• Clinical Research •

Association of interferon regulatory factor 8 dysregulation with dry eye in Sjögren's syndrome

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Abstract

● **AIM:** To investigate the expression of interferon regulatory factors (IRFs) in peripheral blood mononuclear cells (PBMCs) of patients with Sjögren's syndrome-related dry eye (SSDE) and to explore their correlation with clinical features, dendritic cell activation, and serological indicators.

● **METHODS:** A total of 53 SSDE patients and 62 non-Sjögren's syndrome dry eye (NSSDE) patients were enrolled. Demographic and clinical data were collected, and comprehensive ophthalmic examinations were performed, including the ocular surface disease index (OSDI) questionnaires, Schirmer I test (SIT), tear break-up time (TBUT), corneal fluorescein staining score (CFS), and *in vivo* confocal microscopy (IVCM). PBMCs were isolated, and IRFs expression levels were analyzed using Western blotting (WB) and quantitative real-time polymerase chain reaction (qRT-PCR). Serological indicators, including antinuclear antibodies (ANA) and anti-Ro60, anti-Ro52, and anti-La autoantibodies, were detected. Statistical analyses evaluated correlations between IRFs expression and clinical parameters.

● **RESULTS:** Compared to NSSDE, the relative mRNA and protein expression of the IRF-8 was significantly upregulated in patients with SSDE (P<0.001), whereas no significant differences were observed in IRF-1, IRF-3, IRF-5, and IRF-7 (P=0.12, P=0.10, P=0.66, P=0.96). Correlation analysis revealed that IRF-8 expression was positively associated with CFS and OSDI scores (r=0.57, r=0.38, both P<0.05). Moreover, IRF-8 expression correlated with corneal dendritic cell (DC) density and size, and the number of dendrites (r=0.43, r=0.40, r=0.65, all P<0.05). IRF-8 expression was significantly elevated in patients positive for anti-Ro60, anti-Ro52 and anti-La autoantibodies (P<0.05).

● **CONCLUSION:** In SSDE, IRF-8 is upregulated and associated with clinical features, DC activation, and serological indicators. These findings suggest that IRF-8 plays a critical role in SSDE pathogenesis and may serve as a potential therapeutic target for diagnosis and treatment.

● **KEYWORDS:** interferon regulatory factors; Sjögren's syndrome; dry eye

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INTRODUCTION

Sjögren's syndrome (SS) is a chronic autoimmune disorder characterized by mononuclear cell infiltration into exocrine glands, particularly the salivary and lacrimal glands, resulting in xerostomia and xerophthalmia[1]. The presence of autoantibodies, such as antinuclear antibodies (ANA), anti-Ro60, anti-Ro52, and anti-La autoantibodies, is a hallmark of SS and is routinely used as an auxiliary diagnostic criterion in clinical practice[2].

Dry eye, a prominent feature of SS, significantly affects patients' quality of life, manifesting as persistent dryness, discomfort, and visual disturbances that can progress to severe ocular complications, including corneal damage[3]. The pathophysiology of SS-related dry eye (SSDE) involves complex inflammatory and immune-mediated mechanisms, making it a critical area of research[4]. *In vivo* confocal microscopy (IVCM) is a valuable diagnostic tool for evaluating corneal epithelial dendritic cells (DCs) and provides critical insights into local inflammatory status[5]. Beyond conventional diagnostic examinations, IVCM has been widely applied in ocular surface research, including studies on dry eye, contact lens-related complications, keratitis, and corneal transplant rejection[6-9].

Interferon regulatory factors (IRFs) are a family of 10 structurally conserved transcription factors (IRF-1 to IRF-10) characterized by a helix-turn-helix DNA-binding domain[10]. These factors play crucial roles in regulating interferon (IFN)-mediated gene expression and orchestrating complex immune signaling networks[11]. IRFs are involved in diverse biological processes, including immune cell activation, inflammatory responses, cellular proliferation, and apoptosis, thereby bridging innate and adaptive immunity by interacting with pattern recognition receptors (PRRs)[12].

Functionally, IRF family members exhibit distinct regulatory mechanisms. For example, IRF-1, IRF-3, and IRF-7 act as potent transcriptional activators of *IFN* genes, modulating systemic immune responses, whereas IRF-5 and IRF-8 predominantly regulate pro-inflammatory cytokine expression and immune cell differentiation, thereby contributing to autoimmune pathogenesis[13-16]. Prior studies have identified IRF-8 locus variants as critical risk factors for autoimmune diseases, including inflammatory bowel disease, systemic lupus erythematosus (SLE), and rheumatoid arthritis (RA)[17-18]. Additionally, IRF-8 has been implicated in anti-Ro60 autoantibody production, which is highly relevant to SS[19]. Although the roles of IRFs have been extensively studied in other autoimmune diseases, their role in SSDE pathogenesis remains poorly understood. Understanding these expression patterns may provide novel insights into molecular mechanisms and facilitate the development of targeted diagnostic and therapeutic strategies. Therefore, the present study aimed to elucidate the correlation between IRF expression patterns and the systemic and local clinical manifestations of SSDE.

PARTICIPANTS AND METHODS

Ethical Approval This study was conducted in accordance with the principles of the Declaration of Helsinki and approved by the Ethics Committee of the First Affiliated Hospital of Chongqing Medical University (2023-153). Written informed consent was obtained from all participants.

General Information This study enrolled 115 patients from outpatient clinics at the First Affiliated Hospital of Chongqing Medical University between July 1, 2023, and July 1, 2024. Of these, 53 were diagnosed with SSDE, and 62 were diagnosed with non-SS-related dry eye (NSSDE). The diagnosis of SSDE was made according to the 2016 American College of Rheumatology-European League Against Rheumatism (ACR-EULAR) criteria and the 2017 Dry Eye Workshop (DEWS) II Diagnostic Methodology Report[20-21]. Diagnoses were confirmed by both ophthalmologists and rheumatologists. Patients in the NSSDE group had no diagnosis of SS or other autoimmune diseases but presenting with clinically significant dry eye symptoms. The exclusion criteria were as follows: 1) a history of ocular infection, surgery, or trauma within the past six months; 2) a diagnosis of other autoimmune diseases, such as RA, SLE or scleroderma; 3) a history of contact lens use, ocular surgeries, systemic conditions (including diabetes or pregnancy) or use of medications potentially affecting ocular surface homeostasis—specifically systemic corticosteroids or immunosuppressants within the past 3mo.

Patient Symptoms and Clinical Evaluations Demographic data, including age, sex, and medical history, were collected for all participants. Clinical assessments, including the Schirmer I test (SIT), tear break-up time (TBUT), and corneal fluorescein staining score (CFS), were rigorously administered by a single blinded examiner independent of the study grouping protocol. For the SIT, a tear-testing strip was placed in the lower conjunctival sac at the junction of the outer third of the lower eyelid. Participants were instructed to gently close their eyes for 5min, after which the wetted length of the strip was measured and recorded. For TBUT assessment, a fluorescein sodium strip was moistened and applied to the cornea. Participants were asked to blink normally. The time until the first break in the tear film was recorded under cobalt blue light using a slit lamp. The average of three measurements was calculated. CFS was assessed using the SICCA OSS[22] grading system, punctate epithelial erosions (PEEs) are counted and scored: absent=0; 1 to 5 PEEs=1; 6 to 30 PEEs=2, and more than 30 PEEs=3. An additional point is added if PEEs occur in the central 4-mm diameter of the cornea, if any filaments are seen on the cornea, or if any patches of confluent staining including linear stains are found anywhere on the cornea. The maximum possible score is 6. Additionally, participants completed the Ocular Surface Disease Index (OSDI) questionnaires to assess dry eye symptoms and clinical indicators.

***In Vivo* Confocal Microscopy** Corneal imaging was performed using a Heidelberg Retina Tomograph III (HRT-3) confocal microscopy system (Heidelberg Engineering GmbH, Heidelberg, Germany) following standardized protocols. Digital images were captured in sequential mode at a rate of 3 fps, generating a series of 100 images per sequence. Each image represented a corneal cross-section of 400 μm×400 μm (horizontal×vertical), with a 1-μm inter-image spacing. Three representative images of epithelial DCs (Figure 1A) from the right eye were selected for analysis at a depth of 50 to 70 μm. Image selection adhered to stringent quality control criteria, including optimal focus, absence of motion artifacts, and maximal contrast, to ensure reliable representation of a single corneal layer. The density and morphology of DCs were quantified using Image J software based on established methodologies. DCs density was determined using a cell-counting tool (Figure 1B), with manual verification of each

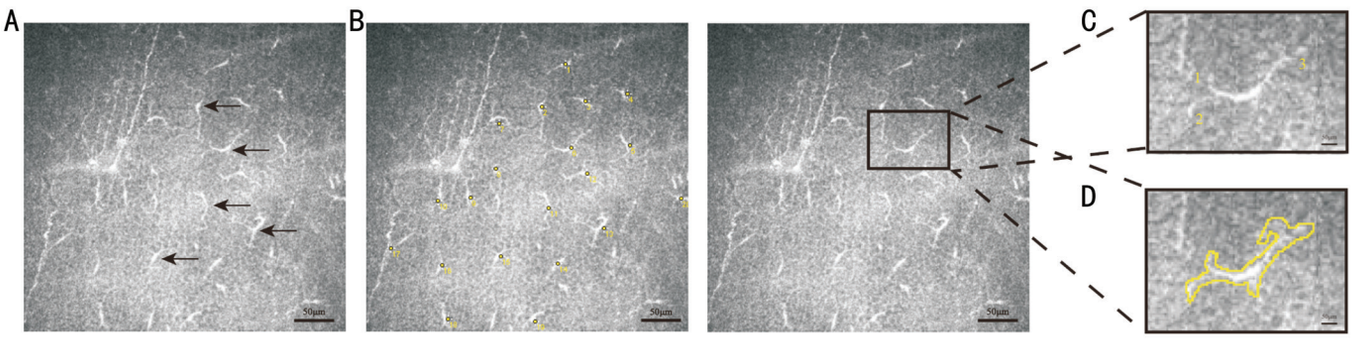

Figure 1 Analysis of dendritic cells (DCs) density and morphology A: Arrows indicate epithelial DCs; B: DC density was measured using the cell-counting tool; C: The number of dendrites per cell was calculated manually; D: DC size was measured.

identified cell. For morphological characterization, ten representative DCs per subject were analyzed according to the standardized protocol described by Kheirkhah *et al*[5]. Morphometric parameters included the number of dendrites (Figure 1C) and cell size (Figure 1D), providing quantitative measures of DC activation status. Statistical analyses were conducted independently by two researchers, and the results were averaged.

Measurement of Serological Indicators Serum samples were collected and processed according to standardized clinical protocols. Comprehensive autoantibodies profiling was conducted in a certified clinical pathology laboratory using validated immunoassays. The analysis included quantitative measurements of ANA, anti-Ro60, anti-Ro52, and anti-La autoantibodies. All assays adhered to strict quality control and standardized calibration procedures to ensure analytical accuracy and reproducibility.

Peripheral Blood Mononuclear Cells Isolation and Preservation Peripheral blood (10 mL) was collected from each participant in heparinized Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ, USA) to prevent coagulation within 2h. Peripheral blood mononuclear cells (PBMCs) were isolated using the Ficoll-Hypaque method according to the manufacturer's protocol. To preserve cellular viability and functionality, the isolated PBMCs samples were stored in liquid nitrogen until further analysis.

Western Blot Analysis Protein extraction from PBMCs was performed using RIPA lysis buffer (P0013B, Beyotime, China) supplemented with protease inhibitors. Cells were lysed in 1 mL of ice-cold RIPA buffer for 30min with periodic vortexing. The lysates were centrifuged at 13 000 rpm for 15min at 4℃ to remove cellular debris, and protein concentration was quantified using a BCA protein assay kit (P0012S, Beyotime, China). According to standard protocols, 10 μg of total protein was loaded onto 4%-20% SDS-PAGE gels for separation and subsequently transferred to a polyvinylidene difluoride (PVDF) membrane. After blocking with 5% non-fat milk in Tris-buffered saline with Tween 20 (TBST) for 1h, the membranes were incubated overnight at 4℃ with primary antibodies against IRF-8 (18977-1-AP, Proteintech, China) and GAPDH (60004-1-Ig, Proteintech, China). The membranes were subsequently washed and incubated with secondary antibody (SA00001-2, Proteintech, China) for 1h at room temperature. The protein blots were visualized with an enhanced chemiluminescence (P0018FS, Beyotime, China) and visualized with a digital chemiluminescence detection system. Band intensity was quantified using Image J software (NIH, Bethesda, MD, USA) and normalized to GAPDH expression levels.

RNA Isolation and Quantitative Real-time PCR Total RNA was extracted from PBMCs using TRIzol reagent (AG21017, Accurate Biology, China) according to the manufacturer's instructions and primers were listed in Table 1. RNA concentration and purity were assessed using a spectrophotometer (NP80-Touch, Agilent Technologie, USA). Samples with A260/A280 ratios between 1.8 and 2.1 and A260/A230 ratios greater than 2.0 were selected for further analysis. The extracted RNA was reverse transcribed into cDNA using the Evo M-MLV RT Kit (AG11728, Accurate Biology, China). A 10 μL reaction mixture containing cDNA, primers, and SYBR Green (AG11701, Accurate Biology, China) was prepared for quantitative real-time polymerase chain reaction (qRT-PCR) amplification, which was conducted on an Applied Biosystems 7500 Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA, USA). The amplification protocol included an initial denaturation at 95℃ for 10min, followed by 40 cycles of 95℃ for 15s and 60℃ for 1min. Melting curve analysis was performed to verify amplification specificity. Gene expression levels of IRF-1, IRF-3, IRF-5, IRF-7, and IRF-8 in chondrocytes were normalized to GAPDH and quantified using $2^{-\Delta\Delta CT}$ method[15,23-25]. All reactions were performed in triplicate.

Statistical Analysis Statistical analyses were conducted using GraphPad Prism 9.5.1 software. The normality of continuous variables was assessed using the Shapiro-Wilk test. Parametric data were expressed as mean±standard deviation (SD), while non-parametric data were expressed as the median with

interquartile range [Md (P25, P75)]. Categorical data were presented as rates [*n* (%)]. Group differences for normally distributed data were evaluated using the unpaired *t*-test, whereas the Mann-Whitney *U* test was used for non-normally distributed data. Categorical variables were analyzed using Pearson's Chi-squared test. Spearman's correlation coefficients were calculated to assess the relationships between the variables. All tests were two-tailed, and statistical significance was defined as $P<0.05$. Reported values were rounded to two decimal places.

RESULTS

Patient Clinical Data This study enrolled 115 participants, comprising 53 patients diagnosed with SSDE and 62 with NSSDE. There were no significant differences in age or sex between the two groups. Detailed demographic characteristics were presented in Table 2.

As shown in Table 2, comparative analysis revealed significant differences in clinical parameters between the SSDE and NSSDE groups. Patients with SSDE exhibited higher CFS scores, shorter TBUT, and significantly reduced SIT. These findings collectively indicate comparable severity of dry eye symptoms between the two groups. Although the SSDE group showed a trend toward higher OSDI scores, this difference was not statistically significant.

Regarding serological characteristics, among the 53 SSDE patients, the positivity rates for autoantibodies were as follows: 45 (84.91%) were ANA-positive, 39 (73.58%) were positive for anti-Ro60 antibodies, 30 (56.60%) for anti-Ro52 antibodies, and 35 (66.04%) for anti-La antibodies.

IVCM Analysis of Dendritic Cells As detailed in Table 3, IVCM analysis revealed significant differences in DC density and morphological characteristics between the two groups. The SSDE group exhibited markedly higher DCs density than the NSSDE group. Morphologically, DCs in the SSDE group were significantly larger and exhibited a greater median number of dendrites per cell. These pronounced morphological and quantitative alterations in corneal epithelial DCs indicate a state of cellular activation, reflecting the severity of corneal immune response and inflammation in SSDE patients.

Increased IRF-8 in PBMCs of SSDE To evaluate the expression of IRFs in PBMCs from patients with SSDE, we quantified IRFs levels using WB and qRT-PCR. As illustrated in Figure 2, there were no significant differences in the mRNA expression levels of IRF-1 ($P=0.12$), IRF-3 ($P=0.10$), IRF-5 ($P=0.66$), or IRF-7 ($P=0.96$) between the SSDE and NSSDE groups. However, the mRNA expression level of IRF-8 was significantly higher in the SSDE group than in the NSSDE group ($P<0.001$). Additionally, Western blot analysis confirmed an increase in the protein expression of IRF-8 in the SSDE group ($P<0.001$), consistent with the mRNA expression results. These findings suggest that IRF-8 may play a specific role in SSDE pathogenesis by contributing to immune cell activation and inflammation.

Table 1 Primers used in this study

Gene	Primer sequence
IRF-1	Forward: 5′-TGCCTCCTGGGAAGATGA-3′
	Reverse: 5′-CCTGGGATTGGTGTTATGC-3′
IRF-3	Forward: 5′-GCACAGCAGGAGGATTTCG-3′
	Reverse: 5′-AGCCGCTTCAGTGGGTTC-3′
IRF-5	Forward: 5′-GGGCTCATCCTCCAGCTAC-3′
	Reverse: 5′-ACAAGGCCCGCTCCAGAA-3′
IRF-7	Forward: 5′-TGCAAGGTGTACTGGGAG-3′
	Revers: 5′-TAACTTCTGCTCCAGCTCCATAG-3′
IRF-8	Forward: 5′-TGGCTGATCGAGCAGATTGACAGT-3′
	Reverse: 5′-AAGGGATCCGGAACATGCTCTTCT -3′
GAPDH	Forward: 5′-AGAAGGCTGGG-GCTCATTTG-3′
	Reverse: 5′-AGGGGCCATCCACACCTTC-3′

Table 2 Demographic and clinical data for SSDE and NSSDE patients

Parameters	SSDE (*n*=53)	NSSDE (*n*=62)	*P*
Number, *n*	53	62	-
Age, y	47.17±13.81	46.11±11.44	0.66
Sex, female (%)	45 (84.91)	52 (83.87)	0.88
Dry eye signs and symptoms			
CFS	4.00 (3.00, 5.00)	2.00 (1.00, 3.00)	<0.05
TBUT, s	3.00 (2.00, 5.00)	5.00 (3.00, 7.00)	<0.05
SIT, mm	4.00 (2.00, 5.00)	6.00 (5.00, 8.00)	<0.05
OSDI	45.75±13.99	43.27±6.95	0.22
Serological indicators, *n* (%)			
ANA	45 (84.91)	-	-
Anti-Ro60	39 (73.58)	-	-
Anti-Ro52	30 (56.60)	-	-
Anti-La	35 (66.04)	-	-

SSDE: Sjögren's syndrome related dry eye; NSSDE: Non-Sjögren's syndrome related dry eye; CFS: Corneal fluorescein staining; TBUT: Tear break-up time; SIT: Schirmer I test; OSDI: Ocular Surface Disease Index; ANA: Antinuclear antibodies; Anti-Ro52: Anti-Ro52 antibody; Anti-Ro60: Anti-Ro60 antibody; Anti-La: Anti-La antibody.

Table 3 IVCM analysis results for SSDE and NSSDE groups

Parameters	SSDE (*n*=53)	NSSDE (*n*=62)	*P*
DC density, cells/mm^2	297.60±37.20	111.20±17.73	<0.05
DC size, μm^2	190.60±46.42	125.20±23.09	<0.05
DC dendrites, number/cell	4.00 (3.00, 5.00)	4.00 (1.00, 5.00)	<0.05

SSDE: Sjögren's syndrome related dry eye; NSSDE: Non-Sjögren's syndrome related dry eye; DC: Dendritic cell.

Correlation Between IRF-8 Expression and Dry Eye Symptoms and Signs in SSDE Patients To explore the role of IRF-8 expression in ocular surface pathology, we analyzed its relationship with clinical dry eye parameters in SSDE patients. As shown in Figure 3, IRF-8 expression positively correlated with CFS and OSDI scores (all $P<0.05$). However, no significant correlation was found between IRF-8 expression and ST or TBUT. These findings suggest that elevated IRF-8 expression is specifically associated with increased ocular surface damage and symptom severity in SSDE.

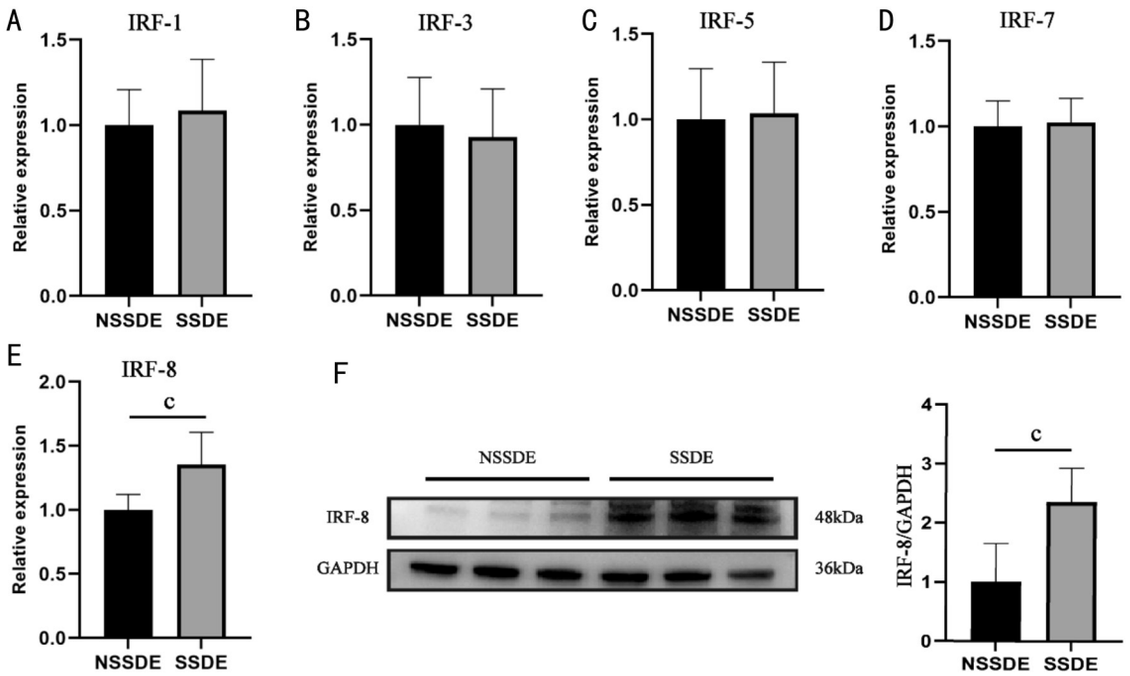

Figure 2 Differential expressions of IRFs in SSDE (*n*=53) and NSSDE (*n*=62) A: mRNA expression levels of IRF-1 in SSDE and NSSDE; B: mRNA expression levels of IRF-3 in SSDE and NSSDE; C: mRNA expression levels of IRF-5 in SSDE and NSSDE; D: mRNA expression levels of IRF-7 in SSDE and NSSDE; E: mRNA expression levels of IRF-8 in SSDE and NSSDE; F: Expression of IRF-8 protein in SSDE and NSSDE was detected by Western blotting. *n*=9, ^{c}P<0.001. SSDE: Sjögren's syndrome related dry eye; NSSDE: Non-Sjögren's syndrome related dry eye; IRF: Interferon regulatory factors.

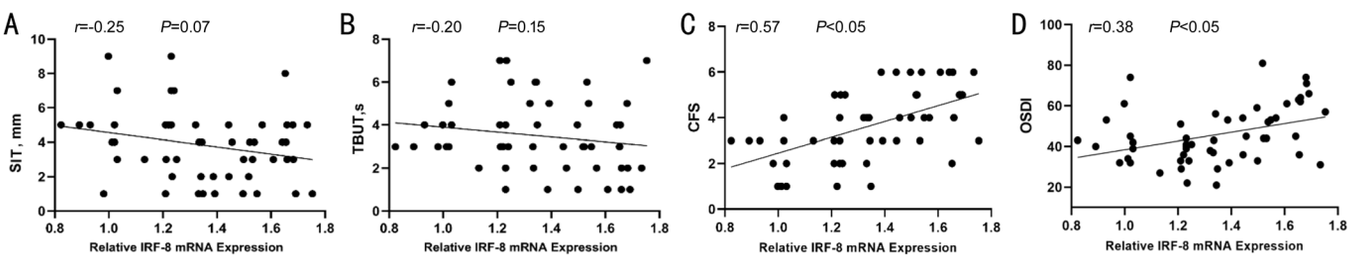

Figure 3 Correlation analysis of IRF-8 expression with clinical dry eye indicators in SSDE patients A: In SSDE, SIT is negatively correlated with the expression IRF-8, but the difference is not statistically significant; B: In SSDE, TBUT is negatively correlated with the expression IRF-8, but the difference is not statistically significant; C: In SSDE, CFS is positive correlated with the expression of IRF-8; D: In SSDE, OSDI is positive correlated with the expression of IRF-8. CFS: Corneal fluorescein staining; TBUT: Tear breakup time; SIT: Schirmer I test; OSDI: Ocular Surface Disease Index; SSDE: Sjögren's syndrome related dry eye; IRF: Interferon regulatory factors.

Correlation Between IRF-8 Expression and DC Density and Morphology in SSDE Patients To evaluate the relationship between IRF-8 expression and corneal DC activation, we conducted a correlation analysis between IRF-8 expression and DC parameters assessed through IVCM. The analysis demonstrated significant positive correlations between IRF-8 expression and DC density, the number of dendrites, and DC size (all P<0.05). These findings indicate that elevated IRF-8 expression is closely associated with increased DC density and morphological changes indicative of DC activation. This further supports the role of IRF-8 in modulating corneal immune responses in SSDE patients (Figure 4).

Correlation Between IRF-8 Expression and Serological Indicators in SSDE Patients To further evaluate the clinical relevance of elevated IRF-8 expression, we analyzed its association with key serological markers, including ANA, anti-Ro60, anti-Ro52, and anti-La autoantibodies. As shown in Figure 5, IRF-8 expression was significantly higher in patients who were positive for anti-Ro60, anti-Ro52, and anti-La autoantibodies compared to their negative counterparts. In contrast, no statistically significant differences in IRF-8 expression were observed in relation to ANA status. These findings suggest that IRF-8 upregulation may be specifically associated with the presence of anti-Ro and anti-La autoantibodies, underscoring its potential role in the autoimmune pathogenesis of SSDE.

DISCUSSION

Over the past few decades, dry eye has gained increasing

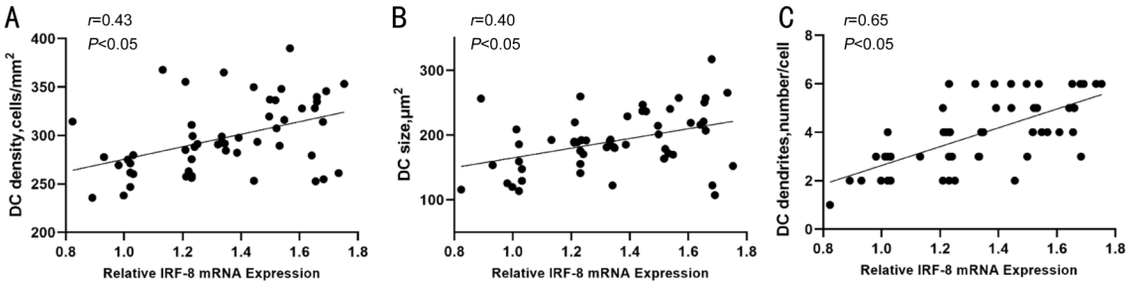

Figure 4 Correlation analysis of IRF-8 expression with DC density and morphology in SSDE patients A: In SSDE, DC density was positive correlated with the expression IRF-8; B: In SSDE, there was a positive correlation between DC size and IRF-8 expression; C: In SSDE, DC dendrites were positive correlated with the expression of IRF-8. DC: Dendritic cell; SSDE: Sjögren's syndrome related dry eye; IRF: Interferon regulatory factors.

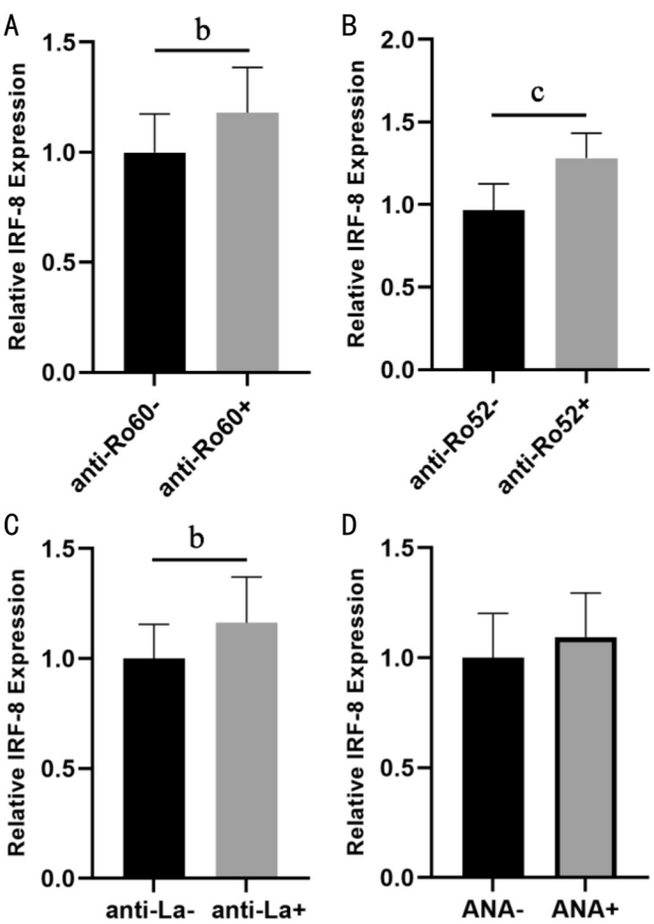

Figure 5 The expression of IRF-8 among different serological indicators A: mRNA expression levels of IRF8 in anti-Ro60$^-$ (n=14) and anti-Ro60$^+$ (n=39); B: mRNA expression levels of IRF8 in anti-Ro52$^-$ (n=23) and anti-Ro52$^+$ (n=30); C: mRNA expression levels of IRF8 in anti-La$^-$ (n=18) and anti-La$^+$ (n=35); D: mRNA expression levels of IRF8 in ANA$^-$ (n=8) and ANA$^+$ (n=45). ANA: Antinuclear antibodies; Anti-Ro52: Anti-Ro52 antibody; Anti-Ro60: Anti-Ro60 antibody; Anti-La: Anti-La antibody; IRF: Interferon regulatory factors. ^bP<0.01, ^cP<0.001.

attention as a common ocular disorder[3]. Dry eye not only affects the quality of life of patients but can also lead to severe ocular conditions[26]. Although numerous studies have explored the various mechanisms of dry eye, research on the differences between different types of dry eye (such as SSDE and NSSDE) remains limited, which poses a challenge to the development of targeted therapies.

Existing research suggests that SSDE patients exhibit significant differences in clinical serum markers and corneal DC characteristics, and the IRFs may play a key role in the pathological mechanisms of SSDE[15]. Through quantitative analysis of IRF-8 expression, this study is the first to confirm its importance in SSDE patients, suggesting that IRF-8 could become a new therapeutic target for dry eye.

Previous studies have revealed significant differences in the pathogenesis of SSDE compared to NSSDE, particularly at the molecular mechanisms and signaling pathways level. Our findings indicate that IRF-8 expression is significantly elevated in SSDE patients and positively correlates with the severity of certain clinical symptoms. This discovery not only provides a new understanding of the pathogenesis of SSDE but also highlights the potential value of IRF-8 as a biomarker and therapeutic target. Similar findings have been reported in myasthenia gravis (MG), where IRF-8 expression in B cells was positively correlated with disease severity[27]. Additionally, our IVCM analysis showed that DC density in SSDE patients was significantly higher than that in NSSDE patients and displayed evident morphological changes. We also observed a positive correlation between IRF-8 expression and changes in DC density and morphology. The role of DCs in immune responses cannot be underestimated, as they are not only antigen-presenting cells but also play a critical role in regulating T-cell activation and differentiation[28-29]. This suggests that IRF-8 plays an important role in regulating immune cell activation, and may influence the pathological process of SSDE by modulating cellular signaling pathways. Future studies can further explore how IRF-8 regulates SSDC function to lead to the activation of corneal immune cells and induce dry eye, offering new insights for intervention and treatment strategies in SSDE.

Finally, we found that in SSDE patients, the expression of IRF-8 in the anti-Ro60$^+$, anti-Ro52$^+$, and anti-La$^+$ groups was significantly higher than in the corresponding antibody-

negative groups, while no significant difference was observed in ANA levels. Anti-Ro and anti-La antibodies are hallmark antibodies of SS and SLE, and their production is closely associated with B cell hyperactivation and abnormalities in the IFN signaling pathway. IRF-8, a member of the interferon regulatory factor family, has been previously suggested to promote plasma cell differentiation and autoantibody secretion[30]. This study further supports the idea that IRF-8 may directly participate in the production of anti-Ro/La antibodies by regulating B cell function. ANA is a nonspecific marker for multiple autoimmune diseases, and the immune response mechanisms targeting its antigens (*e.g.*, double-stranded DNA, histones) may be independent of the IRF-8-dependent B cell pathway. This finding suggests that the upregulation of IRF-8 may specifically drive the autoimmune response associated with SS, rather than a broad-spectrum production of autoantibodies. Therefore, inhibitors targeting IRF-8 or its downstream pathways (*e.g.*, IFN-α/β signaling, such as JAK inhibitors) could represent a precision treatment strategy for SSDE[31].

The limitations of this study mainly lie in the relatively small sample size, which may affect the generalizability and reliability of the results. Furthermore, due to the lack of broader clinical validation, the external validity of the findings requires further confirmation. The diversity within the dataset may also lead to batch effects, influencing the interpretation and applicability of the results. These limitations suggest that future research should consider multi-center studies with larger sample sizes and integrate clinical and experimental data for comprehensive analysis to further validate and expand the findings of this study.

In conclusion, this study reveals that the upregulation of IRF-8 in SSDE patients is closely associated with the enhancement of clinical symptoms and immune responses. This discovery provides a new perspective for understanding the pathological mechanisms of SSDE and lays the foundation for potential therapeutic targets. Future research should further explore the function of IRF-8 and its potential applications in the treatment of SSDE, with the hope of providing more effective intervention strategies for SSDE and improving their quality of life.

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Authors' Contributions: Wang J, Chu GH and Qing QP acquired the data; Wang J and Chu GH wrote the manuscript; Wang ZH, Cai XY and Shi SY analyzed the data; Wang J and Zhang Q conceived the research; Zhang Q supervised the research and revised the manuscript.

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