Journal of Inflammation Research

Dovepress
Taylor & Francis Group

Open Access Full Text Article

ORIGINAL RESEARCH

Long Non-Coding RNA *lncTAF15:1-1* Promotes CCL5 Secretion and Cell Migration of Monocyte-Derived Dendritic Cells via PI3K/AKT/mTOR Pathway in Systemic Lupus Erythematosus

Mengmeng Xiang[1,]*, Zhan Sun[1,]*, Yan Ge[2,]*, Zhixiong Zhang[3], Chenghui Zheng[1], Zhanyan Gao[1], Jie Wang[1], Jinhua Xu[1], Jun Liang[1], Yilun Wang[1]

[1]Department of Dermatology, Huashan Hospital, Fudan University, Shanghai, People's Republic of China; [2]Department of Neurology, Huashan Hospital, Fudan University, Shanghai, People's Republic of China; [3]Department of Clinical Medicine, Shanghai Medical College, Fudan University, Shanghai, People's Republic of China

*These authors contributed equally to this work

Correspondence: Yilun Wang; Jun Liang, Department of Dermatology, Huashan Hospital, Fudan University, No. 12 Middle Wulumuqi Road, Shanghai, 200040, People's Republic of China, Email wangyilun@fudan.edu.cn; Liangjun1976@medmail.com.cn

Purpose: Systemic lupus erythematosus (SLE) is a complex autoimmune disease that seriously endangers human health. Long non-coding RNAs (lncRNAs) have been found to exhibit strong regulatory functions in cell physiology and maturation of dendritic cells (DCs). Hence, this study tried to reveal the underlying roles of one lncRNA, *lncTAF15:1–1*, in modulating DC functions and its involvement with CCL5 secretion in SLE pathogenesis.

Methods: The expression levels of *lncTAF15:1–1* were measured using qPCR in cultivated monocyte-derived dendritic cells (moDCs). Flow cytometry, ELISA, and Transwell chamber experiments were performed to assess various biological functions of moDCs. RNA-seq analysis was conducted to investigate transcriptional alterations in cells overexpressing *lncTAF15:1–1* and negative control cells. Gene Set Enrichment Analysis (GSEA) was utilized to predict potentially involved signaling pathways, which were subsequently confirmed by Western Blotting. Rescue experiments were carried out where the expression of *lncTAF15:1–1* and PI3K/AKT/mTOR pathway were altered simultaneously.

Results: *LncTAF15:1–1* was significantly upregulated in moDCs from SLE patients, and it exhibited a positive correlation with SLE Disease Activity Index (SLEDAI) scores. Additionally, elevated levels of CCL5 were detected in both plasma and moDC supernatants of SLE patients. Overexpression of *lncTAF15:1–1* stimulated moDCs to secrete higher levels of CCL5, and it enhanced the migration ability of moDCs as well as their capacity to attract CD4+ naïve T cells. GSEA analysis of RNA profiles indicated the potential involvement of the PI3K/AKT/mTOR pathway in *lncTAF15:1–1* regulation, which was further validated by Western Blotting. The rescue experiments demonstrated that the effects of *lncTAF15:1–1* on multiple functions of moDCs were attenuated when the PI3K/AKT/mTOR pathway was disrupted.

Conclusion: This study elucidated the role of *lncTAF15:1–1* in orchestrating DC migration and the recruitment of CD4+ T cells by enhancing CCL5 secretion through activating PI3K/AKT/mTOR pathway, which provides insights into potential molecular targets for SLE diagnosis and treatment.

Keywords: systemic lupus erythematosus, monocyte-derived dendritic cells, CCL5, long non-coding RNA

Introduction

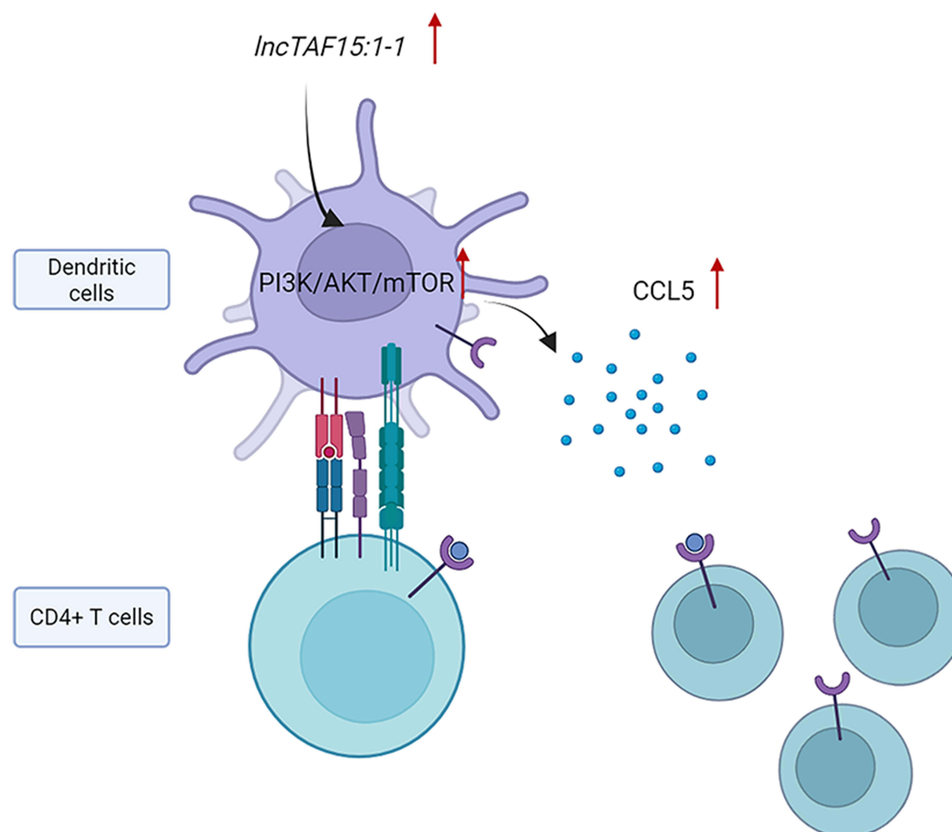
Systemic lupus erythematosus (SLE) is a highly complex and heterogeneous chronic autoimmune disease characterized by the production of autoantibodies and deposition of immune complexes, causing systemic inflammation and tissue damage of multiple organs. Related clinical manifestations such as kidney involvement, and damage to the

Journal of Inflammation Research downloaded from https://www.dovepress.com/
For personal use only.

Received: 17 March 2025
Accepted: 24 July 2025
Published: 3 August 2025

© 2025 Xiang et al. This work is published and licensed by Dove Medical Press Limited. The full terms of this license are available at https://www.dovepress.com/terms.php and incorporate the Creative Commons Attribution – Non Commercial (unported, v4.0) License (http://creativecommons.org/licenses/by-nc/4.0/). By accessing the work you hereby accept the Terms. Non-commercial uses of the work are permitted without any further permission from Dove Medical Press Limited, provided the work is properly attributed. For permission for commercial use of this work, please see paragraphs 4.2 and 5 of our Terms (https://www.dovepress.com/terms.php).

Graphical Abstract



musculoskeletal and cardiovascular system, can be severe or even life-threatening.[1] In the overall population, the estimate from a golden standard model indicates an SLE prevalence of 43.7 (ranging from 15.9 to 108.9) per 100,000 persons, affecting approximately 3.41 million people worldwide.[2] Despite advances in understanding its pathogenesis, the precise mechanisms underlying SLE remain unclear. It is generally believed that environmental factors like infections and exposure to sunlight would trigger or exacerbate the disease in genetically susceptible individuals. Continuous production of autoantibodies, deposition of immune complexes, dysregulated lymphocyte signaling, and epigenetic modifications are all key mechanisms leading to the loss of immune tolerance and subsequent tissue damage in SLE patients.[3–5]

Multiple types of dysfunctional immune cells are proven to initiate and exacerbate the disease,[6,7] while recent studies have highlighted the critical role of dendritic cells (DCs) in the pathogenesis of SLE.[8] Dendritic cells are the most powerful professional antigen-presenting cells (APCs) in the immune system. The composition, localization, and functional alterations of DC subsets, along with the dysregulated activation of myeloid DCs (mDCs) and plasmacytoid DCs (pDCs), are closely associated with the onset and clinical manifestations of SLE.[9] Additionally, the maturation of DCs subsequently facilitates the differentiation of effector T cells and enhances B cell production of autoantibodies in the germinal center (GC), which would consequently lead to the breakdown of immune tolerance and persistent tissue inflammation.[10] Therefore, further elucidating the role of DC abnormalities in the pathogenesis of SLE could significantly enhance our understanding of the underlying mechanisms.

Long non-coding RNAs (lncRNAs) are a class of regulatory RNA molecules that do not code for proteins but have emerged as important regulators of gene expression and cellular function.[11,12] Several lncRNAs, such as *RP11-273G15.2* and *NONHSAT101022.2*, have been identified as promising biomarkers for diagnosis, disease monitoring, and organ

involvement, with some also serving as potential therapeutic targets in SLE.[12,13] Ongoing research is needed to fully understand their mechanisms and clinical applications in SLE. In our preliminary in vitro experiments, we compared the lncRNA expression profiles of monocyte-derived dendritic cells (moDCs) from SLE patients and healthy controls. This analysis revealed significant differences in the expression of several lncRNAs, including *lncTAF15:1–1*, which was notably upregulated in moDCs from SLE patients,[14] but its specific role in immune regulation was unknown. Given the growing recognition of lncRNAs as critical regulators of immune cell function and autoimmune disease progression, this study aimed to investigate the functional impact of *lncTAF15:1–1* on moDCs and its potential involvement in the pathogenesis of SLE. Building on emerging evidence linking lncRNA-mediated regulation to aberrant immune signaling in SLE, we employed molecular assays, including RNA sequencing and bioinformatics analyses, to identify the signaling pathways influenced by *lncTAF15:1–1* and to elucidate its role in immune dysregulation.

Materials and Methods

Study Subjects

15 female SLE patients and 15 age and gender-matched healthy volunteers were recruited consecutively from the Department of Dermatology, Huashan Hospital of Fudan University. All patients met the 2019 American College of Rheumatology (ACR) revised criteria for the classification of SLE. Clinical characteristics including SLE Disease Activity Index (SLEDAI) were collected. The healthy volunteers did not have any history of autoimmune diseases. Written informed consent was obtained before the study initiation, and this study was approved by the Independent Ethics Committee of Huashan Hospital. All the experiments were conducted under the relevant ethical regulations of Huashan Hospital.

Cell Culture

Peripheral blood samples were collected in an EDTA-containing tube. Plasma from both patients and healthy individuals was initially collected by centrifugation, followed by Ficoll-Hypaque density gradient centrifugation to isolate peripheral blood mononuclear cells (PBMCs). CD14+ monocytes were separated through positive selection using EasySep™ Human CD14 Positive Selection Kit II (Cat#17858, STEMCELL, Vancouver, Canada) and were cultured for 7 days in RPMI-1640 supplemented with 1000 U/mL granulocyte-macrophage colony-stimulating factor (GM-CSF) (Cat#300-03, PeproTech, Rocky Hill, NJ, USA), and 1000 U/mL interleukin (IL)-4 (Cat#200-04, PeproTech). Cultured cells were further stimulated with 1 μg/mL lipopolysaccharide (LPS) (Cat#L6529, Sigma, St. Louis, MO, USA) at day 6 for maturation. The matured moDCs and corresponding cell supernatants were collected on day 7 of culture for subsequent experiments.

Cell Transfection

After being cultured for five days, immature moDCs were transfected with the adenovirus carrying the sequence of *lncTAF15:1–1* (multiplicity of infection: 300) for upregulation. The empty adenovirus vector was transfected in parallel as a control. While the expression of *lncTAF15:1–1* was downregulated using the Ribo™ Smart Silencer system (RiboBio, Guangzhou, China). Three siRNAs and three antisense oligonucleotides (ASO) targeting *lncTAF15:1–1* were packed in the Smart Silencer (200 nM) and delivered via Lipofectamine RNAiMAX (3 μL, Thermo Fisher Scientific, Waltham, MD, USA) into 2×10^5 moDCs. Scrambled siRNAs and ASOs transfected were used as the negative controls.

RNA Extraction and Quantitative Real-Time PCR (qRT-PCR)

In brief, total RNA isolation from matured moDCs was conducted with a TRIzol reagent (Thermo Fisher Scientific). PrimeScript™ RT Master Mix kit (Cat#RR036B, Takara, Otsu, Japan) was used for reverse transcription. QRT-PCR was performed using TB Green™ Premix Ex Taq™ II kit (Cat#RR820B, Takara) on ABI 7500 Fast Real-time PCR System (ABI, Foster City, USA) or QuantStudio 6 Flex real-time PCR systems (Thermo Fisher Scientific), as the protocol instructed. All target RNA CT values were then normalized to the housekeeping genes glyceraldehyde-3-phosphate

dehydrogenase (GAPDH) or beta-actin (ACTB) using the ΔΔCT method. The sequences of oligonucleotides are in Supplementary File 1.

Elisa

CCL5 from blood plasma and cell supernatants was detected utilizing the Human CCL5/RANTES DuoSet (Cat#DY278, R&D, USA). All the steps were done following the manufacturer's protocol. A seven-point standard curve using 2-fold serial dilutions was made to determine the protein levels of included samples based on the OD value. A duplicate of each standard and experiment sample was set.

Flow Cytometry

The collected cells were washed twice and then incubated with viability dye FVD780 for 30 minutes (Cat#65-0865-14, eBioscience, San Diego, CA, USA). Fc blocker (Cat#422301, Biolegend, San Diego, California, USA) was added to rule out nonspecific antigen binding. The antibody panel consisted of CD11c-APC (Cat#301613, Biolegend), CD14-PE (Cat#301805, Biolegend), CD40-Alexa Fluor 700 (Cat#334327, Biolegend), CD83-PE/Dazzle (Cat#305327, Biolegend), CD86-Brilliant Violet 510 (Cat#305431, Biolegend) and HLA-DR-PE-Cy7 (Cat#25-9952-42, Thermo Fisher Scientific). All these antibodies were mixed and added into moDCs for 30-minute incubation and then analyzed using a Fortessa cytometer (BD, New Jersey, USA). Aside from this surface marker panel for moDCs, the expression of CCR5-Brilliant Violet 421 (Cat#359117, Biolegend) was stained independently and identified following the same procedures. FlowJo (TreeStar, Inc., Ashland, OR, USA) was used to analyze the data.

Transwell Migration Assay

To evaluate moDCs' migration capability, 600 µL of medium with 10% FBS was added into the lower compartment of 5-µm-pore transwell chambers (Corning, NY, USA), while matured moDCs were added into the upper chamber. After 12h incubation, the migrated cells stained with crystal violet staining solution could be observed under a microscope, and images were captured. The number of migratory moDCs was counted and then normalized to the area. To assess the migration ability of $CD4^+$ naïve T cells, cell supernatants from mature moDCs were added to the lower chamber, while $5\times10^{\wedge 5}$ allogeneic $CD4^+$ naïve T cells were incubated in the upper chamber for 3 hours, and the suspension from the lower chamber was collected. The migration of $CD4^+$ T cells across the insert was quantified using flow cytometry and eBeads™ Counting Beads (Cat# 01–1234-42, Thermo Fisher Scientific). Migratory $CD4^+$ T cells were resuspended and 100 µL of counting beads were added. Given the known number of counting beads, the number of migratory cells was calculated accordingly.

Fluorescence in situ Hybridization (FISH)

The fluorescent probes and a FISH staining kit were designed and obtained from RiboBio. The detailed process included: collection of matured moDCs, PBS wash, fixation with 4% paraformaldehyde at room temperature for 10 minutes, and permeabilization using 0.5% Triton X-100 in PBS. After pre-hybridization at 37°C for 30 minutes, probes were added for incubation overnight. DAPI was utilized for nuclear staining before slide mounting and microscope scanning. U6 and 18s were considered as the nucleus and cytoplasm controls based on their specific intercellular sub-localization.

RNA Sequencing and Bioinformatic Analysis

RNA-seq was performed by OE Biotech Co., Ltd. (Shanghai, China). Total RNA from the vector and overexpressed group was extracted with a TRIzol reagent. CDNA libraries were prepared from isolated RNAs according to the manufacturer's instructions and were sequenced on the Illumina sequencing platform (HiSeqTM 2500). Raw reads were mapped to the human reference genome using HISAT2. Differentially expressed genes (DEGs) were identified using the DESeq R package.[15] A p-value < 0.05 and a fold change > 2 or change < 0.5 were set as the threshold for significantly differential expression. Hierarchical cluster analysis of DEGs was performed to explore gene expression patterns. GO enrichment and KEGG pathway enrichment analysis of DEGs were respectively performed using R based on the hypergeometric distribution.[16] A list of differentially expressed genes present in *lncTAF15:1–1* overexpressed

moDC groups compared to vector groups (P value < 0.05 while fold change > 2 or fold change < 0.5) was shown in Supplementary File 2.

Protein Extraction and Western Blotting

Western blotting was performed under standard procedures. Briefly, cells treated with PI3K activator 740 Y-P (Cat#1236188-16-1, MedChemExpress, Monmouth Junction, NJ, USA) and inhibitor LY294002 (Cat#HY-10108, MedChemExpress) accordingly were lysed in RIPA buffer containing protease inhibitor and phosphatase inhibitor (Beyotime, Nanjing, China). The protein lysate was analyzed by 10% SDS-PAGE, followed by protein transfer to the PVDF membrane. The membrane was then incubated with primary antibodies against AKT (Cat#60203-2-Ig, Proteintech, Chicago, IL, USA), p-AKT (Cat#9271T, Cell Signaling Technology, Danvers, MA, USA), PI3K (Cat#67071-1-Ig, Proteintech), p-PI3K (Cat#17366, CST), mTOR (Cat#66888-1-Ig, Proteintech), and p-mTOR (Cat#5536T, CST) overnight at 4°C, washed, and incubated with appropriate secondary antibodies at room temperature for 1 h. The blots on the membrane were captured using ECL and analyzed by ImageJ. Antibody against GAPDH was used as a loading control.

Statistical Analysis

R software, corresponding R packages, and GraphPad Prism 9 were utilized for statistical analysis. The differences between two groups were assessed using Student's *t*-test (two-tailed) for normally distributed data with equal variances, while for comparisons in three groups, one-way ANOVA was applied. For non-parametric data, the Mann–Whitney test or Kruskal–Wallis test was utilized. Pearson correlation or Spearman correlation was calculated to evaluate the association between two parameters. The results are presented as the mean ± SEM ($p < 0.05$ indicated statistical significance).

Results

LncTAF15:1-1 Was Highly Expressed in moDCs from SLE Patients and Positively Correlated with Disease Activity

In this study, we included a cohort of 15 SLE patients and 15 matched healthy controls. Detailed clinical information for the subjects was summarized in Table 1. It was found that the expression level of *lncTAF15:1–1* in moDCs from SLE

Table 1 Clinical Characteristics of Included SLE Patients

Characteristics	SLE (n=15)
Sex, male/female (n)	0/15
Age (years)	41.3±3.5
Duration (months)	2 (1–15)
RBC (10^12)	3.7±0.2
WBC (10^9)	4.1 (2.42–10.54)
Hemoglobin (g/L)	108.3±6.2
Platelet count (10^9)	170.8±18. 2
ESR (mm/h)	24.9±4.9
Urine protein, yes/no (n)	5/10
SLEDAI score	10.1±1.9
ANA>1:320, yes/no (n)	15/0
Anti-dsDNA (IU/mL)	463.6±83.1
Abnormal (low) complement C3, yes/no (n)	7/8
Abnormal (low) complement C4, yes/no (n)	7/8
Organ involvement, yes/no (n)	13/2
Glucocorticoids, yes/no (n)	14/1
Immunosuppressive drugs, yes/no (n)	0/15

Notes: Data are presented as median (range), and mean±SEM.
Abbreviations: ANA, antinuclear antibody; SLE, systemic lupus erythematosus; SLEDAI, systemic lupus erythematosus disease activity index; RBC, red blood cell; WBC, white blood cell; ESR, erythrocyte sedimentation rate.

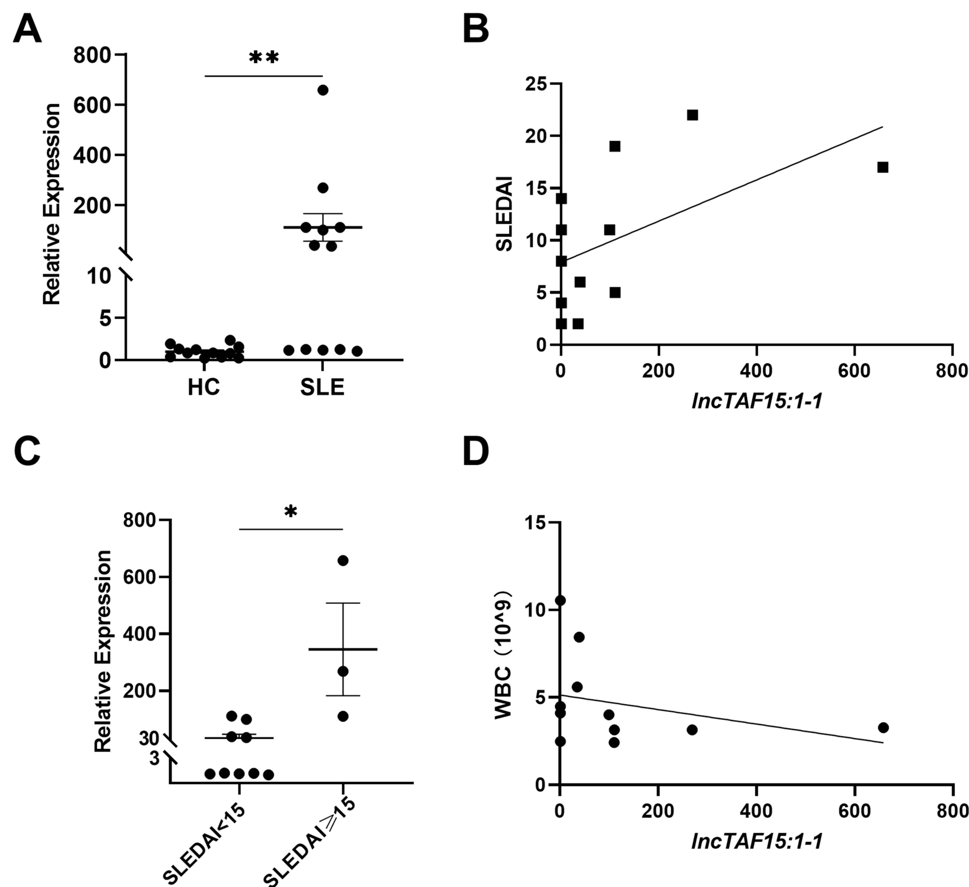


Figure 1 *LncTAF15:1–1* was highly expressed in moDCs from SLE patients and positively correlated with disease activity. (**A**) The expression levels of *lncTAF15:1–1* in moDCs from SLE patients and healthy controls (12 vs 12) were measured by qRT-PCR. (**B**) Correlation of *lncTAF15:1–1* expression levels with SLEDAI scores was assessed (r=0.57; p=0.03). (**C**) The comparison of *lncTAF15:1–1* levels between SLEDAI<15 patients and SLEDAI≥15 patients was made. (**D**) Correlation of *lncTAF15:1–1* expression levels with WBC counts was assessed (r_s=−0.54; p=0.04). Unpaired *t*-test (two-tailed) and Mann–Whitney test were applied. Pearson correlation (r) or Spearman correlation (r_s) was calculated to evaluate the association between two parameters. Data were shown as the mean ± SEM. *p<0.05, **p<0.01.
Abbreviations: MoDCs, monocyte-derived dendritic cells; SLEDAI, systemic lupus erythematosus disease activity index; WBC, white blood cell.

patients was higher and significantly correlated with SLEDAI scores (r=0.57; *p*=0.03; Figure 1A and B). When the patients were divided into two sub-groups based on their SLEDAI scores, increased expression levels of *lncTAF15:1–1* were observed in moDCs from patients with SLEDAI scores above fifteen compared to those with lower scores (Figure 1C). In addition, a negative association between *lncTAF15:1–1* and WBC counts in SLE patients was revealed (r_s=−0.54; *p*=0.04; Figure 1D). Since WBC count is an important indicator for assessing disease activity in SLE, the negative regulatory relationship between *lncTAF15:1–1* and WBC further supported the clinical relevance of *lncTAF15:1–1*. All these findings encouraged us to further explore the possible regulation of *lncTAF15:1–1* on the functions of moDCs.

Regulation of lncTAF15:1-1 Influenced CCL5 Secretion in moDCs

To ascertain the specific role of *lncTAF15:1–1*, its expression level was artificially regulated and verified when the adenovirus or Smart Silencer was transfected into the moDCs (Figure 2A and B). The functional changes of moDCs were then comprehensively assessed, including the levels of pro-inflammatory cytokine secretion, cellular viability, migratory capacity, and the expression of surface markers. It was found that overexpression of *lncTAF15:1–1* led to an increase in CCL5 mRNA expression (Figure 2C). While other associated inflammatory cytokines (IL-2, IL-10, IL-12A, IL-17, TNF-α, IFN-α1, IFN-γ, Blys, and APRIL) did not show any significant rises (Supplementary File 3A). Likewise, when the *lncTAF15:1–1* was downregulated, the expression of CCL5 decreased (Figure 2D). Consistent with transcript levels, CCL5 protein secretion followed a similar pattern (471.9±45.81 vs 859.4±38.47 pg/mL, 657±25.13 vs 353±30.75 pg/mL; Figure 2E and F). The

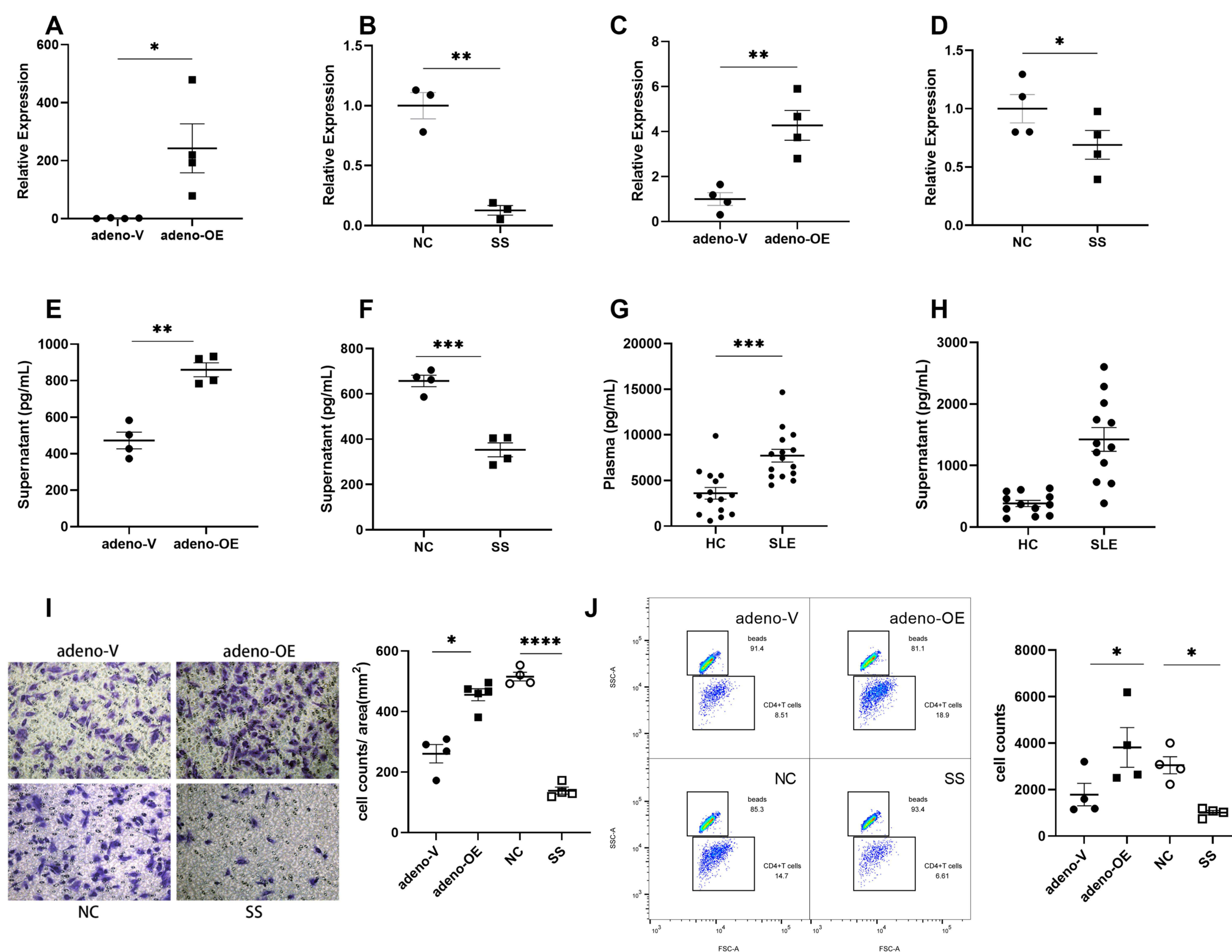


Figure 2 Regulation of *lncTAF15:1–1* influenced CCL5 secretion in moDCs. (**A** and **B**) The expression level of *lncTAF15:1–1* was artificially modulated by the transfection of adenovirus or Smart Silencer and confirmed using qRT-PCR. (**C–F**) Alterations of the expression of *lncTAF15:1–1* resulted in corresponding changes in mRNA expressions and secretion levels of CCL5. (**G** and **H**) The secretion levels of CCL5 in the blood plasma (15 vs 15) and moDCs cell supernatant (12 vs 12) were compared between SLE patients and healthy controls. (**I**) The migratory ability of moDCs was assessed using Transwell chambers, and comparisons were made between adeno-V, adeno-OE, NC, and SS groups. The number of migratory moDCs was counted and normalized to the area. (**J**) The capacity of supernatant from moDCs to attract CD4+T cells was evaluated using Transwell assay. The migratory CD4+T cell numbers were quantified by flow cytometry. Unpaired *t*-test (two-tailed) or Mann–Whitney test was applied. Data were from at least three independent experiments and shown as the mean ± SEM. *p<0.05, **p<0.01, ***p<0.001,****p<0.0001.

Abbreviations: MoDCs, monocyte-derived dendritic cells; OE, overexpression; NC, negative control; SS, Smart Silencer.

secretion levels of CCL5 were then measured in the SLE patients vs healthy controls, and it revealed that the plasma level of secreted CCL5 was significantly higher in SLE patients (7717±699.8 vs 3601±639.6 pg/mL; Figure 2G). A similar increase of CCL5 levels between healthy controls and patients was observed in the supernatant of moDCs (383.1±49.75 vs 1424±193.4 pg/mL; Figure 2H).

Given the potential role of CCL5 in moDC migratory functions, we further assessed moDC migration using the Transwell migration assay. It was found that when *lncTAF15:1–1* was elevated, the number of migratory moDCs rose and vice versa (Figure 2I). Furthermore, the capacity of moDC supernatants to attract CD4$^+$ T cells was assessed. Supernatants from moDCs with overexpressed *lncTAF15:1–1* (adeno-OE) showed a heightened capacity to attract CD4$^+$ T cells compared to those from control moDCs (adeno-V). Conversely, a reduction in CD4$^+$ T cell migration was observed when *lncTAF15:1–1* was downregulated (Figure 2J).

Further assessments of moDC functions were conducted to provide a comprehensive understanding of the role of *lncTAF15:1–1*. The vitality of moDCs was examined after viral transfection to exclude the possibility that adenovirus might alter the cell viability. The results demonstrated that there was no apparent distinction between the vector and overexpression groups (Supplementary File 3B). The surface markers on moDCs were also analyzed, and no significant

changes were found (Supplementary File 3C and D). The expression of CCR5 on matured moDCs was also evaluated by flow cytometry, with no significant changes observed between the vector vs overexpression groups and NC vs SS groups (Supplementary File 3E).

The PI3K/AKT/mTOR Pathway Was Pivotal in the Immune Modulation by lncTAF15:1-1

To further elucidate the specific transcriptomic effects of *lncTAF15:1–1 on* moDCs, we performed RNA-seq analysis on cells overexpressing *lncTAF15:1–1* and their corresponding control group. As shown in Figure 3A and B, 488 differentially expressed genes were identified between the vector and *lncTAF15:1–1* overexpressed moDC groups, including 140 down-regulated and 348 up-regulated genes. In addition, GO function enrichment analysis indicated that these differentially expressed genes in the *lncTAF15:1–1* overexpressed group were related to different pathways (Figure 3C), for example, biological processes such as "regulation of leukocyte activation", "positive regulation of cytokine production", "leukocyte cell-cell adhesion" and so on. The GSEA plot showed that the PI3K/AKT/mTOR signaling pathway was activated in the *lncTAF15:1–1* overexpressed group (Figure 3D). Detailed expression matrix between the overexpressed group and vector was uploaded as Supplementary File 2.

Western blotting was performed to verify the involvement of the PI3K/AKT/mTOR signaling pathway in *lncTAF15:1–1* regulation (Figure 3E). The expression levels of p-PI3K, p-AKT, and p-mTOR were all substantially higher in *lncTAF15:1–1* overexpressed group than in the vector group, which could be reduced by PI3K inhibitor LY294002 (Figure 3E). Meanwhile, the knockdown of *lncTAF15:1–1* inhibited PI3K pathway activity, as evidenced by the downregulation of phosphorylated PI3K, AKT, and mTOR. The inhibition could be restored by PI3K activator 740 Y-P.

LncTAF15:1-1 Regulated moDCs Migration and Their Attraction for CD4+ T Cells Through CCL5 Secretion by Activating the P13K/AKT/mTOR Pathway

Further rescue experiment was carried out when the pathway was disrupted with PI3K activator 740 Y-P and inhibitor LY294002 to demonstrate whether *lncTAF15:1–1* could potentially increase the expression of CCL5 through the PI3K/AKT/mTOR pathway. It was found that the mRNA expression levels and secretions of CCL5 were lowered upon the addition of the PI3K inhibitor compared to the adeno-OE group (Figure 4A and B). Conversely, the introduction of the pathway agonist restored the reduced levels of CCL5 induced by the downregulation of *lncTAF15:1–1* (Figure 4C and D). Similarly, regarding the migratory ability of moDCs, similar trends were observed. The augmented migration capacity of moDCs in the OE group was attenuated with the addition of the inhibitor, whereas the pathway activator could ameliorate the reduced migration capacity in the SS group (Figure 4E). Additionally, the impact of disrupting the PI3K/AKT/mTOR pathway on the ability of moDCs to attract $CD4^+$ T cells was assessed. The results demonstrated that inhibition of the pathway led to a decrease in the number of migratory $CD4^+$ T cells compared to the adeno-OE group. Conversely, induction of the PI3K activator 740 Y-P resulted in an increase in the attraction for $CD4^+$ T cells by moDC-derived supernatant (Figure 4F). These findings support the hypothesis that *lncTAF15:1–1* was involved in regulating CCL5 secretion and migration of moDCs via the PI3K/AKT/mTOR pathway. Understanding the subcellular localization of this lncRNA can further elucidate its functional roles within moDCs. The results of FISH revealed that *lncTAF15:1–1* was sub-localized in both the nucleus and cytoplasm (Figure 4G).

Discussion

This study is the first to reveal the critical role of *lncTAF15:1–1* in the pathogenesis of SLE. We found that *lncTAF15:1–1* activates the PI3K/AKT/mTOR pathway, promoting the secretion of CCL5 by moDCs, enhancing their migratory ability, and recruiting more CD4+ T cells to participate in the immunopathological process of SLE. This finding highlights the role of a novel lncRNA, expands our understanding of its specific function, and provides new insights into the pathogenesis of SLE.

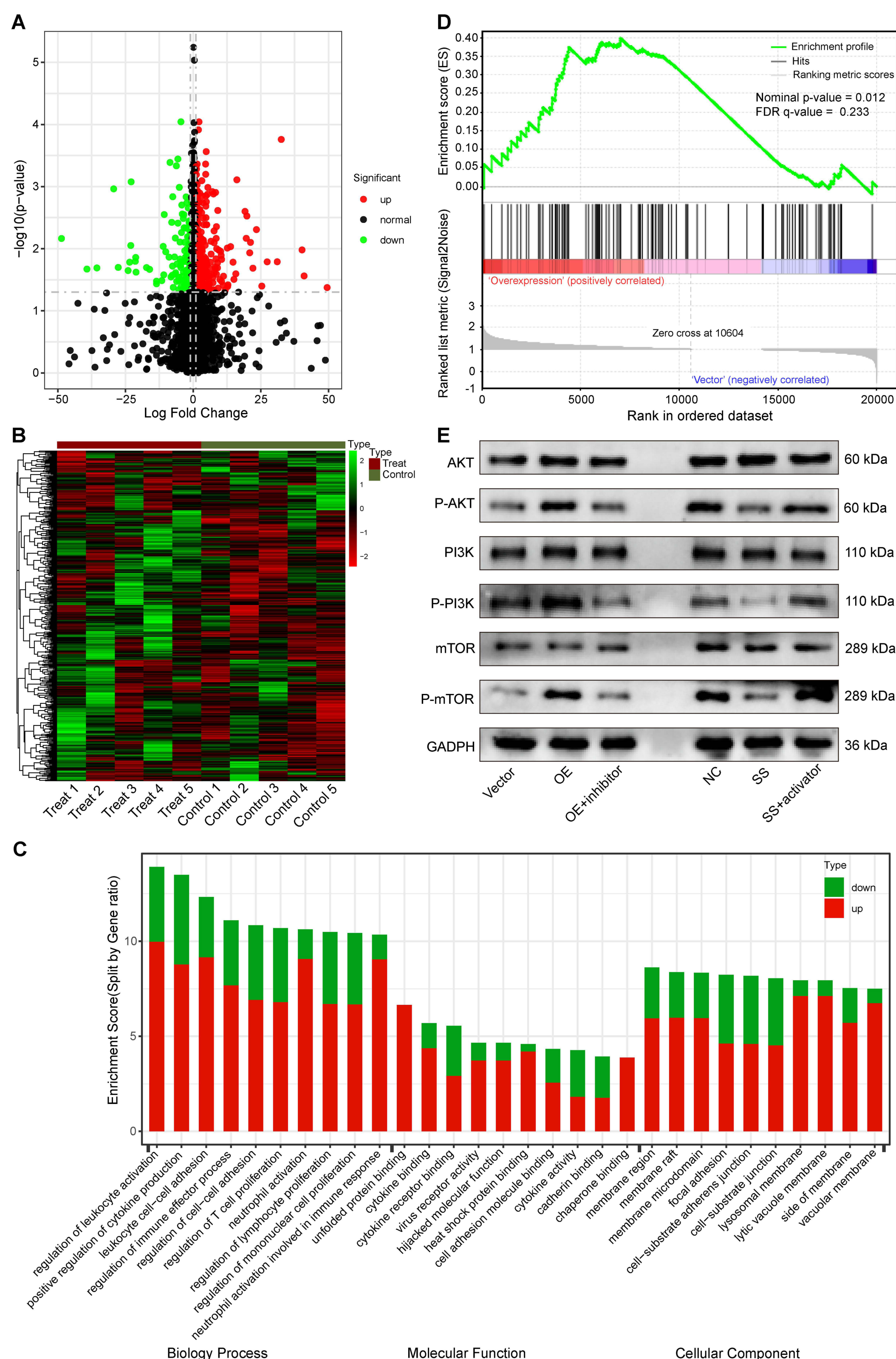


Figure 3 PI3K/AKT/mTOR pathway was pivotal in the immune modulation of *lncTAF15:1–1*. (**A** and **B**) Volcano plot and heatmap suggested the 488 differentially expressed genes between the vector (control) and *lncTAF15:1–1* overexpressed moDC groups (treat), including 140 down-regulated and 348 up-regulated genes. (**C**) GO function enrichment analysis indicated that these differentially expressed genes were related to different pathways. (**D**) GSEA plot showed that the PI3K/AKT/mTOR signaling pathway was activated in the *lncTAF15:1–1* overexpressed group (FDR q-value = 0.233, nominal p-value = 0.012). (**E**) PI3K inhibitor LY294002 and activator 740 Y-P were used to disrupt the PI3K/AKT/mTOR pathway and the levels of AKT, p-AKT, PI3K, p-PI3K, mTOR and p-mTOR were measured and quantified by Western Blotting.
Abbreviations: moDCs, monocyte-derived dendritic cells; OE, overexpression; NC, negative control; SS, Smart Silencer.

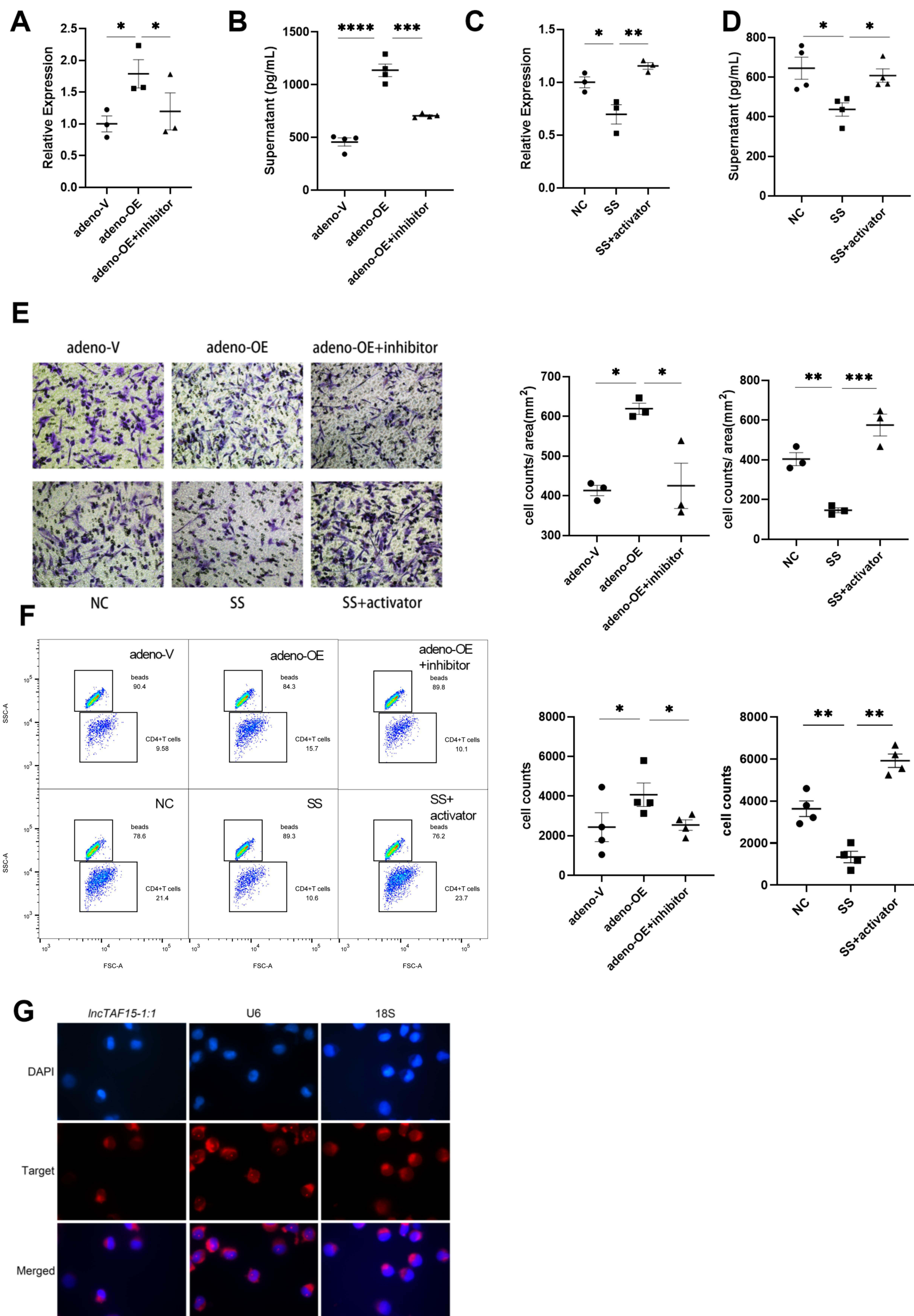


Figure 4 *LncTAF15:1–1* regulated the migration of moDCs and induced CD4+ T cell recruitment through CCL5 secretion by activating the P13K/AKT/mTOR pathway. (**A** and **B**) The PI3K/AKT/mTOR pathway inhibitor LY290042 was administered in conjunction with the adenovirus, followed by the assessment of mRNA expression and secretion levels of CCL5, in comparison with the OE group. (**C** and **D**) The PI3K/AKT/mTOR pathway activator 740 Y-P was added together with the Smart Silencer and then the mRNA expression and secretion of CCL5 were measured compared with the SS group. (**E**) Transwell assay was performed to compare the migratory capacity of moDCs when the PI3K/AKT/mTOR signaling pathway was disrupted. The number of migratory moDCs was counted and normalized to the area. (**F**) The capacity of supernatant from moDCs to attract CD4+ T cells was compared when the *lncTAF15:1–1* expression and the PI3K/AKT/mTOR pathway were regulated. The migratory CD4+T cell numbers were quantified by flow cytometry. (**G**) Intercellular sublocalization of *lncTAF15:1–1* was determined by FISH. 18s and U6 were considered as the positive control of cytoplasm and nucleus. One-way ANOVA or Kruskal–Wallis test was applied. Data were from at least three independent experiments and shown as the mean ± SEM. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Abbreviations: MoDCs, monocyte-derived dendritic cells; OE, overexpression; NC, negative control; SS, Smart Silencer.

Recent advances in non-coding RNAs (ncRNAs) have expanded our understanding of epigenetic regulation of both innate and adaptive immune responses and inflammatory diseases.[17] Non-coding RNAs are generally defined as a heterogeneous group of transcripts that cannot encode proteins, including long non-coding RNAs (lncRNAs) with at least 200 nucleotides in length,[18] microRNAs (miRNAs), and others. Accumulating evidence has highlighted the pivotal regulatory roles of ncRNAs in the loss of immune tolerance and tissue damage in individuals with SLE.[19] For instance, studies have found that *linc00513* is increased in PBMCs and renal tissues of SLE, which could positively regulate the type I IFN pathway by promoting the phosphorylation of STAT1 and STAT2.[20] Another lncRNA, *growth arrest-specific transcript 5* (*Gas5*), located on chromosome 1q25.1, has been identified as an SLE-susceptibility locus.[21] Functional studies have revealed that *Gas5* binds to the DNA-binding domain of glucocorticoid receptors and blocks its binding to glucocorticoid response elements, which could influence the metabolic state of cells and induce apoptosis, thus leading to antigen exposure and autoantibody production in SLE.[22] Therefore, identifications of abnormal expression patterns of lncRNAs in SLE patients would provide new insights into the diagnosis and therapeutic targets for SLE.

To date, this is the only article that elucidated the potential function of *lncTAF15:1–1* in autoimmune disease. This study confirmed the upregulation of *lncTAF15:1–1* in moDCs derived from SLE patients and identified its impact on the involvement of moDCs in SLE. *LncTAF15:1–1* was located on chromosome 17, which was 662bp in length. The study findings also revealed that *lncTAF15:1–1* functioned as an upstream modulator inducing the secretion of CCL5. In SLE, several studies have demonstrated that an increased level of CCL5 is one of the prominent immune characteristics in patients. Serum CCL5 levels show strong associations with clinical parameters in SLE patients, and persistently elevated urinary levels of CCL5 serve as predictors of renal flares.[23,24] Notably, in CCL5-deficient MRL/lpr lupus mice, lupus-related peribronchial pulmonary lesions were significantly relieved and a reduction in the size of lymph nodes was also observed.[25] The migratory cDCs exhibit heightened production of CCL5, which is crucial for recruiting and guiding CCR5-expressing monocytes to the draining lymph nodes.[26] Furthermore, CCL5 plays a significant role in promoting the migratory capacity of cDCs, thereby facilitating their accumulation within the tumor microenvironment. This accumulation is associated with the execution of anti-tumor functions by cDCs and correlates with favorable patient outcomes.[27] These findings collectively suggest that CCL5 is a pivotal chemokine in DC biology, particularly in the context of DC migration. Our study extends these findings to moDCs, revealing that increased secretion of CCL5 promotes moDC migration, which might represent a novel target for intervention in SLE. Additionally, we identified that the lncRNA *lncTAF15:1–1* regulated the secretion of CCL5, offering a more comprehensive understanding of the lncRNA/chemokine/moDCs axis. The maturation of activated DCs usually involves migration to a draining lymph node and the priming of T cells. In this process, the abundant chemokines secreted by matured DCs could increase the circulating numbers and recruitment of proinflammatory $CD4^+$T cells. For instance, in our previous study, we found that moDCs in patients with SLE in their active phase secreted higher levels of IL-6, CCL2, and CCL5, and the enforced expression of miRNA *miR-142-3p* in moDCs could affect moDC-$CD4^+$ T cell interaction through inhibiting the proliferation of $CD4^+CD25^+Foxp3^+$ Tregs.[28] The activated $CD4^+$ T cells would subsequently provide cognate help to self-reactive B cells, leading to the production of pathogenic autoantibodies and continuously amplified autoimmunity of SLE.[29]

Our study identifies *lncTAF15:1–1* as an upstream regulator that influences the migration and pro-inflammatory function of moDCs through the PI3K/AKT/mTOR pathway. This finding aligns with growing evidence that non-coding RNAs are key regulators in immune responses and may serve as therapeutic targets.[30] Targeting *lncTAF15:1–1* may thus offer a novel strategy for SLE treatment, adding translational relevance to our work. Additionally, the PI3K/AKT/mTOR pathway is a well-recognized inflammatory signaling axis involved in various autoimmune diseases, including SLE.[31,32] While current studies in SLE primarily focus on its role in disease pathogenesis, numerous small-molecule inhibitors targeting this pathway are already under development in fields such as oncology and neurodegenerative diseases.[33] Our findings further support the therapeutic potential of modulating this pathway in SLE, highlighting its promise as a target for future intervention.

Despite the novel findings of this study, several limitations should be acknowledged. First, the sample size used in this study was relatively small, which may limit the generalizability of the results. Future studies with larger cohorts, particularly involving SLE patients at different disease stages, are necessary to validate our findings. Second, the current experiments were primarily conducted in vitro, which may not fully recapitulate the complex microenvironment in vivo. Therefore, additional in vivo studies, such as using SLE mouse models, are essential to confirm the functional role of *lncTAF15:1–1* in disease pathogenesis. Third, while we identified the PI3K/AKT/mTOR pathway as a key downstream target of *lncTAF15:1–1*, the precise molecular mechanisms by which *lncTAF15:1–1* regulates this pathway remain unclear. Further investigations, including RNA-protein interaction assays and epigenetic analyses, are needed to elucidate the detailed regulatory mechanisms. Addressing these limitations in future research will not only strengthen the validity of our findings but also pave the way for developing *lncTAF15:1-1*-based diagnostic and therapeutic strategies for SLE.

Conclusion

Our study has revealed that the elevated levels of *lncTAF15:1–1* in SLE patients can enhance the CCL5 secretion of moDCs via PI3K/AKT/mTOR signaling pathway, and subsequently augment the migratory capabilities of moDCs with attraction for CD4+ T cells recruitment downstream. These findings can assist in understanding the pathophysiology of SLE along with offering novel insights into potential diagnosis and therapy strategies.

Abbreviations

SLE, Systemic lupus erythematosus; RANTES, regulated upon activation normal T cell expressed and secreted; DC, dendritic cell; mDC, myeloid DCs; pDC, plasmacytoid DCs; APCs, antigen-presenting cells; GC, germinal center; IFN-I, type I interferons; ncRNAs, non-coding RNAs; SLEDAI, SLE Disease Activity Index; tolDCs, tolerogenic dendritic cells; moDCs, monocyte-derived dendritic cells; ACR, American College of Rheumatology; PBMCs, peripheral blood mononuclear cells; LPS, lipopolysaccharide; ASO, antisense oligonucleotides; qRT-PCR, Quantitative real-time PCR; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; ACTB, beta-actin; FISH, Fluorescence In Situ Hybridization; lncRNAs, long non-coding RNAs; miRNAs, microRNAs; Gas5, growth arrest-specific transcript 5.

Data Sharing Statement

The datasets used and/or analyzed during the current study are available from the corresponding author Yilun Wang upon reasonable request.

Ethics Approval and Consent to Participate

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Huashan Hospital (HIRB-2021-879). Informed consent was obtained from all individual participants included in the study.

Funding

The research was supported by the National Natural Science Foundation of China (No. 82073443 and No.81703122), Shanghai Municipal Commission of Health and Family Planning (No. 2023ZZ02018), Shanghai Municipal Key Clinical Specialty (No. shslczdzk01002).

Disclosure

The authors declare that they have no competing interests.

References

1. Durcan L, O'Dwyer T, Petri M. Management strategies and future directions for systemic lupus erythematosus in adults. *Lancet*. 2019;393 (10188):2332–2343. doi:10.1016/S0140-6736(19)30237-5

2. Tian J, Zhang D, Yao X, Huang Y, Lu Q. Global epidemiology of systemic lupus erythematosus: a comprehensive systematic analysis and modelling study. *Ann Rheumatic Dis*. 2023;82(3):351–356. doi:10.1136/ard-2022-223035
3. Tanaka Y. Systemic lupus erythematosus. *Best Pract Res Clin Rheumatol*. 2022;36(4):101814. doi:10.1016/j.berh.2022.101814
4. Lou H, Ling GS, Cao X. Autoantibodies in systemic lupus erythematosus: from immunopathology to therapeutic target. *J Autoimmun*. 2022;132:102861. doi:10.1016/j.jaut.2022.102861
5. Xiao G, Zuo X. Epigenetics in systemic lupus erythematosus. *Biomed Rep*. 2016;4(2):135–139. doi:10.3892/br.2015.556
6. Liao X, Pirapakaran T, Luo XM. Chemokines and chemokine receptors in the development of lupus nephritis. *Mediators Inflamm*. 2016;2016:6012715. doi:10.1155/2016/6012715
7. Aringer M. Inflammatory markers in systemic lupus erythematosus. *J Autoimmun*. 2020;110:102374. doi:10.1016/j.jaut.2019.102374
8. Amo L, Kole HK, Scott B, et al. CCL17-producing cDC2s are essential in end-stage lupus nephritis and averted by a parasitic infection. *J Clin Invest*. 2021;131(11):e148000. doi:10.1172/JCI148000
9. Klarquist J, Zhou Z, Shen N, Janssen EM. Dendritic cells in systemic lupus erythematosus: from pathogenic players to therapeutic tools. *Mediators Inflammation*. 2016;2016:1–12. doi:10.1155/2016/5045248
10. Liu J, Zhang X, Cao X. Dendritic cells in systemic lupus erythematosus: from pathogenesis to therapeutic applications. *J Autoimmun*. 2022;132:102856. doi:10.1016/j.jaut.2022.102856
11. Sigdel KR, Cheng A, Wang Y, Duan L, Zhang Y. The emerging functions of long noncoding RNA in immune cells: autoimmune diseases. *J Immunol Res*. 2015;2015:848790. doi:10.1155/2015/848790
12. Cheng Q, Chen M, Chen X, et al. Novel long non-coding RNA expression profile of peripheral blood mononuclear cells reveals potential biomarkers and regulatory mechanisms in systemic lupus erythematosus. *Front Cell Dev Biol*. 2021;9:639321. doi:10.3389/fcell.2021.639321
13. Wu H. LncRNA expression profiles in systemic lupus erythematosus and rheumatoid arthritis: emerging biomarkers and therapeutic targets. *Front Immunol*. 2021;12:12. doi:10.3389/fimmu.2021.792884
14. Wang Y, Chen S, Chen S, et al. Long noncoding RNA expression profile and association with SLEDAI score in monocyte-derived dendritic cells from patients with systematic lupus erythematosus. *Arthritis Res Ther*. 2018;20(1):138. doi:10.1186/s13075-018-1640-x
15. Anders S, Huber W. Differential expression of RNA-Seq data at the gene level – the DESeq package. *Heidelberg Germany Eur Mol Biol Lab*. 2012;10:1000research.
16. Kanehisa M, Araki M, Goto S, et al. KEGG for linking genomes to life and the environment. *Nucleic Acids Res*. 2008;36(Database issue):D480–484. doi:10.1093/nar/gkm882
17. Chen YG, Satpathy AT, Chang HY. Gene regulation in the immune system by long noncoding RNAs. *Nat Immunol*. 2017;18(9):962–972. doi:10.1038/ni.3771
18. Esteller M. Non-coding RNAs in human disease. *Nat Rev Genet*. 2011;12(12):861–874. doi:10.1038/nrg3074
19. Shen Y, Qu B, Shen N. Expanding roles of noncoding RNAs in the pathogenesis of systemic lupus erythematosus. *Curr Rheumatol Rep*. 2022;24(3):64–75. doi:10.1007/s11926-022-01058-6
20. Xue Z, Cui C, Liao Z, et al. Identification of LncRNA Linc00513 containing lupus-associated genetic variants as a novel regulator of interferon signaling pathway. *Front Immunol*. 2018;9:2967. doi:10.3389/fimmu.2018.02967
21. Suarez-Gestal M, Calaza M, Endreffy E, et al. Replication of recently identified systemic lupus erythematosus genetic associations: a case-control study. *Arthritis Res Ther*. 2009;11(3):R69. doi:10.1186/ar2698
22. Kino T, Hurt DE, Ichijo T, Nader N, Chrousos GP. Noncoding RNA gas5 is a growth arrest- and starvation-associated repressor of the glucocorticoid receptor. *Sci Signal*. 2010;3(107):ra8. doi:10.1126/scisignal.2000568
23. Lu -M-M, Wang J, Pan H-F, et al. Increased serum RANTES in patients with systemic lupus erythematosus. *Rheumatol Int*. 2012;32(5):1231–1233. doi:10.1007/s00296-010-1761-2
24. Tian S, Li J, Wang L, et al. Urinary levels of RANTES and M-CSF are predictors of lupus nephritis flare. *Inflamm Res*. 2007;56(7):304–310. doi:10.1007/s00011-007-6147-x
25. Tsukahara T, Makino Y, Fujii T, et al. Role of RANTES in the development of autoimmune tissue injuries in MRL-Fas lpr mice. *Clin Immunol*. 2002;103(1):89–97. doi:10.1006/clim.2001.5198
26. Rawat K, Tewari A, Li X, et al. CCL5-producing migratory dendritic cells guide CCR5+ monocytes into the draining lymph nodes. *J Exp Med*. 2023;220(6):e20222129. doi:10.1084/jem.20222129
27. Böttcher JP, Bonavita E, Chakravarty P, et al. NK cells stimulate recruitment of cDC1 into the tumor microenvironment promoting cancer immune control. *Cell*. 2018;172(5):1022–1037.e14. doi:10.1016/j.cell.2018.01.004
28. Wang Y, Liang J, Qin H, et al. Elevated expression of miR-142-3p is related to the pro-inflammatory function of monocyte-derived dendritic cells in SLE. *Arthritis Res Ther*. 2016;18(1):263. doi:10.1186/s13075-016-1158-z
29. Rawlings DJ, Metzler G, Wray-Dutra M, Jackson SW. Altered B cell signalling in autoimmunity. *Nat Rev Immunol*. 2017;17(7):421–436. doi:10.1038/nri.2017.24
30. Yuan L, Xu Z-Y, Ruan S-M, et al. Long non-coding RNAs towards precision medicine in gastric cancer: early diagnosis, treatment, and drug resistance. *Mol Cancer*. 2020;19(1):96. doi:10.1186/s12943-020-01219-0
31. Zhao C, Gu Y, Chen L, Su X. Upregulation of FoxO3a expression through PI3K/Akt pathway attenuates the progression of lupus nephritis in MRL/lpr mice. *Int Immunopharmacol*. 2020;89(Pt A):107027. doi:10.1016/j.intimp.2020.107027
32. Stylianou K, Petrakis I, Mavroeidi V, et al. The PI3K/Akt/mTOR pathway is activated in murine lupus nephritis and downregulated by rapamycin. *Nephrol Dial Transplant*. 2011;26(2):498–508. doi:10.1093/ndt/gfq496
33. Huang J, Chen L, Wu J, et al. Targeting the PI3K/AKT/mTOR signaling pathway in the treatment of human diseases: current status, trends, and solutions. *J Med Chem*. 2022;65(24):16033–16061. doi:10.1021/acs.jmedchem.2c01070

Journal of Inflammation Research

Dovepress
Taylor & Francis Group

Publish your work in this journal

The Journal of Inflammation Research is an international, peer-reviewed open-access journal that welcomes laboratory and clinical findings on the molecular basis, cell biology and pharmacology of inflammation including original research, reviews, symposium reports, hypothesis formation and commentaries on: acute/chronic inflammation; mediators of inflammation; cellular processes; molecular mechanisms; pharmacology and novel anti-inflammatory drugs; clinical conditions involving inflammation. The manuscript management system is completely online and includes a very quick and fair peer-review system. Visit http://www.dovepress.com/testimonials.php to read real quotes from published authors.

Submit your manuscript here: https://www.dovepress.com/journal-of-inflammation-research-journal