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ORIGINAL RESEARCH

Exosomal miR-493-3p Promote the Secretion of CXCL10 by Suppressing Keratinocyte Autophagy in Vitiligo

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Background: Vitiligo is a dermatological disorder characterized by the destruction of melanocytes, resulting from a complex interplay of genetic, immune and environmental factors. Recent studies have highlighted the involvement of keratinocytes in the pathogenesis of vitiligo; however, the precise mechanisms underlying this process remain to be fully elucidated. This study aimed to elucidate the specific role of keratinocytes in vitiligo pathogenesis, investigate autophagic changes in lesional keratinocytes, validate miR-493-3p's regulatory effect on keratinocyte autophagy.

Methods: Initially, the role of keratinocytes in vitiligo lesions were evaluated by reanalyzing single-cell transcriptome data from public databases. Subsequently, a comprehensive analysis of differentially expressed genes was conducted, focusing particularly on changes in the autophagy within keratinocytes. Finally, in vitro studies, including Western Blotting, ELISA, and immunofluorescence assays, were utilized to validate the effects of miR-493-3p on the autophagy, which are implicated in the secretory dysfunction of keratinocytes.

Results: Our study revealed a reduction in the number of keratinocytes in vitiligo lesions, and autophagy was significantly enriched in both WGCNA and KEGG analyses. Additionally, we demonstrated that miR-493-3p directly inhibits the expression of LC3, leading to a decrease in autophagy. Ultimately, we confirmed that autophagy dysfunction results in an increase in CXCL10 levels in keratinocytes, which may be associated with immune-mediated damage to melanocytes.

Conclusion: Our research illustrates the role of keratinocytes in vitiligo and clarifies how miR-493-3p induces secretion dysfunction in keratinocytes by modulating autophagy.

Keywords: vitiligo, keratinocyte, scRNA-seq, miR-493-3p, autophagy

Introduction

Vitiligo is an acquired chronic depigmenting disorder of the skin, which affects 0.5~1% of the world's population.[1] Its pathogenesis involves complex interactions between oxidative stress, environmental and genetic factors that ultimately lead to melanocyte destruction in epidermis, resulting in disfiguring white patches.[2] The primary cause of vitiligo is the decrease in the number of melanocytes and the loss of function.[3] Emerging evidence implicates multifaceted cellular mechanisms in this process, including oxidative stress-mediated melanocyte damage, dysregulated secretory functions of fibroblasts and keratinocytes, and $CD8^+$ T cell-driven immune responses.[4] Collectively, these findings underscore the intricate cellular crosstalk underlying vitiligo pathogenesis, necessitating further mechanistic exploration.

Keratinocytes and melanocytes form melanocyte units within the epidermis, playing a pivotal role in regulating melanocyte function.[5] Disruptions in keratinocyte secretory activity have been identified as a key driver of melanocyte

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dysfunction by altering the microenvironment.[6] The secretory phenotype of keratinocytes is intricately associated with their autophagy function. Under oxidative stress conditions, impaired keratinocyte autophagy triggers cell apoptosis and induces an aging-associated secretory phenotype.[7] Autophagy is a vital physiological process that sustains cellular homeostasis and is crucial for the optimal functioning of cells.[8] In vitiligo, the role of autophagy dysregulation has been preliminarily explored. For instance, autophagy disorder leads to increased secretion of CCL2 and CCL8 by fibroblasts, thereby amplifying the immune response they mediate.[9] While our previous work identified elevated serum miR-493-3p levels in patients with active segmental vitiligo and its regulatory effects on keratinocyte secretory functions,[6] the broader implications of autophagy dysregulation in epidermal keratinocytes remain underexplored. This knowledge gap is particularly notable given miR-493-3p's established role in modulating cell survival pathways in oncological contexts,[10] suggesting potential conserved mechanisms in melanocyte homeostasis. Further in-depth investigations into its diverse regulatory targets could offer novel perspectives for the development of vitiligo treatments.

In this study, we employed an integrative approach combining single-cell transcriptomics with weighted gene co-expression network analysis (WGCNA) to delineate the keratinocyte-specific molecular landscape in vitiligo. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis further identified key signaling networks potentially modulated by miR-493-3p. Through subsequent in vitro functional validation, we elucidate a novel mechanism through which this microRNA contributes to vitiligo pathogenesis via autophagy regulation. This work provides critical insights into the interplay between epidermal cellular communication and vitiligo pathogenesis, offering a framework for future translational research targeting autophagy pathways.

Materials and Methods

Patient Recruitment and Sampling

This study was carried out from February to October 2023 at the Dermatology Outpatient Department of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology. The participants included patients diagnosed with advanced vitiligo based on the criteria outlined in the "Consensus on the Diagnosis and Treatment of Vitiligo (2021 Edition)", as well as a control group matched by gender and age. All subjects were of Han ethnicity and voluntarily participated in the study after providing written informed consent. The information of all patients is listed in Supplementary Table S1. And the activity of vitiligo was evaluated by Vitiligo Area Scoring Index (VASI) and Vitiligo Disease Activity Score (VIDA). The research protocol was approved by the Ethics Committee of Tongji Hospital, Huazhong University of Science and Technology (Ethics Approval Number: TJ-IRB20220150).

Data Source and Bioinformatic Analysis

miRNA sequence data can be downloaded in the Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo/) under accession number GSE198956 and GSE141655. Data from the dataset GSE203262, accessed from the GEO, was from vitiligo patient matched lesional and nonlesional skin using the 10x genomics platform. Data from dataset PRJCA006797, accessed from the National Genomics Data Center at (https://ngdc.cncb.ac.cn/bioproject/browse/PRJCA006797), was from vitiligo patient and healthy control skin using the 10x genomics platform. R software (http://www.r-project.org, version 4), Seurat 4, Harmony and Bioconductor packages were utilized for analyses. The Weighted Gene Co-expression Network Analysis (WGCNA), Gene Set Enrichment Analysis (GSEA) and Kyoto Encyclopedia of Genes and Genomes (KEGG) database were used for annotation and enrichment analysis to obtain pathway information.

Cell Culture and Treatment

Human HaCaT keratinocyte cell line (Sunncell, Wuhan, China) was employed. The cells were grown in Dulbecco's Modified Eagle's Medium (DMEM) (Invitrogen, Carlsbad, CA, USA), enriched with 10% fetal bovine serum (FBS) (Hyclone, South Logan, UT, USA) and 1% penicillin-streptomycin mixture (Sigma-Aldrich, St. Louis, MO, USA). The culture was maintained at 37°C in a humidified incubator containing 5% CO_2. To establish an in vitro model of vitiligo, HaCaT cells were exposed to 100 ng/mL of IFN-γ (ABclonal, Wuhan, China) for 24 hours. Rapamycin (MedChemExpress, New Jersey, USA) was used at a concentration of 10 nM with an incubation time of 12 hours.

Real-Time Quantitative PCR

RNA was isolated from HaCaT cells using RNA-easy Isolation Reagent (Vazyme, Nanjing, China) after treatment. Complementary DNA (cDNA) was generated from 1.5 μg of purified total RNA with the HiScript III RT SuperMix for quantitative PCR (Vazyme, Nanjing, China). The expression levels of messenger RNA in HaCaT cells were assessed via quantitative real-time PCR employing SYBR Green (TB Green® Premix Ex Taq™ II, TaKaRa, Shiga, Japan). Data were calculated using the comparative Ct method and normalized relative to β-actin expression. The primer sequences used are provided in Supplementary Table S2.

Western Blotting Assay

Following experimental treatment, HaCaT cells were washed thrice with ice-cold PBS. Total protein lysates were prepared using IP lysis buffer (Servicebio, Wuhan, China) on ice for 15 minutes and protein concentrations were determined via BCA protein quantitation assay (Servicebio, Wuhan, China). Equal quantities of protein lysates from each group were combined with 2× SDS loading buffer and boiled at 100°C for 15 minutes to denature. Protein samples and molecular weight markers were separated by electrophoresis on 12% Bis-Tris polyacrylamide gels. Following SDS-PAGE, proteins were electrotransferred onto PVDF membranes (BD Biosciences, San Jose, CA, USA). Membranes were blocked for 90 minutes in TBST containing 5% non-fat milk, then incubated overnight at 4°C with primary antibodies against LC3 (ABclonal, Wuhan, China), CXCL10 (ABclonal, Wuhan, China), and β-actin (ABclonal, Wuhan, China) diluted in 5% BSA. After three 15-minute TBST washes, membranes were probed with horseradish peroxidase-conjugated secondary antibodies for 1.5 hours at room temperature.[11] Protein bands were visualized using an enhanced chemiluminescence detection system (ABclonal, Wuhan, China) and quantified with Bio-Rad Chemi-Doc XRS imaging platform (Bio-Rad, Hercules, CA, USA), with β-actin serving as loading control.

Enzyme-Linked Immunosorbent Assay (ELISA)

The levels of CXCL10 were assessed by using ELISA Kit from ABclonal (Wuhan, China). First, culture supernatants of HaCaT were collected following experimental interventions, with 100 μL aliquots of calibration standards and biological samples dispensed into pre-coated 96-well microplates. Following sequential incubation with biotinylated detection antibodies, bound analytes were amplified through streptavidin-HRP conjugate binding and developed using TMB substrate solution.[11] Signal intensity was determined by measuring absorbance at 450 nm using a BioTek Cytation 5 multimode reader (Winooski, VT, USA).

Luciferase Reporter Assay

The wild-type and mutant 3'-UTR sequences of MAP1LC3B were chemically synthesized and directionally inserted into the pGL6 luciferase reporter vector (Beyotime, Shanghai, China). Following this procedure, HaCaT keratinocytes were co-transfected with miRNA mimics, the constructed pGL6 plasmids, and a Renilla luciferase-expressing control plasmid.[6] The Renilla reporter served as an internal normalization standard for transfection efficiency. Luciferase activity was quantitatively measured using a Spark 10 M multimode microplate reader (Thermo Fisher Scientific, Waltham, MA) after a 48-hour incubation period.

Immunofluorescence

Human skin tissue sections were deparaffinized in xylene and rehydrated through graded ethanol to distilled water. Antigen retrieval was performed via microwave treatment (5 min at 95°C) in citrate buffer (pH 6.0). Endogenous peroxidase activity was blocked with 3% hydrogen peroxide for 15 min. Sections were then incubated with a blocking solution containing 5% goat serum in PBS with 0.1% Triton X-100 for 1 hour at room temperature to prevent nonspecific binding. Primary antibodies against LC3B (1:200, rabbit monoclonal, Cell Signaling Technology) and p62/SQSTM1 (1:100, mouse monoclonal, Abcam) were applied together and incubated overnight at 4°C in a humidified chamber. After three 5-min washes with PBS-T, sections were incubated with Alexa Fluor 488 anti-rabbit IgG (1:500) for LC3 and Alexa Fluor 594 anti-mouse IgG (1:500) for p62 (Thermo Fisher Scientific) for 1 hour at 37°C in the dark. Nuclei were

stained with DAPI (1 μg/mL) for 5 min. Fluorescence was visualized using a Zeiss microscope with appropriate filters (488 nm/525 nm for FITC, 594 nm/615 nm for TRITC, and 358 nm/461 nm for DAPI).

Statistical Analysis

All the experiments were performed in at least three independent trials. Data analysis was performed using GraphPad Prism, version 9.0.0 (GraphPad Software, San Diego, CA), and expressed as the mean SD. For all quantitative data, two-tailed Student's *t*-test analysis was used when comparing two groups, and one-way ANOVA with correction was used for multiple datasets. Statistical significance was set at $P < 0.05$.

Results

Single-Cell Transcriptional Profiling of Cells in Vitiligo Lesion

The single-cell transcriptional gene expression profiles and clinical data of GSE203262 and PRJCA006797 data sets were downloaded from GEO database and GSA database respectively. All single-cell transcriptome RNA sequencing data were obtained from a skin lesion cohort of vitiligo patients and control participants. The "Seurat" and "Harmony" software packages are used for data integration and filtering, 64,748 single cells were obtained and 33,694 genes represented (Figure 1A). Eleven types of major cells were annotated by expressions of canonical gene markers,[12] which included melanocytes, keratinocytes, fibroblasts, endothelial cells, epithelial cells, smooth muscle cells, T cells, B cells, mono/macro phagocytes, Langerhans cells, dendritic cells (DCs) (Figure 1B). t-distributed stochastic neighbor

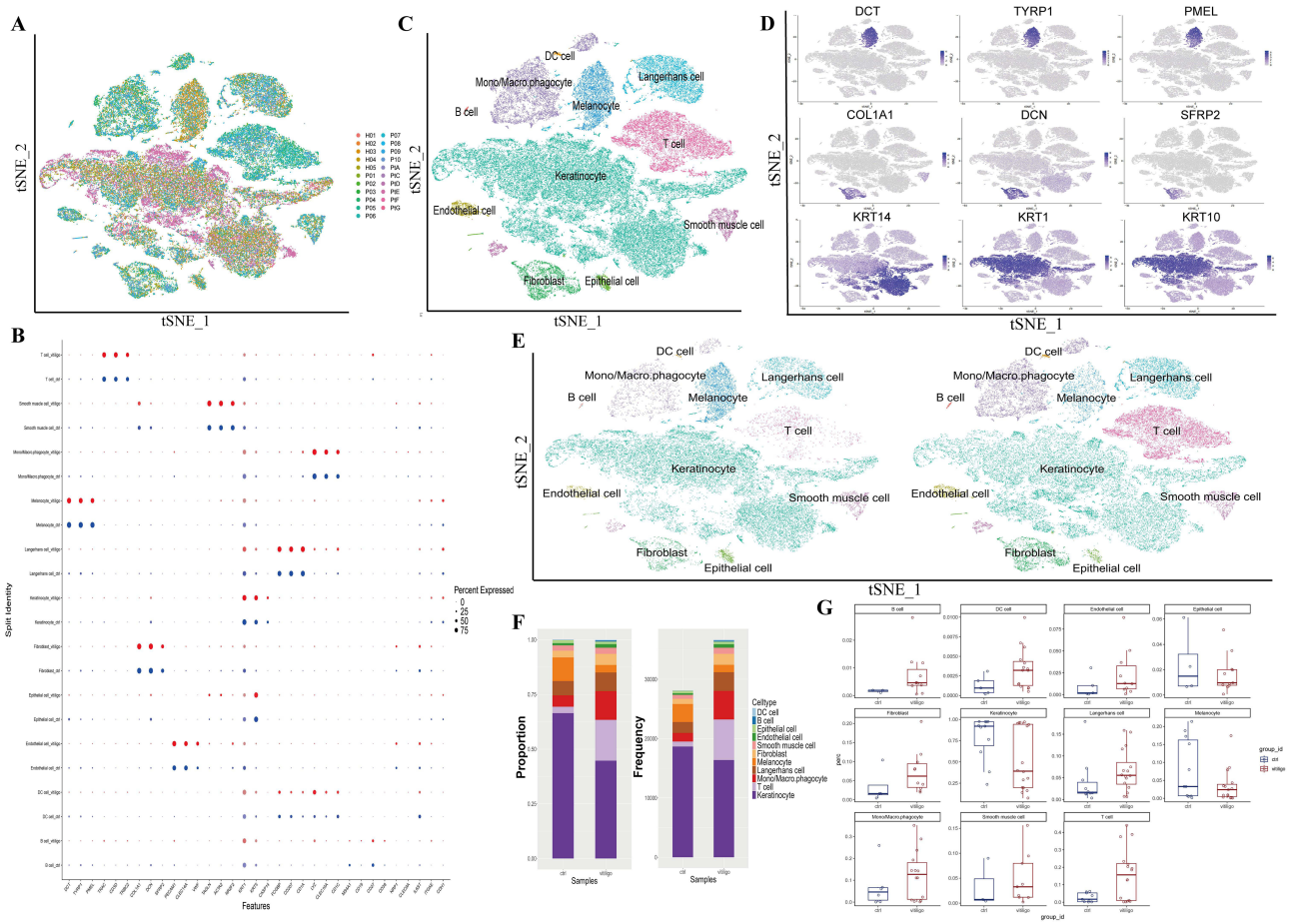

Figure 1 Single-cell transcriptional profiling of cells in vitiligo lesion. (**A**) t-SNE plot showed no batch effect between two datasets. (**B** and **C**) t-SNE and Dotplot showed cell clusters with integrated data. (**D**) Dotplot showed the clusters of melanocytes, fibroblasts and keratinocytes. (**E–G**) t-SNE plot, histogram and box diagram showed the changes of cell number and proportion of different groups in vitiligo compared with normal control group.

embedding (t-SNE) visualization confirmed distinct cellular clustering patterns between lesional and control samples (Figures 1C and D). We further explored the change of composition of cells in vitiligo lesions according to the scRNA-seq data analysis. The cell counts in the lesion area of vitiligo patients exhibited significant alterations compared to that of normal controls (Figure 1E). Comparative analysis revealed significant compositional alterations in vitiligo lesions, characterized by increased proportions of immune cells (T cells, B cells, DCs, Langerhans cells, mononuclear phagocytes) and stromal components (endothelial cells, epithelial cells, fibroblasts, smooth muscle cells), alongside decreased melanocyte and keratinocyte populations (Figures 1F and G).

Autophagy-Related Gene Dysregulation in Vitiligo Keratinocytes

Given the observed keratinocyte depletion (Figure 1G), we focused subsequent analyses on this critical epidermal population (Figure 2A). By conducting a comparative and analytical investigation of the differential genes expressed in keratinocytes from vitiligo patients and the control group, we successfully identified a total of 12,074 differentially expressed genes (DEGs). Subsequently, these DEGs underwent comprehensive enrichment analysis employing GSEA methodology (Figure 2B). In comparison to the control group, there was a significant downregulation of genes associated with keratinocyte formation and melanin synthesis in the lesional skin of vitiligo patients, while genes related to cytokine secretion showed a significant upregulation, consistent with previous findings (Figure 2B and C). Notably, we discovered concomitant suppression of autophagy-related genes and activation of apoptosis pathways (Figure 2C), suggesting potential mechanisms underlying keratinocyte loss. To investigate the potential role of autophagy in regulating keratinocyte numbers, we employed a well-established approach developed by F. Cecconi et al to monitor transcriptional activity of genes associated with autophagy.[13] Utilizing a comprehensive genetic toolbox consisting of 604 ATGs covering diverse signal transduction pathways related to autophagy, we performed single-cell transcriptomic analysis

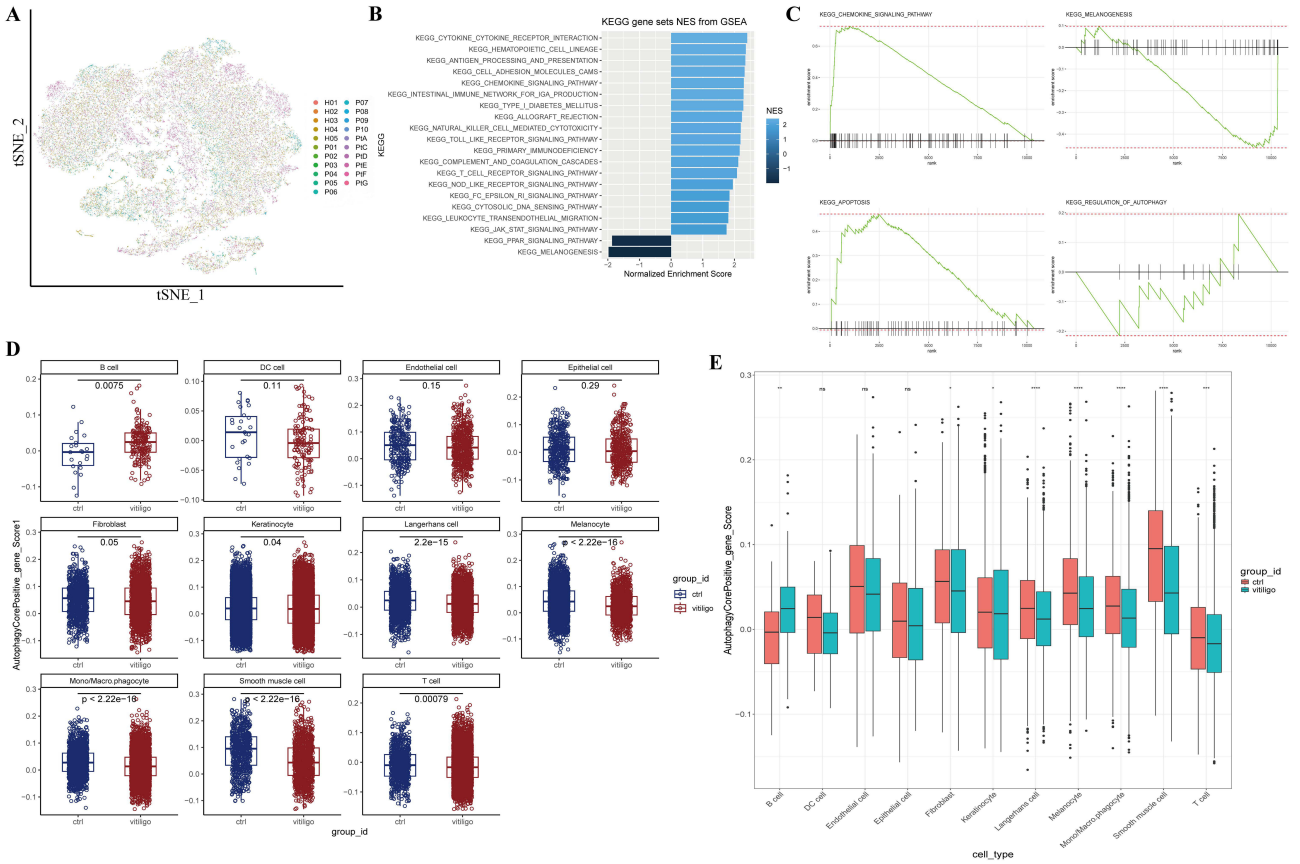

Figure 2 Expression profiling of autophagy-related genes in cells in vitiligo lesion. (**A**) t-SNE plot showed the cell cluster of keratinocytes. (**B** and **C**) KEGG analyses of NES from GSEA. (**D** and **E**) Autophagy score of different cell types. *p*-value: * $p< 0.05$; ** $p< 0.01$;*** $p< 0.001$; **** $p< 0.0001$. ns: not significant.

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to assess alterations in autophagy within different type cells.[13] Our findings demonstrated significantly decreased expression levels of genes involved in regulating autophagy in vitiligo patients' keratinocytes (Figures 2D and E).

Suppressed Autophagy in Vitiligo Keratinocytes

To systematically evaluate autophagy dysregulation in vitiligo pathogenesis, we first quantified autophagic markers in lesional epidermis. Comparative histopathological analysis revealed marked suppression of epidermal autophagy in vitiligo specimens relative to healthy controls (Figure 3A). Weighted gene co-expression network analysis (WGCNA) was performed to categorize DEGs into different gene modules, and 11 gene modules were randomly assigned color labels (Figure 3B). The turquoise module (M10) was significantly correlated with Autophagy Gene Score in keratinocyte ($r = 0.82$, $p < 0.05$) (Figures 3C and D). In terms of KEGG enrichment analysis, DEGs in the turquoise module were significantly associated with metabolic pathway and cell death pathways (Figure 3D). This finding aligns with our prior observation of autophagy-related pathway alterations (Figure 2C), with M10 particularly enriched in core autophagy processes (Figure 3E). Given the established elevation of IFN-γ in vitiligo lesions and its pathogenic significance,[14] in vitro stimulation of keratinocytes with IFN-γ is commonly employed to investigate the underlying mechanisms of vitiligo.[15,16] Therefore, our in vitro experiment was conducted to provide evidence that under IFN-γ stimulation, there was a notable decrease observed in LC3 mRNA levels within keratinocytes (Figure 3F).

miR-493-3p-Mediated Autophagy Suppression in Vitiligo Keratinocytes

While the molecular regulation of keratinocyte autophagy remains partially characterized, our prior findings established microRNAs as key modulators of vitiligo pathogenesis through secretory regulation. To further investigate their autophagic regulatory potential, we reanalyzed the miRNA sequencing on epidermal samples from 6 vitiligo patients

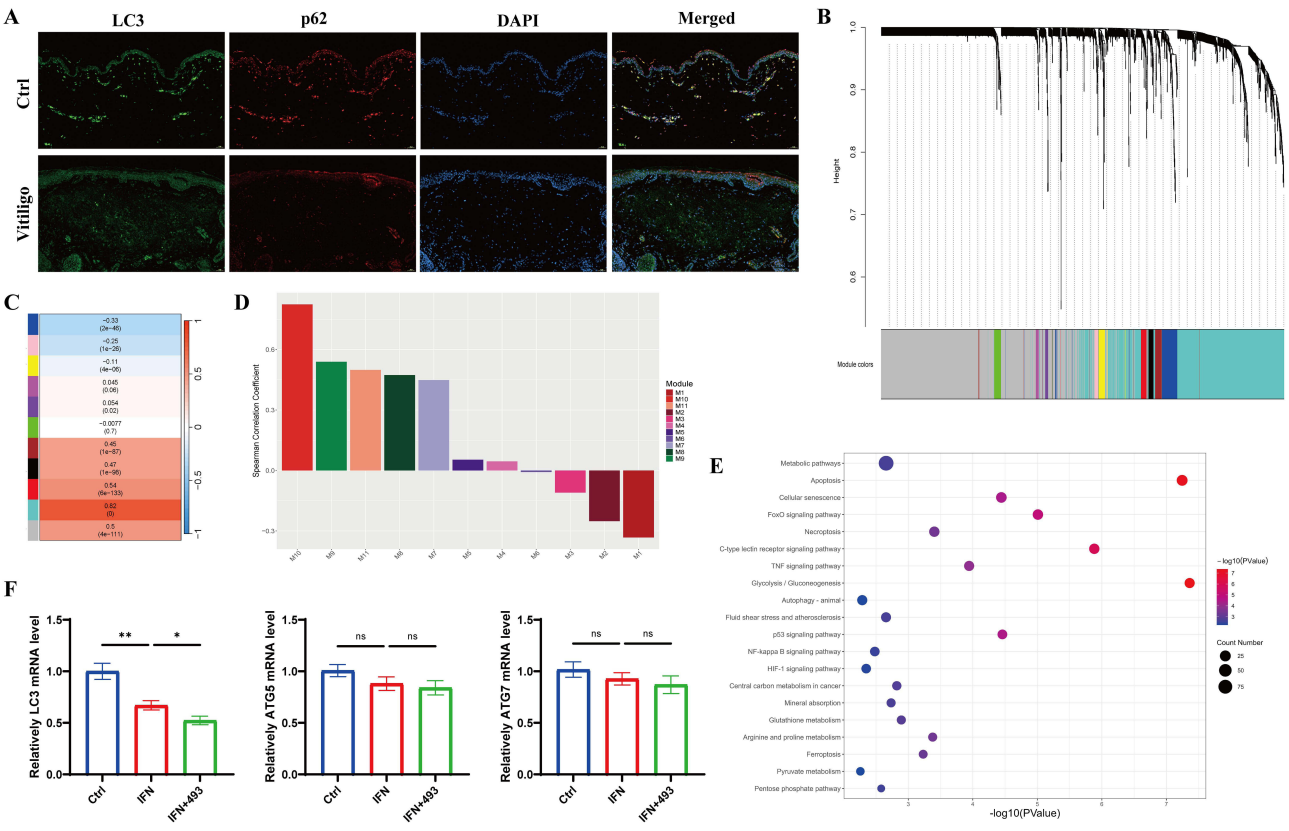

Figure 3 Inhibited autophagy in keratinocyte. (**A**) Changes of autophagy in patients with skin lesions of magnification factor of 20×. (**B**–**D**) WGCNA showed the cluster of keratinocytes genes, and the correlation score of each cluster of autophagy genes. (**E**) KEGG showed the enriched pathways of cluster M11. (**F**) The changes of LC3, ATG5 and ATG7 mRNA were verified in HaCaT cells. *p*-value: * $p < 0.05$; ** $p < 0.01$. ns: not significant.

and 6 matched controls (Figure 4A and Supplementary Table S3) which has been mentioned in our previous research.[6] Consistent with our previous findings,[6] miR-493-3p levels were significantly increased in blood samples from patients with active segmental and non-segmental vitiligo compared to healthy controls (Figure 4B). By predicted in 4 different target prediction tools, LC3 is the sole target of miR-493-3p (Figure 4C). Functional validation demonstrated that miR-493-3p mimics significantly attenuated IFN-γ-induced autophagic activity in keratinocytes (Figure 4D). The results of luciferase reporter gene experiment have shown that miR-493-3p can bind to the 3'UTR region of LC3 and inhibit the generation of mRNA (Figure 4E). The expression of LC3 and autophagy in keratinocytes was suppressed by miR-493-3p mimics in vitro, exhibiting a concentration-dependent trend where the inhibitory effect became more pronounced with increasing concentrations of IFN-γ (Figures 4F and G).

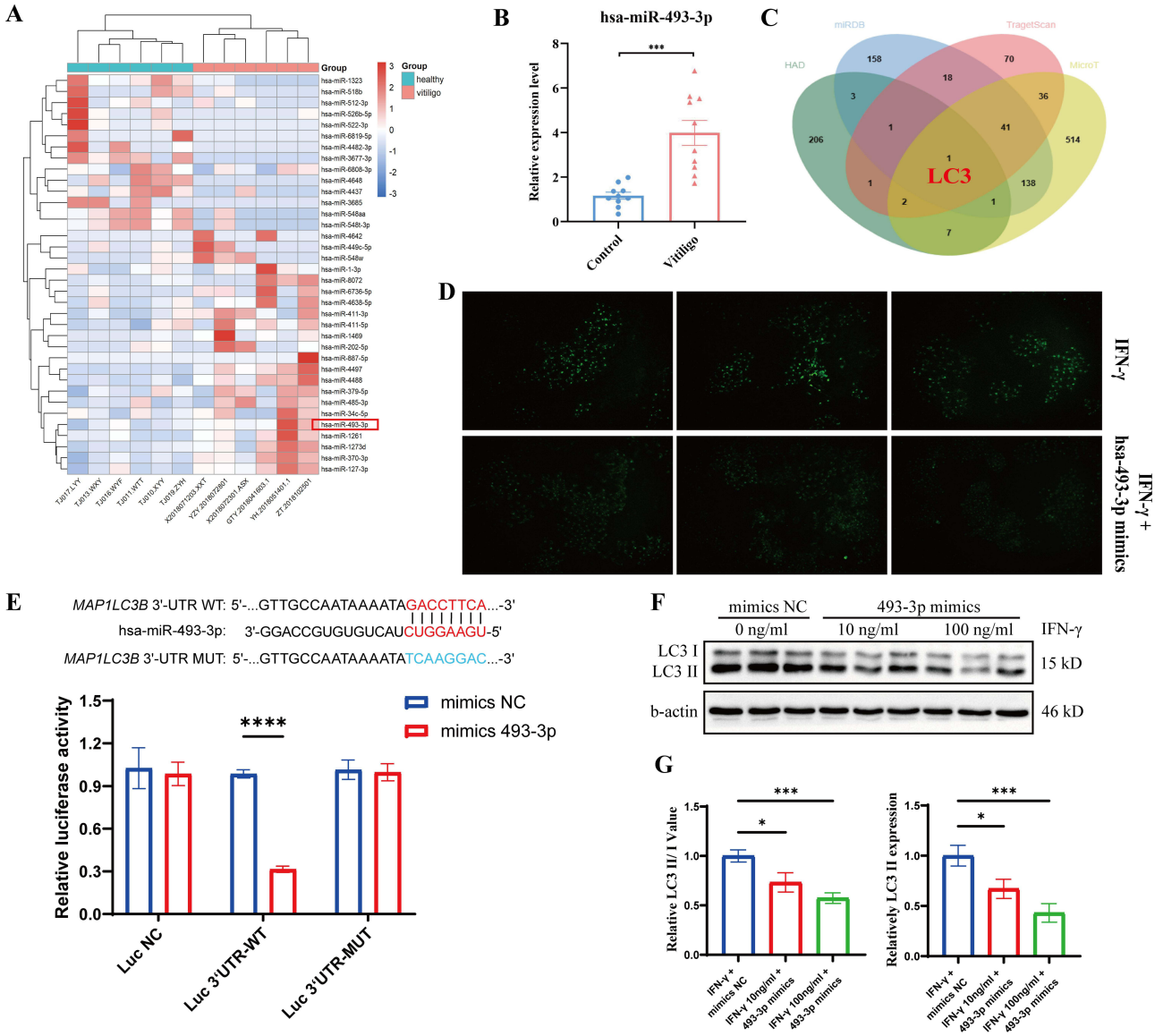

Figure 4 miR-493-3p increased in vitiligo epidermis and inhibited autophagy in keratinocyte. (**A**) Heat map analysis of differential miRNAs in patients with vitiligo (red) and healthy CTRLs (blue). (**B**) RT-qPCR were used to confirm the changes of miR-493-3p in plasma between patients with vitiligo and normal controls. (**C**) Venn diagram displaying one target genes of miR-493-3p, as predicted from four databases (miRBD/ TargetScan/ miRTarBase/ HAD). (**D**) miR-493-3p inhibiting autophagy of HaCaT cells was verified by Autophagy Staining Assay Kit with MDC in vitro of magnification factor of 20×. (**E**) Dual-luciferase assay in HaCaT cells co-transfected with the miR-493-3p mimic and a construct containing the wild-type or MAP1LC3B 3'-UTR MUT. (**F** and **G**) The expression of LC3 in IFN-γ and mimics 493–3p treated HaCaT cells was evaluated by Western blot. *p*-value: * $p < 0.05$; *** $p < 0.001$; **** $p < 0.0001$. The red box highlights miR-493-3p.

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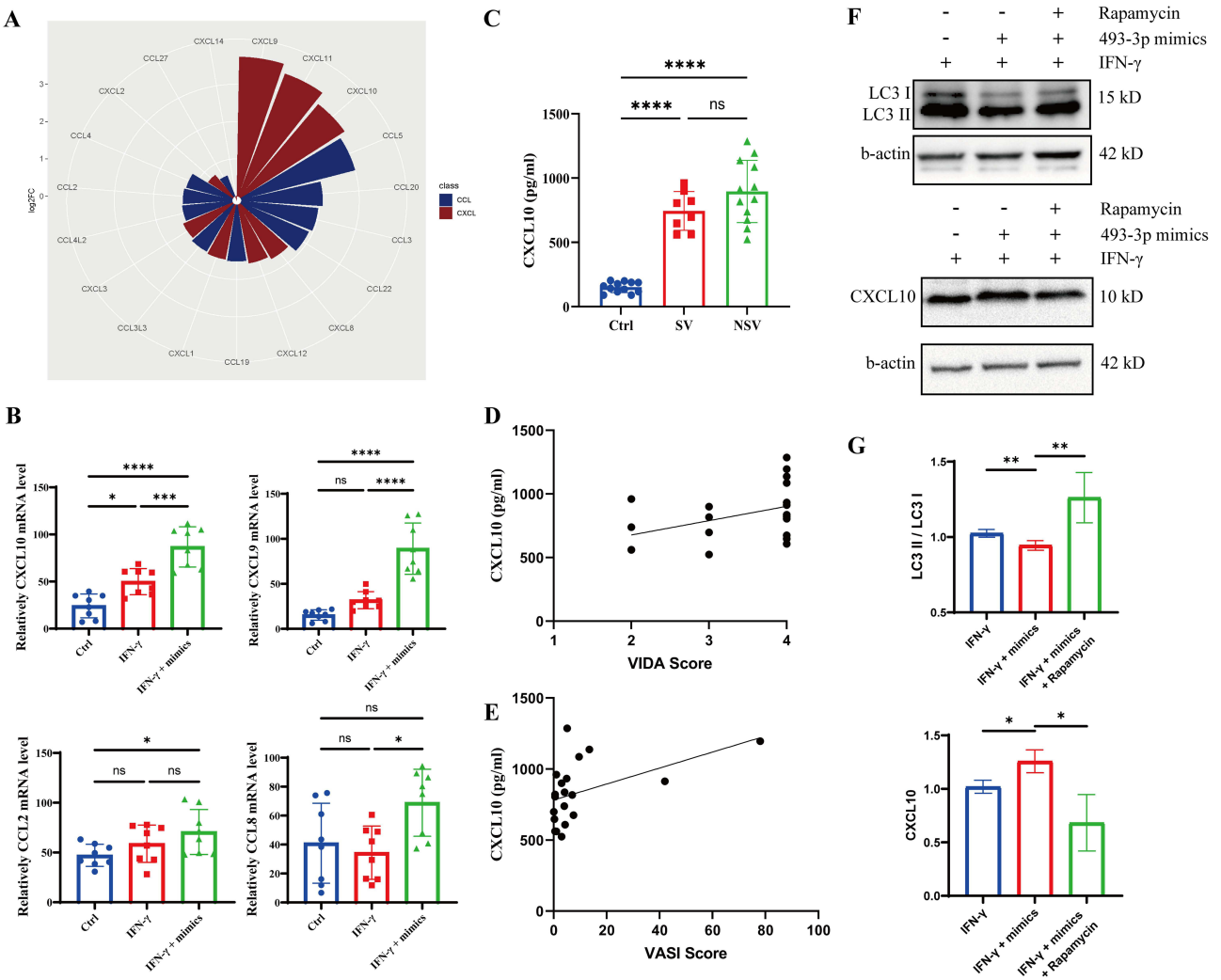

Figure 5 CXCL10 increased in vitiligo keratinocyte. (**A**) scRNA-seq analysis showed the change of CXCL and CCL family in keratinocytes. (**B**) The mRNA level of CXCL9, CXCL10, CCL2, CCL8 were tested by RT-qPCR. (**C**) ELISA assay estimated the level of CXCL10 in vitiligo patient and healthy control plasm. (**D** and **E**) The relationship between CXCL10 and severity of vitiligo. (**F** and **G**) Western blotting analysis of LC3 and CXCL10 protein expression in HaCaT cells treated with IFN-γ, transfected with mimic-493-3p for 24 hours, or treated with rapamycin for 12 hours. *p*-value: * $p< 0.05$; ** $p< 0.01$;*** $p< 0.001$; **** $p< 0.0001$. ns: not significant.

miR-493-3p Promote CXCL10 Secretion in Vitiligo Keratinocyte

CXCL, CCL family are the most important chemokines keratinocyte secreted, the analysis of scRNA-seq has shown that cytokine and metabolic process are enriched in DEGs of keratinocytes (Figure 5A). The expression of CXCL and CCL family was significantly increased, and CXCL9, CXCL11, CXCL10 are the top 3 gene among others. Considering that CXCL10 is the most critical chemokines secreted by keratinocytes,[9,12,15] the q-PCR results revealed a substantial elevation in CXCL10 mRNA levels in the IFN-γ + 493–3p mimics group (Figure 5B). In the blood of vitiligo patients, CXCL10 levels were significantly elevated and positively correlated with both the VIDA and VISA scores, which are indicators of vitiligo severity (Figure 5C–E). In vitro, autophagy activators rapamycin can effectively reduce the increased secretion of CXCL10 induced by miR-493-3p in the presence of IFN-γ (Figures 5F and G).

Discussion

Vitiligo represents a complex immune-mediated dermatosis characterized by progressive melanocyte loss, yet its precise pathomechanisms remain partially defined. Currently, the depigmentation observed in vitiligo is primarily attributed to melanocyte dysfunction and subsequent loss, serving as the central mechanism of the disease. Keratinocytes, as essential components of the epidermal melanin unit, significantly influence melanocyte survival and function through the secretion of

cytokines and chemokines. Despite extensive research, effective therapeutic strategies have yet to be fully developed; therefore, further investigation into the role of keratinocytes in vitiligo is critically important. In this study, we conducted comprehensive single-cell transcriptome analysis and WGCNA to gain novel insights into the keratinocyte landscape in vitiligo patients. Our findings revealed a previously unknown mechanism involving miR-493-3p regulation of CXCL10 release in keratinocytes, which is implicated in autophagy dysfunction. These findings highlight the therapeutic potential of targeting exosomal miR-493-3p and suggest that modulating keratinocyte function could form the basis for novel precision therapies.

Keratinocytes are central regulators of melanocyte viability through paracrine signaling.[4] The role of keratinocytes in the development of vitiligo is well-established, as they contribute to the pathogenesis by impairing the production of survival factors, disrupting the adhesion of melanocytes in lesional skin, presenting melanocyte antigens, and facilitating the recruitment of pathogenic T cells.[16–18] However, more current studies focus primarily on oxidative stress-induced dysfunctions, leaving a gap in understanding how epigenetic and post-transcriptional mechanisms shape keratinocyte pathogenicity. Integrating comprehensive single-cell RNA sequencing datasets can enhance the accuracy of analysis and facilitate a deeper exploration of the underlying mechanisms.[19] To investigate new avenues of inquiry, we acquired single-cell RNA sequencing data from public databases pertaining to vitiligo lesional skin. We conducted a standardized integration and reanalysis of these data, which revealed a substantial decrease in keratinocytes within the affected skin. Through WGCNA and KEGG analyses, we identified potential associations between alterations in autophagy and secretion functions of keratinocytes with vitiligo pathogenesis. Nevertheless, the precise mechanisms underlying the alterations in the autophagy and secretory functions of keratinocytes remain unclear and require further investigation.

Recent studies have revealed that exosomal microRNAs are closely associated with skin physiological and pathophysiological processes by regulating the secretion of proinflammatory cytokines and bioactive molecules.[20–23] Previous studies on miR-493-3p have predominantly focused on its implications in the field of oncology, specifically investigating its impact on cell survival and proliferation. For instance, miR-493-3p has been shown to induce cellular apoptosis by directly inhibiting the expression of AKT2, HMGA2, and other molecules[10] or to suppress tumor cell proliferation via PTEN inhibition.[24] In addition, our previous work has shown that circulating exosomal miR-493-3p can cross the basement membrane, be internalized by keratinocytes, and thereby modulate cellular secretory function.[6] As evidenced by our previous research, miR-493-3p upregulation in keratinocytes increases dopamine levels in the melanocyte microenvironment, thereby promoting vitiligo progression.[6] In the present study, we have identified a novel mechanism by which miR-493-3p regulates keratinocyte secretion. These findings highlight the intricate and diverse regulatory effects of miR-493-3p on keratinocytes, emphasizing the importance of elucidating its underlying mechanisms for targeted therapeutic interventions in vitiligo.

Autophagy plays a pivotal role in maintaining cellular homeostasis by orchestrating intricate catabolic processes to eliminate and renew intracellular components, thereby assuming critical significance in both physiological and pathological contexts.[25,26] Previous study reported that autophagy was suppressed by oxidative stress in vitiligo melanocytes. Dysregulation of melanocyte autophagy results in the accumulation of substances that require cellular clearance, thereby increasing its susceptibility to oxidative stress and exacerbating oxidative damage.[27,28] This factor directly contributes to melanocyte dysfunction and reduced numbers. Additionally, autophagy dysregulation in neighboring cells upregulates the secretion of chemotactic factors, which facilitates immune cell recruitment and subsequent immune-mediated melanocyte damage. Recent studies have demonstrated that oxidative stress in keratinocytes enhances the TRPM2-dependent autophagy inhibition process, leading to an upregulation of CXCL16 secretion.[29] Another study showed that autophagy dysregulation in fibroblasts increases the secretion of CCL2 and CCL8.[9] In the current study, we integrated multiple single-cell sequencing datasets, providing a substantial amount of data for comprehensive reanalysis. Our results confirmed that autophagy is inhibited in keratinocytes from vitiligo patients. Furthermore, our findings demonstrated that miR-493-3p directly downregulates LC3 expression, thereby suppressing autophagy level in keratinocytes. Restoring autophagy function in keratinocytes can reduce CXCL10 secretion. Therefore, therapeutic development targeting the miR-493-3p/LC3/CXCL10 axis could involve miRNA inhibition strategies using locked nucleic acid antagomirs,[30] autophagy enhancement through topical rapamycin analogs,[31] or CXCL10 neutralization with monoclonal antibodies.

However, the precise mechanism by which autophagy dysfunction leads to abnormal elevation of CXCL10 secretion in vitiligo remains to be elucidated. Prior studies have indicated that autophagy modulates secretion through multiple

pathways. For instance, studies in inflammatory bowel disease demonstrate that autophagy-related proteins such as p62/SQSTM1 and ATG16L1 regulate chemokine expression via NF-κB activation.[32] Similarly, in neurodegenerative disorders, autophagy dysfunction correlates with elevated levels of IL-1β and IL-6 due to impaired clearance of damaged mitochondria, which leads to mitochondrial DNA leakage and subsequent activation of the NLRP3 inflammasome.[33–36] Conversely, enhancing autophagy via PPARα agonists or rapamycin suppresses inflammatory cytokine production by restoring mitochondrial homeostasis and reducing ROS accumulation, as observed in liver injury models.[37] In vitiligo, these mechanisms may converge to shape the autoimmune microenvironment of the skin. While our study identified miR-493-3p/LC3-mediated autophagy dysfunction as a pivotal driver of CXCL10 secretion, accumulating evidence indicates that autophagy defects may amplify inflammatory responses via interconnected pathways. Specifically, impaired clearance of apoptotic melanocytes may lead to the release of damage-associated molecular patterns, which subsequently activate dendritic cells and initiate immune signaling cascades.[38] Concurrently, dysregulation of NLRP3 inflammasome activity, potentially triggered by excessive mitochondrial ROS accumulation, could further exacerbate inflammation by promoting pro-inflammatory cytokine production.[39] Additionally, metabolic reprogramming in T cells or macrophages toward pro-inflammatory phenotypes may synergistically perpetuate immune activation.[40,41] However, the precise crosstalk between autophagy impairment in keratinocytes and melanocytes and systemic immune activation in vitiligo remains to be investigated. Future studies utilizing single-cell transcriptomics and spatial proteomics may clarify the cell-type-specific roles of autophagy in chemokine gradient formation and tissue-resident memory T cell recruitment, thereby offering novel therapeutic strategies to restore immunological tolerance in vitiligo.

In summary, our findings show that miR-493-3p exacerbates autophagy dysfunction in keratinocytes by potentially directly inhibiting LC3 protein expression, which in turn leads to increased CXCL10 secretion (Figure 6). To the best of our knowledge, this study is the first to reveal the role of keratinocyte autophagy dysfunction in vitiligo pathogenesis and underscores the regulatory role of miR-493-3p in autophagy, thus providing a novel therapeutic target and research direction for vitiligo treatment.

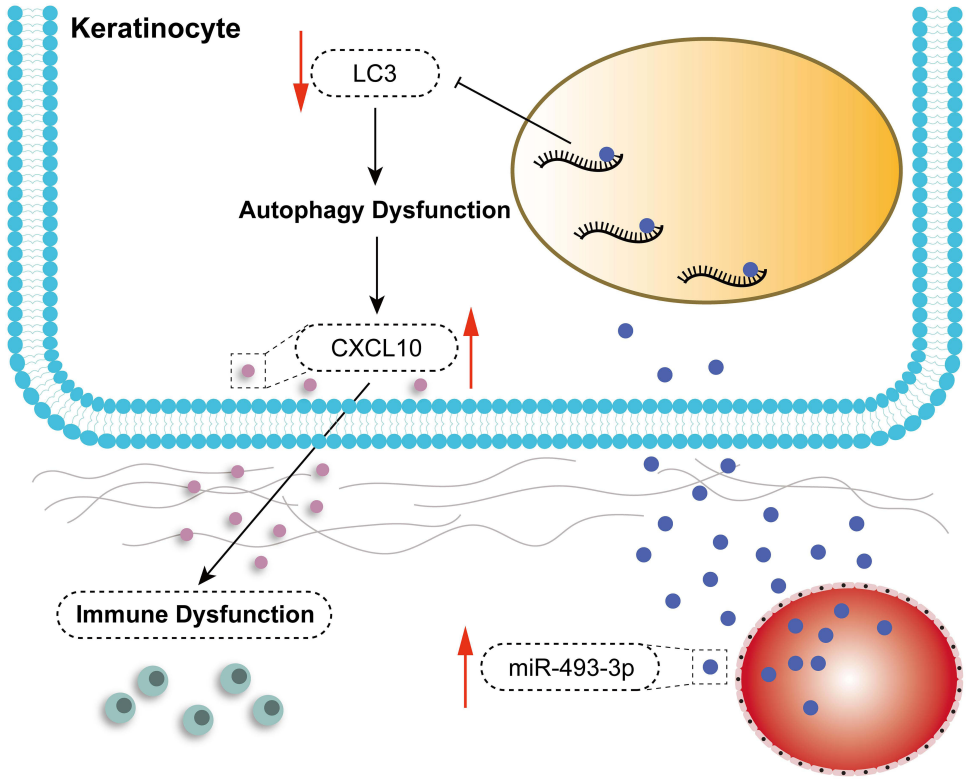

Figure 6 Proposed working model of exosomal miR-493-3p regulated secretion of CXCL10 by modulating keratinocyte autophagy in vitiligo. Red upward arrows: increased expression/levels; red downward arrows: decreased expression/levels or inhibition.

Conclusion

This study demonstrated that miR-493-3p may exert a potential direct effect on suppressing LC3 expression, thereby impairing autophagy and enhancing CXCL10 secretion, which is known to be associated with autoimmune melanocyte destruction through the recruitment of CD8+ T cells. These findings deepen our understanding of the molecular mechanisms underlying vitiligo and underscore the miR-493-3p/LC3 axis as a potential therapeutic target for modulating autophagy-related pathways, though further studies are needed to confirm this direct regulatory relationship. Further investigations into the precise interplay between keratinocyte autophagy dysfunction and immune cell activation, along with the development of targeted interventions aimed at restoring autophagy homeostasis, may pave the way for innovative treatments to attenuate disease progression and improve clinical outcomes in vitiligo patients.

Abbreviations

ATGs, Autophagy Related Genes; DEGs, Differentially Expressed Genes; GEO, Gene Expression Omnibus; GSA, Genome Sequence Archive; GSEA, Gene Set Enrichment Analysis; KEGG, Kyoto Encyclopedia of Genes and Genomes; LC3, Microtubule-Associated Protein 1 Light Chain 3; scRNA-seq, single-cell transcriptome RNA sequencing; t-SNE, t-distributed Stochastic Neighbor Embedding; WGCNA, Weighted Gene Co-expression Network Analysis.

Data Sharing Statement

The miRNA sequence data that support the findings of this study are openly available in Gene Expression Omnibus (GEO) at http://www.ncbi.nlm.nih.gov/geo/, project numbers GSE198956 and GSE141655. The scRNA-seq data that support the findings of this study are openly available in GEO at http://www.ncbi.nlm.nih.gov/geo/, project number GSE203262, and in the National Ge-nomics Data Center at https://ngdc.cncb.ac.cn/bioproject/browse/PRJCA006797, project number PRJCA006797.

Ethics Statement

The study was conducted in accordance with the Declaration of Helsinki, and approved by the ethics committee of Tongji Hospital affiliated with Tongji Medical College of Huazhong University of Science & Technology, China (TJ-IRB20220150).

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work.

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