

88375\_Auto\_Edited.docx

***Basic Study*****Single-Cell Analysis Reveals Activation of Endothelial Cell and Identifies Phospholysine Phosphohistidine Inorganic Pyrophosphate Phosphatase as a Target in Ulcerative Colitis**

LHPP- a ulcerative colitis target

Yanfei Wang, Ruoyu He, Chan Xu, Xiaoling Li, Yanfei Cao

**Abstract****BACKGROUND**

Ulcerative colitis (UC) is a chronic gastrointestinal disorder characterized by inflammation and ulceration, representing a significant predisposition to colorectal cancer (CRC). Recent advances in single-cell RNA sequencing (scRNA-seq) technology offer a promising avenue for dissecting the complex cellular interactions and molecular signatures driving UC pathology.

**AIM**

To utilize scRNA-seq technology to dissect the complex cellular interactions and molecular signatures that underlie UC pathology.

**METHODS**

In this research, we integrated and analyzed the scRNA-seq data from UC patients. Moreover, we conducted mRNA and protein level assays as well as pathology-related staining tests on clinical patient samples.

## RESULTS

In this study, we identified the sustained upregulation of inflammatory response pathways during UC progression, characterized the features of damaged endothelial cells in colitis. Furthermore, we uncovered the downregulation of phospholysine phosphohistidine inorganic pyrophosphate phosphatase (LHPP) has a negative correlation with signal transducer and activator of transcription 3 (STAT3). Significant downregulation of LHPP in UC patient tissues and plasma suggests that LHPP may serve as a potential therapeutic target for UC. This paper highlights the importance of LHPP as a potential key target in UC and unveils its potential role in inflammation regulation.

## CONCLUSION

The findings suggest that LHPP may serve as a potential therapeutic target for UC, emphasizing its importance as a potential key target in UC and unveiling its role in inflammation regulation.

**Key Words:** Ulcerative colitis, Single-cell RNA sequencing, LHPP

Wang Y, He R, Xu C, Li X, Cao Y. Single-Cell Analysis Reveals Activation of Endothelial Cell and Identifies Phospholysine Phosphohistidine Inorganic Pyrophosphate Phosphatase as a Target in Ulcerative Colitis. *World J Gastroenterol* 2023; In press

**Core Tip:** Ulcerative colitis (UC), a chronic inflammatory bowel disease linked to colorectal cancer, was investigated using single-cell RNA sequencing (scRNA-seq) technology. The study unveiled sustained upregulation of inflammatory response pathways and characterized damaged endothelial cells during UC progression. Notably, the downregulation of phospholysine phosphohistidine inorganic pyrophosphate phosphatase (LHPP) exhibited a negative correlation with signal

transducer and activator of transcription 3 (STAT3). LHPP's significant downregulation in UC patient tissues and plasma suggests its potential as a therapeutic target. The findings highlight LHPP as a key target in UC and emphasize its role in inflammation regulation, offering insights for potential therapeutic interventions.

## **INTRODUCTION**

2

UC is a chronic inflammatory bowel disease (IBD) characterized by mucosal inflammation and ulceration primarily affecting the colon and rectum<sup>[1]</sup>. It is a multifactorial <sup>[2, 3]</sup>. Individuals with UC often experience debilitating symptoms such as abdominal pain, bloody diarrhea, diarrhea, rectal bleeding, and an increased risk of CRC, severely impacting their quality of life. Extensive research over the years has yielded valuable insights into the pathogenesis of UC, highlighting the importance of aberrant immune responses and the gut microbiome in disease development and progression<sup>[4-6]</sup>. However, a comprehensive understanding of the cellular and molecular mechanisms underlying UC development and progression remains elusive, and there is a growing need for more precise and targeted therapeutic interventions<sup>[7]</sup>.

Recent advances in scRNA-seq technology have revolutionized our ability to dissect the heterogeneity of cell populations within complex tissues<sup>[8-10]</sup>. This revolutionary technique empowers researchers to discern distinct cell subsets, unveil novel cellular pathways, and delve into the dynamics of immune responses with unparalleled resolution<sup>[11, 12]</sup>. In the context of UC, scRNA-seq offers a unique opportunity to decipher the intricate cellular interactions and molecular signatures driving disease pathology<sup>[13-17]</sup>.

LHPP, a histidine phosphatase protein, has been implicated in diverse biological processes, including tumor suppression in hepatocellular carcinoma<sup>[18]</sup>, increasing the expression of cleaved-PARP and cleaved-Casp3 protein to promote apoptosis<sup>[19]</sup>, and inflammation regulation and immune response modulation. Recent studies have suggested its potential correlation with survival of colorectal cancer patients <sup>[20]</sup>. Another study reports that the loss of LHPP in intestinal epithelial cells correlate with

colitis in mice, suggests the involvement of LHPP in IBD<sup>[21, 22]</sup>. However, the precise involvement and impact of LHPP in the context of UC remain an unexplored territory, offering a promising avenue for further investigation.

In this study, we integrated the current single-cell sequencing data of UC<sup>[15, 16]</sup>, shedding light on the specific cell types, transcriptional profiles, and immune signaling pathways that play pivotal roles in UC pathogenesis. By interrogating the single-cell landscape of UC-affected colonic tissues, we identified sustained upregulation of inflammatory response pathways during the progression of UC and characterized the features of damaged endothelial cells in colitis. Through integrated analysis of UC disease databases utilizing single-cell data, we uncovered that *LHPP* exhibits sustained downregulation in UC, displaying a negative correlation with *STAT3*. Notably, our experimental results in UC patients corroborated that when *STAT3* expression is upregulated, *LHPP* expression is downregulated, suggesting a potential role for *STAT3* in transcriptionally inhibiting *LHPP* expression. Furthermore, *LHPP* not only exhibited decreased expression in the intestinal tissue of UC patients but also displayed reduced expression in their plasma samples. This observation suggests that *LHPP* may serve as a critical factor in the pathogenesis of UC.

## **MATERIALS AND METHODS**

### **Construction of cell atlas of healthy and ulcerative colitis human clonal tissues.**

The raw data of healthy and ulcerative colitis human clonal tissues were from GSE125527 in GEO database. The R package Seurat (version 4.0.2) was used for construction of cell atlas of human clonal tissues. In brief, the function “CreateSeuratObject” was used to load gene expression matrix of each sample. The function ‘SCTransform’ was used for finding high variable genes, normalization and scaling of the gene expression matrix for each sample, the ‘PrepSCTIntegration’ and ‘FindIntegrationAnchors’ functions were used for selecting the anchors for integration all samples. The function “IntegrateData” was used for the following integration. After integration, the function “ScaleData” was used to scale the integrated expression

matrix, then the principle component analysis and Dimensionality reduction of dataset were performed by the functions “RunPCA” and “RunUMAP”, the functions “FindNeighbors” and “FindClusters” were used to cell clustering and identification. The function ‘FindAllMarkers’ ( $|\text{avg\_log2FC}| \geq 0.5$  and  $p\_val\_adj \leq 0.05$ ) was used to calculate marker genes for each cell type.

#### **Identification of DEGs between healthy and ulcerative colitis group across tissues and cell compartments.**

The ‘FindMarkers’ function in Seurat was used to identify differentially expressed genes in diseased *vs* healthy group (diseased/healthy) of each cell type, which were based on normalized data and the Wilcoxon test. The screening criteria for significantly differentially expressed genes were selected by BH-adjusted  $P$  value  $< 0.05$  and  $|\log_2\text{FC}| > 0.25$ .

#### **Pseudo-time trajectory inference.**

To characterize the mobilization and of EC during ulcerative colitis, the R package Monocle2 was used to perform pseudotime trajectory inference for EC of healthy and ulcerative colitis tissues. The top 3000 high variable genes were used to calculate the pseudotime. The functions “plot\_pseudotime\_heatmap” and “plot\_genes\_in\_pseudotime” were used to perform time-related gene analysis.

#### **Gene set score analysis.**

The gene sets were downloaded from MSigDB (<https://www.gsea-msigdb.org/gsea>) and the ‘AddModuleScore’ function of Seurat was used to calculate gene set scores.

#### **Patients and tissues.**

The biopsy samples were collected from 12 healthy control subjects and 15 ulcerative colitis patients from the Department of Gastroenterology, The Third Affiliated Hospital of Zhejiang Chinese Medical University (Hangzhou, China, ZSLL-KY-2023-031-01). The protocol was approved by the Institutional Ethics Committee of The Third Affiliated Hospital of Zhejiang Chinese Medical University and was in concordance with the Helsinki Declaration. Biopsy specimens were collected and washed twice with phosphate-buffered saline (PBS), then frozen by liquid nitrogen.

### Quantitative real-time PCR.

Colonic samples were subjected to physical homogenization and total RNA extracted by TRIzol Reagent (Invitrogen). Then <sup>2</sup> cDNA was synthesized using the high-capacity reverse transcription kit (Thermo Fisher) following the manufacturer's instructions. The qPCR reactions were performed using TaqMan gene expression and assays on ABI QuantStudio 5 (Applied Biosystems, Thermo-Fisher).

### Western blotting

<sup>6</sup> Tissues were lysed using SDS lysis buffer (containing 100 mmol/L Tris-HCl (pH = 7.0), 1% SDS and 2% 2-mercaptoethanol, supplemented with 1 x protease inhibitors from Roche) and incubated at 105°C for 10 minutes. Total protein was extracted and quantified using the BCA Kit (Abcam). Equal amounts proteins were subjected to <sup>3</sup> SDS-PAGE electrophoresis and subsequently transferred onto PVDF membranes (Millipore). After blocking with 5% skim milk, the membranes were treated overnight at 4°C with the following antibodies: mouse monoclonal antibody against GAPDH (1:2,000 dilution; 0411; sc47724); Rabbit monoclonal antibody against STAT3 (1: 1,000 dilution; abcam; ab68153). Rabbit monoclonal antibody against STAT3 (1: 500 dilution; abcam; ab254788). Following incubation with HRP-conjugated secondary antibodies, the PVDF membrane was visualized using an enhanced chemiluminescence (ECL) kit (Thermo).

### Enzyme-linked Immunosorbent Assay (ELISA)

The protein levels of IL6 (Biolegend) and LHPP (Abbexa) in the blood were determined using ELISA according to the manufacturer's instructions. Following incubation with the detection kit, the plate was analyzed at 450 nm using a chemiluminescence immunoassay system (Dxl800Access, Beckman, USA).

### <sup>1</sup> Statistical analysis

All data were statistical analyzed using GraphPad Prism software version 8.0. The data were presented as the mean  $\pm$  SEM. Comparisons were conducted using the two-tailed student's t test or one-way ANOVA. *P* values < 0.05 were considered statistically significant.

## **RESULTS**

### **Single-cell transcriptomics identified major cell types in human colon microenvironment.**

To systematically elucidate the microenvironmental changes occurring during the development of ulcerative colitis, we conducted an analysis utilizing publicly available single-cell datasets from healthy individuals and UC patients. Analyzing a total of 64,643 cells from healthy ( $n = 8$ ) and UC ( $n = 8$ ) individuals, we identified eight major cell types, which were CD4<sup>+</sup> T cells (CD4<sup>+</sup> TC) (37.44%), CD8<sup>+</sup> T cells (CD8<sup>+</sup> TC) (4.70%), natural killer T cells (NKT) (15.00%), natural killer cells (NK) (7.98%), B cells (BC) (13.81%), myeloid immune cells (Mye) (8.80%), and endothelial cells (EC) (2.45%) (Figures 1A & S1). The accuracy of cell identification was confirmed by the expression of canonical marker genes and top marker gene annotation of each cell type (Figures 1B-D).

Regarding alterations in cellular composition, we observed that during UC development, the proportions of CD8<sup>+</sup> T cells and NKT cells were increased (Figures 1F & G). This suggests a pronounced activation response by the host immune system in the face of inflammation, mobilizing these immune cell populations to target and eliminate diseased cells. The heightened presence of CD8<sup>+</sup> T cells, renowned for their cytotoxic capabilities, signifies a concerted effort by the immune system to target and eliminate inflamed UC tissues. NKT cells, with their unique properties bridging the innate and adaptive immune responses, may also play a crucial role in anti-inflammatory. This bolstering of cytotoxic immune cells within the colon microenvironment is an encouraging sign, suggesting that the immune system is actively engaged in combating the UC. However, the observed decline in the proportions of CD4<sup>+</sup> T cells and B cells is a matter of concern (Figures 1F & G). CD4<sup>+</sup> T cells play a vital role in mediating diverse immune responses, including antigen presentation to cytotoxic CD8<sup>+</sup> T cells and regulation of immune tolerance. A reduction in their presence could suggest a potential weakening of the immune system's ability to recognize inflammatory cells. Similarly, B cells, which are instrumental in antibody production and antigen presentation, also



exhibited diminishing proportions. This decline might impede the cytotoxic ability against UC of CD8<sup>+</sup> T and NKT cells. The reduction in CD4<sup>+</sup> T cells and B cells might be indicative of an evolving immune evasion strategy employed by UC cells, where the colon microenvironment becomes less conducive to an effective immune response and gains a tendency of the chronic, long-term inflammation.

Concurrently, we observed a progressive increase in the proportion of endothelial cells within the colon microenvironment (Figures 1F & G). This phenomenon underscored the significant role of angiogenesis in the ulcerative colitis's progression. The increasing angiogenesis has been proved a strong link with inflammation in various inflammatory diseases. Angiogenesis and inflammation cooperative with each other, and hypoxia acts as a common stimulus for both<sup>[23]</sup>. Furthermore, a robust vascular network can facilitate the intravasation and extravasation of inflammation factors, enabling metastasis to distant sites within the body.

These findings offer valuable insights into the alterations taking place within the microenvironment during the progression of UC. The body responds to inflammation through a series of immune cell activities. The augmented angiogenesis underscores the significance of vascularization in the inflammation microenvironment.

#### **Characterization of cell-type-specific transcriptomic changes in UC progression.**

We systematically investigated the differential gene expression profiles across all cell types during the process of UC development. Overall, we identified 706 upregulated and 1,471 downregulated differential genes. Notably, endothelial cells exhibited the highest number of both upregulated and downregulated differential expression genes (DEGs). These trends in differential gene expression to some extent reflect the inflammatory response of these cells during the progression of ulcerative colitis (Figures 2A-D).

Upon examining the upregulated differential genes within each cell type, we identified a substantial association with the regulation of the innate immune response, such as "Regulation of innate immune response", "Cytokine Signaling in the Immune system", and "Neutrophil degranulation". These findings suggested an elevated immune

response and immune cell infiltration within the inflamed tissue. During the UC, the body recruited immune cells to the inflamed tissue for the purpose of clearing infections, necrotic cells, or other pathological cells. Notably, the upregulation of the "Epithelial to mesenchymal transition" pathway was also observed, hinting at the possibility that sustained inflammation may induce tissue fibrosis. Additionally, the upregulation of the "Angiogenesis" pathway signifies the presence of neovascularization within the inflamed tissue, which aligns with our previous observation of an increased proportion of endothelial cells. These findings underscored the dynamic response of the body to inflammation, involving the recruitment of immune cells to the site of inflammation, concurrent local tissue fibrosis, and angiogenesis.

In the realm of downregulated genes, a notable decrease in the expression of genes associated with various metabolic processes, including "Xenobiotic metabolic process", "Oxidative phosphorylation", and "Fatty acid metabolic process". These findings indicated that inflammation may lead to a functional decline in affected tissues. Moreover, the downregulation of genes related to "Chromatin organization", "Cell activation" and "Intracellular protein transmembrane transport" indicated alterations in cellular states within the inflamed tissue. These observations collectively imply that inflammation induced substantial changes in cellular function and identity.

These findings of differential gene expression profiles across diverse cell types during UC process has unveiled intriguing insights into the dynamic molecular changes occurring within the inflammation microenvironment. The activation of inflammation-related pathways, immune cell recruitment, tissue fibrosis, and angiogenesis collectively reflect the body's response to inflammation. Concurrently, the downregulation of metabolic pathways and changes in cellular state suggest a functional decline within the affected tissues. These differential gene transcription profiles provide a valuable landscape for assisting in the identification of potential therapeutic targets for UC.

### **Compromised endothelial mobility during UC progression**

Prior research has emphasized the regulatory role of endothelial cells in immune responses, encompassing functions like filtration, endocytosis, antigen presentation, and leukocyte recruitment [24, 25]. Aberrant crosstalk between endothelial cells and immune cells were established in injury tissues [26, 27]. Furthermore, endothelial cells undergo significant alterations themselves, contributing to inflammation [28]. These findings not only underscore the intricate interplay between endothelial cells and the inflammatory microenvironment but also accentuate the potential of targeting endothelial cell-specific mechanisms as a promising avenue for UC therapy.

To gain a deeper understanding of the specific cellular changes that endothelial cells undergo during UC, we conducted pseudotime analysis to elucidate the progressive changes in endothelial cell states. Notably, we identified a distinct differentiation trajectory from healthy to UC-associated endothelial cells. Healthy endothelial cells were predominantly situated at the front end of the differentiation trajectory, while UC-associated subpopulations were concentrated toward the middle and end, providing compelling evidence that endothelial cells undergo a series of alterations in their cellular states during UC (Figures 3A and B).

We then focused on the transcriptomic profiles alterations during UC by conducting gene expression patterns that gradually evolved along the pseudotime trajectory. These genes were then categorized into two distinct groups based on their expression trends: the cluster 1 displayed increasing expression as pseudotime advanced, while the cluster 2 exhibited decreasing expression (Figure 3C).

The upregulated genes offer insights into the transcriptional alterations occurring in endothelial cells during the inflammatory process. Notably, they were primarily associated with critical signaling pathways, including The PI3K-Akt signaling pathway, known as a key regulator in multiple inflammations. the TGF-beta pathway, known for its role in cellular proliferation and differentiation, as well as tumor genesis. The blood vessel development pathway, indicated endothelial cells' active engagement in inflammation-related angiogenesis processes. Additionally, the ALK in cancer pathway,

suggested that endothelial cells possibly implicating them in a potential predisposition towards carcinogenesis (Figures 3C and D). Intriguingly, these observations underscored the active involvement of endothelial cells in angiogenesis and their responsiveness to various signals driving inflammation. Moreover, it hints at a proclivity of endothelial cells themselves towards malignant transformation. Conversely, the downregulated genes were predominantly linked to pathways such as the Hippo signaling pathway, which plays a pivotal role in regulating cell proliferation and organ size. The apoptosis pathway, responsible for programmed cell death and damaged cell clearance. Furthermore, cellular homeostasis pathways, indicated the disorder of cell state (Figures 3C and E). Collectively, the suppression of these pathways suggests that endothelial cells might undergo functional changes, contributing to their altered roles during inflammation progression.

Among genes whose expression exhibited the most significant changes along the pseudotime trajectory, *TGFB3*, a member of the transforming growth factor-beta (TGF- $\beta$ ) family, is well-known for its role in regulating cell growth, differentiation, and immune responses. Its upregulation in endothelial cells implies its potential involvement in the activation of signaling pathways critical for inflammation-related angiogenesis and microenvironment remodeling. Similarly, the upregulation of *EGFR* and *FGFR2* suggests the heightened responsiveness of endothelial cells to growth factor signaling, possibly fueling their angiogenic activities and interaction with inflammation microenvironment. The increased expression of *EPHB4*, a receptor involved in cell-cell communication and tissue patterning, hints at its role in endothelial cell differentiation and response to inflammation. Moreover, the upregulation of *ILA* and *COL1A2* highlights the active participation of endothelial cells in immune responses and extracellular matrix remodeling within the UC microenvironment (Figure 3F). Conversely, we observed a downregulation of *LHPP*, a phosphatase implicated in cell cycle regulation, may suggest a reduced control over endothelial cell proliferation and differentiation during UC progression. *HMOX1*, a stress-responsive enzyme, exhibited decreased expression, possibly indicating altered oxidative stress responses in

endothelial cells within the UC microenvironment. The decreased expression of *ATP5PD* may implicate alterations in energy metabolism within endothelial cells during inflammation. The downregulation of *ANXA2*, a protein involved in cellular processes such as membrane trafficking and cell adhesion, suggests potential changes in cell-cell interactions. The downregulation of *BCL2* may indicate alterations in apoptosis regulation in endothelial cells within the UC pathological process (Figure 3G).

#### **LHPP was identified as a core regulator for Ulcerative Colitis**

To deepen our insight of the correlation among dysregulated genes in the UC transcriptome profile and uncover key regulator into the molecular mechanisms underlying UC pathogenesis, we conducted an integrated analysis of UC by combining data from disease databases and scRNA-seq DEGs. We identified 13 commonly upregulated genes, including *CFB*, *SPINK4*, *FOXP1*, *TIMP1*, *CSF3R*, *STAT3*, *DUOXA2* and *MMP1*, and 7 downregulated genes (*LHPPP*, *CK1*, *CLDN8*, *ABCG2*, *FMO5*, *CKB*, *GUCA2A*) across these datasets (Figure 4A). Notably, apart from pathways associated with inflammation, we observed the upregulation of pivotal transcription factors such as *STAT3* and *FOXP1*. Correlation analysis of common regulatory factors revealed a negative association between *LHPP* and the expression of *STAT3* and *FOXP1*. *LHPP*, known for its role in cell proliferation and tumor suppression, displayed a significant negative correlation with *STAT3* and a less significant correlation with *FOXP1* (Figures 4B & C).

To further substantiate our findings, we recruited a cohort of 15 UC patients and 12 healthy controls, from whom we obtained intestinal tissue samples. Histological assessment (HE staining) of these tissue specimens indicated a significantly elevated presence of chronic inflammation in UC patients (Figure 4D,  $P < 0.05$ ). Subsequent qPCR analysis of these tissue specimens demonstrated elevated mRNA expression levels of inflammation-related genes, including *IL6*, *IL1A*, *CRP*, consistent with our preceding analyses (Figures 2A & C). Notably, in comparison to the healthy control group, we observed a significant reduction in *LHPP* mRNA expression and an increase in *STAT3* expression within the UC patient group ( $P < 0.05$ ), although the increase in *FOXP1*

expression did not reach statistical significances (Figure 4E). Furthermore, western blot analysis of the enrolled individuals provided further confirmation, showing a substantial decrease in LHPP expression and an increase in STAT3 expression among UC patients (Figures 4F & G,  $P < 0.05$ ). These findings suggest a potential transcriptional inhibitory role of STAT3 on LHPP expression.

Finally, we conducted ELISA testing on blood samples collected from 23 matched pairs of healthy individuals and UC patients. Our analysis revealed a noteworthy elevation in IL6 Levels among UC patients, underscoring the systemic inflammatory effects associated with UC (Figure 4H,  $P < 0.01$ ). Furthermore, the diminished levels of plasma LHPP in UC patients hint at its potential as a predictive biomarker for UC (Figure 4H,  $P < 0.05$ ). These findings emphasize the systemic impact of UC-related inflammation and point towards the promising prospect of utilizing LHPP as a potential predictive marker for this condition.

## **DISCUSSION**

Ulcerative colitis (UC) is a complex and chronic inflammatory bowel disease that predominantly affects the colon and rectum. Despite significant progress in understanding the pathogenesis of UC, there remains a need for more precise and targeted therapeutic interventions. Our study leveraged single-cell RNA sequencing (scRNA-seq) technology to delve into the intricate cellular and molecular landscape of UC. We observed a notable upregulation of inflammatory response pathways during UC progression, indicating an active immune response against inflamed tissues. The increased presence of cytotoxic CD8<sup>+</sup> T cells and NKT cells suggests an encouraging antitumor response, but the declining proportions of CD4<sup>+</sup> T cells and B cells raise concerns about potential immune evasion strategies employed by UC cells.

A particularly intriguing finding in our study was the sustained downregulation of *LHPP* in UC, coupled with its negative correlation with *STAT3*. Our experimental data supported the notion that STAT3 may transcriptionally inhibit LHPP expression, both in intestinal tissue and plasma samples of UC patients. LHPP's multifaceted roles in

tumor suppression, apoptosis regulation, and immune response modulation make it a promising candidate for further investigation in the context of UC pathogenesis.

Moreover, the heightened angiogenesis observed in UC underscores the importance of vascularization in disease progression. Enhanced vascularization provides UC cells with access to vital nutrients and oxygen, facilitating their survival and proliferation. This finding may open avenues for targeted therapies aimed at disrupting angiogenic processes in UC.

In summary, our study sheds light on the cellular and molecular mechanisms driving UC pathogenesis. These insights provide a foundation for future research into potential therapeutic interventions, with LHPP emerging as a key player in UC regulation and the immune response. Ultimately, unraveling the complexities of UC at the single-cell level holds promise for more effective treatments and improved outcomes for patients with this challenging condition.

## **CONCLUSION**

This study suggests that LHPP may serve as a potential therapeutic target for UC, emphasizing its importance as a potential key target in UC and unveiling its role in inflammation regulation.

## **ARTICLE HIGHLIGHTS**

### ***Research background***

Ulcerative colitis (UC) is a chronic intestinal condition characterized by inflammation and ulceration, and it is a significant risk factor for colorectal cancer (CRC). Recent advances in single-cell RNA sequencing (scRNA-seq) technology offer a promising avenue for dissecting the complex cellular interactions and molecular signatures driving UC pathology.

### ***Research motivation***

A comprehensive understanding of the cellular and molecular mechanisms underlying UC development and progression remains elusive, and there is a growing need for more precise and targeted therapeutic interventions.

### ***Research objectives***

The object of this study is to utilize scRNA-seq technology to dissect the complex cellular interactions and molecular signatures that underlie UC pathology.

### ***Research methods***

We integrated and analyzed the scRNA-seq data from UC patients. Moreover, we conducted mRNA and protein level assays as well as pathology-related staining tests on clinical patient samples.

### ***Research results***

We identified the sustained upregulation of inflammatory response pathways during UC progression, characterized the features of damaged endothelial cells in colitis. Furthermore, we uncovered the downregulation of phospholysine phosphohistidine inorganic pyrophosphate phosphatase (LHPP) has a negative correlation with signal transducer and activator of transcription 3 (STAT3). Significant downregulation of LHPP in UC patient tissues and plasma suggests that LHPP may serve as a potential therapeutic target for UC. This paper highlights the importance of LHPP as a potential key target in UC and unveils its potential role in inflammation regulation.

### ***Research conclusions***

This study suggests that LHPP may serve as a potential therapeutic target for UC, emphasizing its importance as a potential key target in UC and unveiling its role in inflammation regulation.

### ***Research perspectives***



This study sheds light on the cellular and molecular mechanisms driving UC pathogenesis. These insights provide a foundation for future research into potential therapeutic interventions, with LHPP emerging as a key player in UC regulation and the immune response. Ultimately, unraveling the complexities of UC at the single-cell level holds promise for more effective treatments and improved outcomes for patients with this challenging condition.

#### **ACKNOWLEDGEMENTS**

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

3%

SIMILARITY INDEX

PRIMARY SOURCES

1	Yusheng Cai, Muzhao Xiong, Zijuan Xin, Chengyu Liu et al. "Decoding aging-dependent regenerative decline across tissues at single-cell resolution", Cell Stem Cell, 2023 <small>Crossref</small>	37 words — 1%
2	academic.oup.com <small>Internet</small>	29 words — 1%
3	assets.researchsquare.com <small>Internet</small>	19 words — < 1%
4	id.nii.ac.jp <small>Internet</small>	15 words — < 1%
5	new.esp.org <small>Internet</small>	15 words — < 1%
6	www.ncbi.nlm.nih.gov <small>Internet</small>	15 words — < 1%
7	res.mdpi.com <small>Internet</small>	13 words — < 1%

