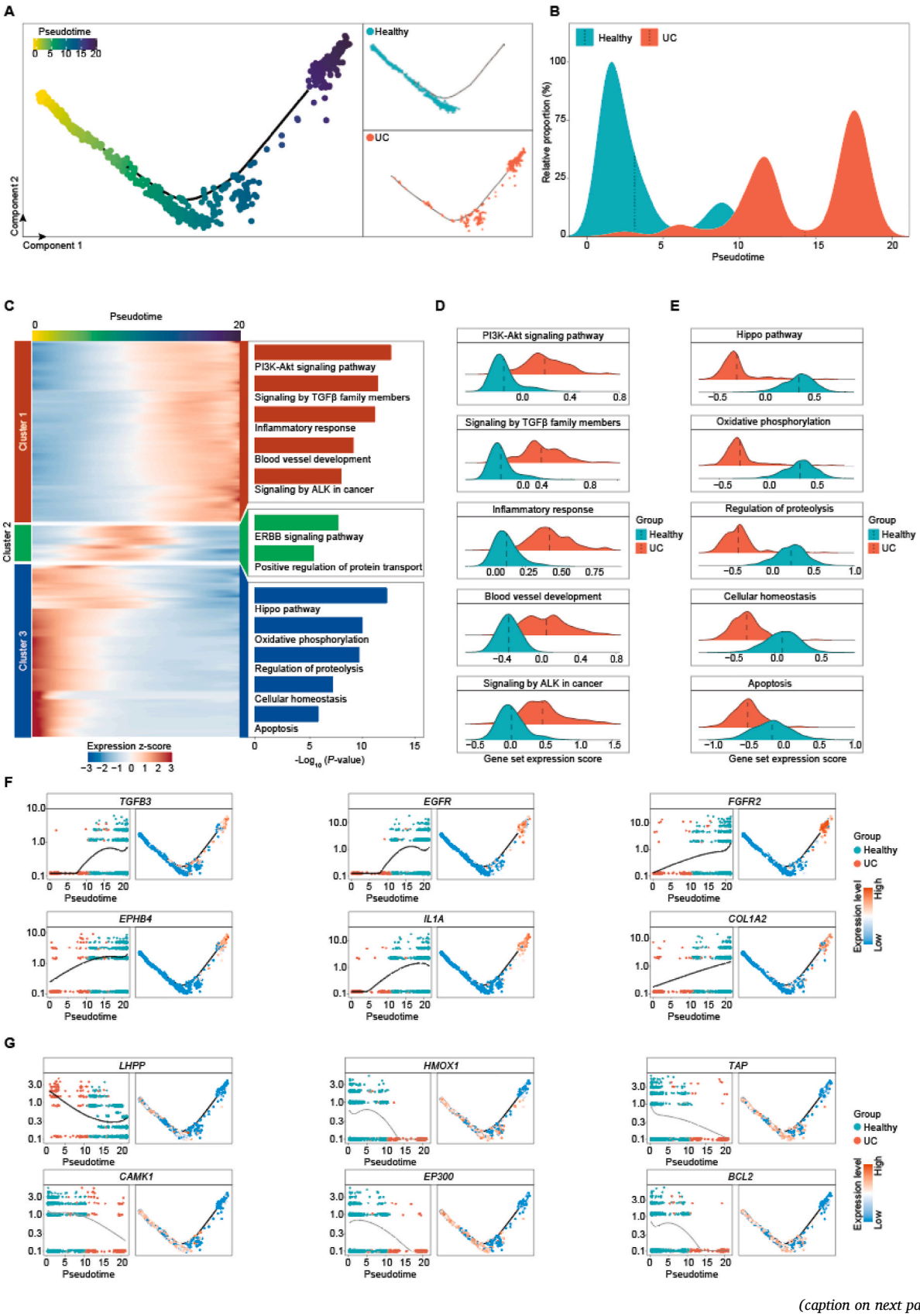

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Fig. 3. Pseudotime trajectory analysis of Endothelial cells during UC progression. A. Pseudotime trajectory plot showing the differentiation path of endothelial cells along pseudotime, from a starting point (yellow) to terminal states (purple), compared between healthy and Ulcerative Colitis (UC) conditions. Insets show detailed progression paths for healthy and UC cells, indicating divergence in cell fate decisions. B. Ridge plot illustrating the distribution of EC cell numbers along the pseudotime trajectory for both healthy individuals and those in UC, based on the analysis in Fig. 3A. C. Heatmap showcasing time-evolving gene expression profiles in UC, with each row representing a gene and columns indicating progression through pseudotime. Gene function annotations are provided on the right, offering insights into the biological roles of these genes during UC progression. D. Ridge plots detailing the expression scores of genes within cluster 1 from Fig. 3C for both healthy and UC-affected groups. E. Ridge plots presenting the expression scores for genes within cluster 3 from Fig. 3C, comparing healthy to UC conditions. F. Scatter plots and trajectory analyses depicting the expression levels of top genes from cluster 1 in Fig. 3C. G. Scatter plots and trajectory analyses showing the expression trends of key genes within cluster 3 from Fig. 3C.

angiogenesis indicated endothelial cells' active engagement angiogenesis associated with inflammation. Additionally, the ALK in cancer pathway was upregulated, suggested a potential role of endothelial cells in carcinogenesis (Fig. 3C and D). These findings highlight endothelial cells' proactive role in angiogenesis and their response to inflammation-driving signals, while also hinting at a possible inclination toward malignant transformation. Pattern 2 genes, which initially rose and then fell in expression, were linked to ERBB signaling and positive regulation of protein transport, reflecting a transient activation of growth signaling and cellular trafficking during certain stages of UC. Conversely, genes classified under Pattern 3 predominantly interacted with pathways such as the Hippo signaling pathway, crucial for regulating cell proliferation and organ size [33]. The apoptosis pathway, essential for cell proliferation and organ size regulation [34]. The consistent downregulation of these genes suggests a gradual shift in endothelial cell functionality, potentially leading to a diminished capacity for tissue maintenance and repair in the inflamed state.

Within the pseudotime trajectory analysis, certain genes stood out for their pronounced alterations, *TGFB3*, part of the TGF-β family, plays a pivotal role in cell growth, differentiation, and immune modulation [35]. Its increased expression in EC implies its involvement in activating pathways linked to inflammation-driven angiogenesis and remodeling of the microenvironment. The elevated levels of *EGFR* and *FGFR2* indicates a heightened responsiveness of ECs to growth factor signals, potentially amplifying their roles in angiogenesis and their engagement with the inflamed microenvironment. The receptor *EPHB4*, integral to cellular communication and tissue architecture [36], also exhibited an upsurge in expression. This elevation hints at its importance in the differentiation of endothelial cells and their response to inflammatory stimuli. The upregulation of *ILA* and *COL1A2* further points to ECs' active involvement in immune modulation and the restructuring of the extracellular matrix within the UC context (Fig. 3F). Conversely, the observed downregulation of LHPP, known for its regulatory role in the cell cycle, suggests a reduction in the control over EC proliferation and differentiation, a crucial aspect of UC's progression. The diminished expression of *HMOX1*, a stress-responsive enzyme, might reflect an altered oxidative stress response in ECs within the UC microenvironment. The downregulation of *CAMK1*, known for its role in calcium signaling and potentially affecting endothelial cell functions like vasodilation and inflammation, implies changes in intracellular signaling pathways [37]. *EP300*, a gene crucial in regulating gene expression and chromatin remodeling, is also downregulated, suggesting potential shifts in gene transcription and cellular responses [38]. The reduction in *BCL2*, crucial for regulating apoptosis, indicates alterations in cell survival mechanisms in endothelial cells amid UC's pathological landscape (Fig. 3G).

2.4. Identification of low LHPP level and its role during UC progression

To enhance our understanding of the complex interactions among deregulated genes within the UC transcriptomic landscape and pinpoint critical mediators of UC pathogenesis, we next embarked on a comprehensive integrated analysis, combining data from disease-specific databases with DEGs identified through scRNA-seq. Through this meticulous integration, we discerned 13 genes consistently upregulated, including *STAT3*, *SPINK4*, *FOXP1*, *CFB*, *TIMP1*, *DUOXA2*, *CSF3R* and *MMP1*, and 7 consistently downregulated, such as *LHPP*, *CLDN8*, *CK1*, *ABCG2*, *CKB*, *FMO5*, *GUCA2A* (Fig. 4A). Intriguingly, this analysis not only underscored genes integral to the inflammatory response but also illuminated the significant upregulation of crucial transcription factors *STAT3* and *FOXP1*. Subsequent correlation analyses between these regulatory factors and other genes from our dataset revealed a notable negative correlation between *LHPP* and the levels of *STAT3* and, to a lesser degree, *FOXP1* (Fig. 4B and C). This inverse relationship between *LHPP* and *STAT3*, particularly, suggests that *LHPP*'s downregulation may play a significant role in UC's molecular underpinnings, potentially through a pathway mediated by *STAT3*'s transcriptional activity.

To validate these insights, we enlisted a cohort of 12 healthy individuals and 15 UC patients, obtaining intestinal tissue samples for future analysis. Histological assessment (HE staining) of the tissue samples showed more chronic inflammation in UC patients compared with healthy individuals (Fig. 4D and E). Moreover, qPCR analysis revealed higher mRNA levels of inflammation-related genes like *IL6* and *IL1A*, aligning with previous findings (Fig. 2A and C). A marked decrease in LHPP mRNA and an uptick in STAT3 expression were noted in UC patients compared to healthy individuals, with FOXP1 expression changes not statistically significant (Fig. 4F and G). Western blot analysis of intestinal tissue samples further verified a significant drop in LHPP expression and a rise in STAT3 levels in UC subjects. These results suggest a potential hypothesis of STAT3 might transcriptionally inhibit LHPP expression.

Additionally, we carried out ELISA test on blood samplesfrom 23 matched UC patients and healthy individual pairs. Our data showed a significant increase in IL6 levels among UC patients, underlining the disease's systemic inflammatory impact (Fig. 4J). The reduced plasma LHPP levels in UC patients indicate its possible utility as a predictive biomarker for UC (Fig. 4H). These observations underscore the extensive nature of UC-related inflammation and suggest LHPP as a potential early detection marker for the disease.

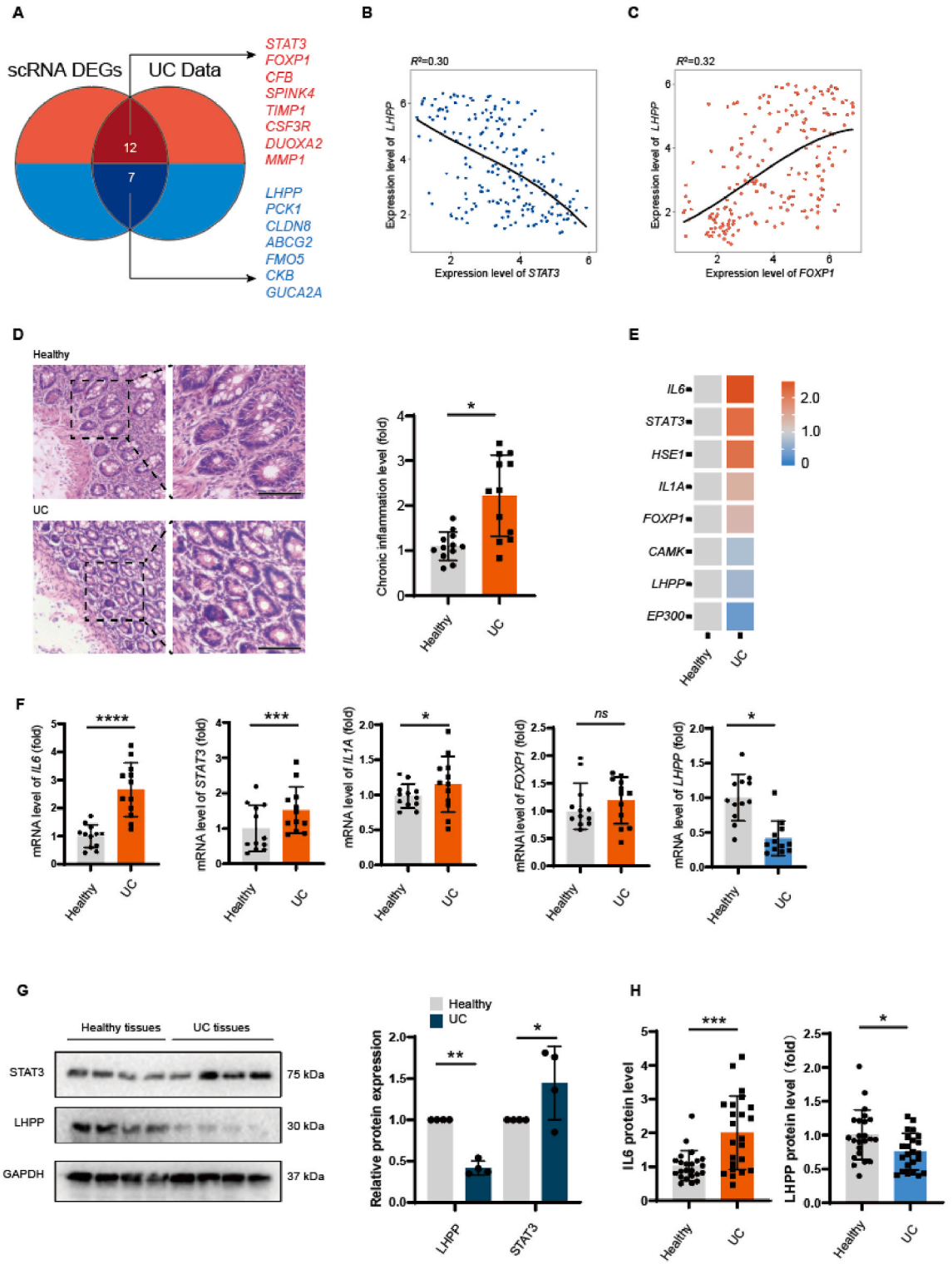

Fig. 4. Identification of low LHPP level and its role during UC progression A. Venn diagram illustrating the intersection between DEGs identified through single-cell RNA sequencing analysis and genes highlighted in publicly available ulcerative colitis datasets. B. Scatter plot detailing the correlation between the expression levels of LHPP and STAT3. C. Scatter plot displaying the relationship between expression levels of LHPP and FOXP1. D. H&E staining of colon samples from healthy participants (n = 12) and UC-diagnosed patients (n = 12). Representative images were taken at 20x magnification, with scale bars measuring 5 μm. E. QPCR analysis of the relative mRNA expression levels of indicated genes as *IL6*, *STAT3*, *HESE1*, *IL1A*, *FOXP1*, *CAMK*, *LHPP*, and *EP300* were measured in tissues from in 12 paired of heathy individuals and UC patients. F. A comparative

plot of the mRNA levels of *IL6*, *STAT3*, *IL1A*, *FOXP1* and *LHPP* in 12 paired of heathy individuals and UC patient. G The relative protein expression of STAT3 and LHHP in 4 paired heathy individuals and UC patient tissues. left: western blot detection for STAT3 and LHPP in intestinal tissue samples from heathy individuals and UC patients, loading control: GAPDH; right: quantitative evaluation of the expression levels of STAT3 and LHPP. H. ELISA analysis of IL6 and LHPP protein levels in 23 paired heathy individuals and UC patient Serum. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.005$; $^{****}P < 0.001$, *ns* represents as not significant.

3. Discussion

Ulcerative colitis embodies a complex, chronic inflammatory condition predominantly impacting the colon and rectum. Despite substantial advancements in deciphering UC's pathogenesis, the quest for more refined and targeted therapeutic approaches remains paramount. Utilizing the capabilities of single-cell RNA sequencing, our investigation delved into the cellular and molecular intricacies of UC. Notably, we documented a marked increase in inflammatory response pathways during UC within the human colon microenvironment, reflective of a vigorous immune counteraction against the inflamed tissues. The observed augmentation in the populations of cytotoxic $CD8^{+}$ T cells and NKT cells denotes a potential bolstering of anti-tumor mechanisms. However, the diminished ratios of $CD4^{+}$ T cells and B cells signal a potential adaptation mechanism by UC cells to evade immune detection.

A remarkable discovery in our study was the consistent reduced expression level of *LHPP* in both intestinal tissue and plasma samples from UC patients, exhibiting a negative correlation with *STAT3* expression levels. Our empirical findings proposed that STAT3 might play a role in the transcriptional downregulation of LHPP. Considering the diverse functions of LHPP in modulating the immune response, inhibiting tumor growth, and regulating apoptosis, it emerges as a potent subject for further research within the context of UC pathogenesis.

Furthermore, our study investigation brought to light the phenomenon of increased angiogenesis within the context of UC, thereby underscoring the critical role of vascular development in disease progression. The process of enhanced vascularization ensures that UC-affected cells are well-supplied with essential nutrients and oxygen, which are crucial for their continued survival and proliferation. This insight into the angiogenic landscape of UC presents the potential for developing targeted therapeutic strategies that aim to inhibit these angiogenic pathways, offering a novel approach to combat the disease.

In conclusion, our research provides a detailed exploration of the cellular and molecular dynamics underpinning the pathogenesis of UC. These revelations lay the groundwork for subsequent research into potential therapeutic avenues, positioning LHPP as a pivotal factor in UC regulation and the immune response. By dissecting the complexities of UC through single-cell analysis, our study opens new pathways for advancing treatment modalities, ultimately contributing to enhanced patient care and outcomes in facing this formidable disease.

4. Materials and methods

4.1. Construction of the cell atlas for healthy and ulcerative colitis human colon tissues

To map the cellular landscape of the human colon in both healthy states and those affected by ulcerative colitis (UC), we sourced our raw data from the GSE125527 dataset within the Gene Expression Omnibus (GEO) database. Utilizing the Seurat package (version 4.0.2) in R, we embarked on constructing a comprehensive cell atlas of these tissues. The initial step involved creating a Seurat object for each sample using the CreateSeuratObject function, which loaded the gene expression matrices.

Subsequently, the function 'SCTransfrom' function was applied to each sample's data, allowing for the identification of high variable genes, as well as normalization and scaling of the gene expression matrices. The integration of samples was facilitated by the 'PrepSCTIntegration' and 'FindIntegrationAnchors' functions, which were instrumental in selecting integration anchors across all samples. This was followed by the application of the IntegrateData function to achieve a cohesive dataset integration.

Post-integration, the function "ScaleData" was employed to scale the integrated expression matrix, preparing it for further analysis. Principal component analysis and dimensionality reduction were then conducted using the "RunPCA" and "RunUMAP" functions, respectively. Cell clustering and identification were accomplished through the functions "FindNeighbors" and "FindClusters", categorizing cells into distinct groups based on their gene expression profiles.

To identify marker genes characteristic of each cell type, the 'FindAllMarkers' function was utilized, with selection criteria set to an average log fold change (|avg_log2FC|) of $\geq$0.5 and an adjusted p-value (p_val_adj) of $\leq$0.05. The visualization of these marker genes for each cell type was achieved using the VlnPlot function, highlighting the differential expression patterns that distinguish various cell populations within the colon tissue samples.

4.2. Identification of differentially expressed genes between ulcerative colitis and healthy group across cell compartments

For the delineation of differentially expressed genes (DEGs) across cell types between UC affected and healthy tissue samples, we employed the 'FindMarkers' function available within the Seurat package. The selection criteria for DEGs were stringently set to ensure robustness: only genes exhibiting a Benjamini-Hochberg (BH)-adjusted P value below 0.05 and an absolute log2 fold change (|log2FC|) exceeding 0.25 were considered significant.

4.3. Pseudotime trajectory inference

To investigate the mobilization and behavior of endothelial cells (EC) during ulcerative colitis, we employed the R package Monocle2 to conduct pseudotime trajectory analysis for ECs derived from both healthy and ulcerative colitis tissues. This analysis involved selecting the top 3000 highly variable genes as a basis for calculating the pseudotime. Utilizing functions such as "plot_pseudotime_heatmap" and "plot_genes_in_pseudotime" within Monocle2, we were able to perform detailed time-related gene expression analyses.

4.4. Gene set score analysis

For the analysis of gene set scores within our study, gene sets were procured from the Molecular Signatures Database (MSigDB) available at https://www.gsea-msigdb.org/gsea. Utilizing the AddModuleScore function provided by the Seurat R package, we computed gene set scores for each cell identified in our dataset.

4.5. Patients and tissues

In this study, the biopsy samples were systematically collected from a cohort comprising 12 healthy control subjects (8 males and 4 females) and 15 patients diagnosed with ulcerative colitis (12 males and 3 females). These participants were recruited from the Department of Gastroenterology, The Third Affiliated Hospital of Zhejiang Chinese Medical University (Hangzhou, China). The age range of the patients spanned from 21 to 56 years, and all were residents of the local Han Chinese population. Notably, they had not undergone any prior surgical treatments before the sample collection. Histopathological analysis of excised tumors or biopsy samples was used to confirm ESCC diagnoses. Prior to tumor removal, none of the patients underwent chemotherapy or radiotherapy treatments. The protocol (ZSLL-KY-2023-031-01) received approval from the Institutional Ethics Committee of The Third Affiliated Hospital of Zhejiang Chinese Medical University, aligning with the Helsinki Declaration. Fresh biopsy specimens were obtained, rinsed twice with 1x phosphate-buffered saline (PBS), and promptly frozen by liquid nitrogen.

4.6. Quantitative real-time PCR

The frozen colonic specimens underwent physical homogenization using a mortar and pestle, followed by total RNA extracted with TRIzol Reagent (Invitrogen). The cDNA synthesis was carried out utilizing the high-capacity reverse transcription kit (Thermo Fisher) according to the manufacturer's guidelines. The qPCR reactions were executed with TaqMan gene expression assays on the ABI QuantStudio 5 (Applied Biosystems, Thermo-Fisher).

4.7. Western blotting

Frozen samples were crushed using a mortar and pestle, and then lysed inlysis buffer (100 mM Tris-HCl (pH = 7.0), 2% 2-mercaptoethanol, 1% SDS, plus 1 x cOmplete™ Protease Inhibitor Cocktail, Roche) and heated at 100 °C for more than 10 min. Protein was isolated and quantified using Abcam's BCA Kit. Equal protein samples underwent SDS-PAGE, then transferred to PVDF (Millipore). After a blocking step with 5% skim milk, the membranes were incubated overnight at 4 °C with primary antibodies: LHPP (1: 500 dilution; abcam; ab254788), STAT3 (1: 1000 dilution; abcam; ab68153) and GAPDH (1:2000 dilution; 0411; sc47724). After HRP-linked secondary antibody treatment, detection used Thermo's enhanced chemiluminescence.

4.8. Enzyme-linked immunosorbent assay (ELISA)

Protein concentrations of IL6 (Biolegend) and LHPP (Abbexa) in blood sample were quantified utilizing ELISA kits, adhering to the manufacturer's instructions. After incubation with detection reagents, absorbance was measured at 450 nm using a chemiluminescence immunoassay system (Dxl800Access, Beckman, USA).

4.9. Statistical analysis

Experimental data underwent statistical analysis with GraphPad Prism software version 8.0, presented as mean ± SEM. Two-tailed student's *t*-test or one-way ANOVA were employed for comparisons. A P value < 0.05 was deemed to denote statistically significant. (Significance levels were marked in figures and legends as follows: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.005$; $^{****}P < 0.001$).

Data availability statement

All data collected and analyzed in this study are openly available in the Gene Expression Omnibus (GEO) database, under the public repository accession number GSE125527.

Ethics declarations

The study protocol (ZSLL-KY-2023-031-01, 2023) was reviewed and approved by the Institutional Ethics Committee of The Third Affiliated Hospital of Zhejiang Chinese Medical University.

CRediT authorship contribution statement

Ruoyu He: Writing – original draft, Project administration. **Yanfei Wang:** Writing – original draft, Methodology. **Chen Shuang:** Writing – review & editing, Conceptualization. **Chan Xu:** Writing – review & editing. **Xiaoling Li:** Writing – review & editing. **Yanfei Cao:** Writing – original draft, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Yanfei Cao reports financial support was provided by Zhejiang Province Traditional Chinese Medicine Science and Technology Program Project. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was supported by Zhejiang Province Traditional Chinese Medicine Science and Technology Program Project (2023ZF114).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e29163.

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