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KLF2 controls the apoptosis of neutrophils and is associated with disease activity of systemic lupus erythematosus

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Abstract

Background Neutropenia is more common in patients with systemic lupus erythematosus (SLE) and is a major cause of life-threatening infections. The increased apoptosis of neutrophils is likely to be an essential cause of neutropenia in SLE. However, the detailed mechanisms of increased neutrophil apoptosis in SLE remain unknown. This study focused on the role of Krüppel-like factor 2 (KLF2) in the regulation of neutrophil apoptosis and its association with SLE disease activity.

Methods The levels of KLF2 in neutrophils from SLE patients and healthy controls (HCs) were detected by RT-PCR and western blot. The relationship between the levels of KLF2 and the apoptosis levels of neutrophils in SLE patients was analyzed. The KLF2 inhibitor Geranylgeranyl pyrophosphate (GGPP) and the KLF2 inducer geranylgeranyl transferase inhibitor (GGTI-298) were used to incubate with neutrophils to investigate the role of KLF2 in the regulation of neutrophil apoptosis. To clarify whether serum from SLE patients affects neutrophil KLF2 expression and apoptosis, sera from SLE patients were collected and used to incubate with neutrophils from HCs, followed by the detection of KLF2 levels and apoptosis levels of neutrophils. Additionally, the correlation between KLF2 levels and SLE disease activity index (SLEDAI) was analyzed.

Results The expression of KLF2 in neutrophils of SLE patients was significantly suppressed, and the decreased KLF2 was associated with the upregulation of neutrophil apoptosis. Moreover, newly diagnosed SLE patients, SLE patients with higher serum IgG and positive anti-Smith antibodies had lower KLF2 expression. Furthermore, we demonstrated that modulating the expression of KLF2 can regulate the apoptosis of neutrophils. The levels of KLF2 in neutrophils were associated with the SLEDAI. In addition, we found that serum from SLE patients could induce apoptosis in neutrophils by down-regulating the expression of KLF2.

Conclusions KLF2 controls the apoptosis of neutrophils and is associated with SLEDAI, which suggests that KLF2 in neutrophils may be involved in the occurrence and development of SLE.

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Keywords Systemic lupus erythematosus, Krüppel-like factor 2, Neutrophil, Apoptosis, SLE disease activity index

Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterized by a waxing and waning course, a loss of self-tolerance, production of autoantibodies, B-cell hyperreactivity, aberrant formation of immune complexes, enhanced type I interferon signaling, accumulated cellular debris from various forms of cell death and multiorgan impairment [1–3]. Increased neutrophil death and subsequent ineffective debris clearance in SLE patients contribute to the release of stable autoantigens, amplified inflammatory responses, and promoted tissue damage [4]. Neutropenia and lymphopenia are common features of SLE [5]. However, the detailed molecular mechanisms of increased neutrophil death in SLE have not been fully elucidated.

As the first line of defense against infectious pathogens, mature neutrophils are considered terminally differentiated cells that have a short lifespan and usually die by apoptosis [6]. Neutrophils are the most abundant immune cells and can modulate immune responses by synthesizing many important proinflammatory cytokines and chemokines [7, 8]. Neutrophils from patients with SLE exhibit increased apoptosis, altered phagocytosis, disordered oxidative metabolism, and accelerated cell death [9–11].

KLF2, a zinc-finger transcription factor (KLF) that belongs to the Krüppel-like family, was found to be initially enriched in the lungs and contributed to the maintenance of quiescence in many cell types, including macrophages, monocytes, and T cells [12, 13]. KLF2 was found to decrease the pro-inflammatory activity of nuclear factor kappa B (NF-κB) and negatively regulate inflammation [13, 14]. Induced KLF2 deficiency was reported to lead to T cell apoptosis in spleen and lymph nodes [15]. In addition, KLF2 can regulate neutrophil development and inflammatory activity in atherosclerosis [16]. A recent genome-wide association study identified *klf2* as a novel Asian-specific locus for susceptibility to SLE, and lower KLF2 expression may be associated with an increased risk of SLE [17, 18]. However, the correlation between KLF2 expression in neutrophils from SLE patients and disease activity and cell apoptosis has not been addressed. In this study, we aimed to characterize the expression of KLF2 in neutrophils of SLE patients and investigate the correlation between KLF2 levels and the disease activity of SLE.

Materials and methods

Patients

A total of 68 patients who fulfilled the 1997 revised American College of Rheumatology classification criteria for the SLE [19] were recruited from the First Affiliated Hospital of Nanchang University from 2020.1 to 2024.6. Disease activity was assessed by the SLEDAI-2000 index [20]. Individuals with other autoimmune or rheumatic diseases, kidney diseases other than lupus nephritis, pregnancy, HIV, or malignancy were excluded from this study. 29 rheumatoid arthritis (RA) patients, and 26 Sjogren's syndrome (SS) patients were recruited as disease controls, and 30 age- and sex-matched healthy subjects who were free of autoimmune diseases were recruited as healthy controls (HCs). Characteristics of SLE patients and controls enrolled in this study are included in Table 1. Peripheral neutrophil counts of each subject were determined. The study had approval from the Ethics Committee of the First Affiliated Hospital of Nanchang University (2014003) and complied with the Helsinki Declaration.

Neutrophil preparation and culture

Whole blood was collected in EDTA vacutainer tubes. According to the manufacturer's instructions, neutrophils were isolated from whole blood by Ficoll density gradient centrifugation. The purity of the isolated neutrophil was confirmed to be greater than 95% by flow cytometry. After isolation, neutrophils were cultured in RPMI 1640 medium (Solarbio, Beijing, China), supplemented with 10% fetal bovine serum (FBS, Gibco, Australia), at 37℃ in a humidified atmosphere of 5% CO_2 and 95% air. When indicated, neutrophils were incubated with 10% fresh human serum from SLE patients or HCs, 10 μM of KLF2 inducer GGTI-298 (MedChemExpress, NJ, USA, HY-15871) or KLF2 inhibitor GGPP (Sigma Aldrich, St. Louis, MO, USA, G6025) for 4 h.

Apoptosis analysis by flow cytometry

The neutrophils were incubated in a solution containing Annexin V-FITC and PI (Bestbio, Nanjing, China, BB-4101-50T) according to the manufacturer's instructions. The apoptosis of cells was analyzed by flow cytometry using a Beckman Coulter Cytomics FC 500 Flow Cytometer. The data was analyzed using the Flow Jo software.

Western blot analysis

Cells were washed twice with cold PBS, lysed with lysis buffer (Solarbio, China, BC3710), and centrifuged at 4 ℃ 12,000 g for 30 min. The supernatant was pipetted into a new E.P. tube, and the protein concentration was measured using the BCA Protein Concentration Assay Kit (Sosarbio, Beijing, China, Solarbio, China, PC0020) according to the manufacturer's instructions. An equal amount of protein (20 μg) for each sample and protein

Table 1 Clinical and laboratory characteristics of patients with SLE and controls

Categories	SLE (68)	Controls (85)	*P* value
Females/males, n (%)	61/7 (89.71)	78/7 (91.76)	0.66
Age, mean (SD), years	37.32 ± 12.33	39.51 ± 10.05	0.23
SLEDAI score, mean (SD.)	9.4 ± 7	-	-
new-onset SLE, n (%)	34 (50)	-	-
ANA, ≥ 1:320/ ≤1:100 (%)	41/8 (83.67)	-	-
Anti-dsDNA, P n/N n (%)	24/34 (41.38)	-	-
Anti-nRNP/Sm, P n/N n (%)	31/18 (63.27)	-	-
Anti-Sm, P n/N n (%)	17/23 (42.5)	-	-
Anti-RIB-P, P n/N n (%)	14/19 (42.42)	-	-
ANUA, P n/N n (%)	24/16 (60)	-	-
Anti-SSA, P n/N n (%)	36/13 (73.47)	-	-
Anti-SS-A52, P n/N n (%)	25/14 (64.1)	-	-
Anti-SSB, P n/N n (%)	14/20 (41.18)	-	-
C3, mean (SD.)	0.6 ± 0.22	-	-
C4, mean (SD.)	0.13 ± 0.07	-	-
IgG, mean (SD.)	19.41 ± 11.79	-	-
ESR, mean (SD.)	47.4 ± 36.13	-	-
CRP, mean (SD.)	10.5 ± 23.23	-	-
WBC, mean (SD.)	4.46 ± 1.69	5.78 ± 1.12	< 0.0001
RBC, mean (SD.)	3.79 ± 0.8	4.37 ± 0.3	< 0.0001
HGB, mean (SD.)	108.84 ± 23.19	132.73 ± 9.81	< 0.0001
HCT, mean (SD.)	0.33 ± 0.07	0.41 ± 0.27	< 0.0001
PLT, mean (SD.)	189.22 ± 89.77	235.95 ± 60.05	< 0.001
L, mean (SD.)	1.23 ± 0.59	1.81 ± 0.6	< 0.0001
L%, mean (SD.)	26.60 ± 9.22	31.14 ± 8.03	0.001
M, mean (SD.)	0.41 ± 0.25	0.73 ± 3.32	0.002
M%, mean (SD.)	9.32 ± 4.2	6.41 ± 1.59	0.429
N, mean (SD.)	2.79 ± 1.27	3.49 ± 0.8	< 0.0001
N%, mean (SD.)	61.72 ± 11.28	59.87 ± 10.05	0.287

Abbreviations: SLEDAI, SLE disease activity index; ANA, antinuclear antibody; Anti-dsDNA, anti-double stranded DNA antibody; Anti-nRNP/Sm, anti-nuclear ribonucleoprotein/anti-smith; Anti-RIB-P, anti-ribosomal P protein antibody; ANUA, anti-nucleosome antibody; Anti-SSA, anti-Sjgren syndrome A antibody; Anti-SS-A52, anti-Sjgren syndrome A 52 kDa antibody; Anti-SSB, anti-Sjgren syndrome B antibody; C3, Complement 3; C4, Complement 4; IgG, immunoglobulin G; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; WBC, white blood cell; RBC, red blood cell; HGB, hemoglobin; HCT, hematocrit; PLT, platelet; L, lymphocyte count; L%, lymphocyte percentage; M, monocyte count; M%, monocyte percentage; N, neutrophil percentage; N%, neutrophil percentage

marker were loaded on 12% SDS-PAGE gels, and proteins were separated electrophoretically and then transferred toImmobilon®-P^{SQ}Membrane (PVDF MilliporeMassachusetts, USA, 0000278926). The PVDF membrane was then blocked with 5% non-fat skim milk for 2 h at room temperature. After blocking, the membranes were incubated overnight with the primary antibody to KLF2 (1:1000; Abcam, USA, Ab236507) and GAPDH (1:1000; Proteintech, USA, 00110344) diluted in 5% skim milk (dissolved in Tris-buffered saline with 0.1%Tween 20 detergent, TBST, Sosarbio Beijing China) at 4 °C. After three times washing with TBST, membranes were incubated in species-specific horseradish peroxidase (HRP)-coupled secondary antibody for 1 h at room temperature.

Table 2 The amplification primer sequences

Gene name	Sequence (5′-3′)
KLF2	F: CTACACCAAGAGTTCGCATCTG
	R: CCGTGTGCTTTCGGTAGTG
Caspase-3	F: AGAGGGGATCGTTGTAGAAGTC
	R: ACAGTCCAGTTCTGTACCACG
GAPDH	F: TGCACCACCAACTGCTTAGC
	R: GGCATGGACTGTGGTCATGAG

Abbreviations: KLF2, Krüppel-like factor 2; GAPDH, glyceraldehyde-3-Phosphate Dehydrogenase

Membranes were rewashed and exposed to SuperKine^{TM}-enhanced ECL Luminous Liquid (Abbkine, Wuhan, China, ATWL22081). Protein bands were visualized using Image J software and band intensities were normalized to the internal control GAPDH.

RT-PCR

The total RNA of neutrophils was isolated using Trizol (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. The sequences of the primers used in this study were listed in Table 2. The integrity and concentration of the RNA was assessed by agarose gel electrophoresis and a NanoDrop ND-1000 spectrophotometer (Invitrogen Bio, Waltham, MA, United States), respectively. RT-PCR were carried out with the PrimeScript™ RT kit (Takara Bio Inc., Kyoto, Japan) and SYBR Premix Ex Taq™ II (Takara Bio Inc., Kyoto, Japan) in an ABI 7500 Real-Time PCR System (Invitrogen, California, USA) with the following PCR thermocycler protocol: initial denaturation step at 95℃for 5 min, followed by 40 cycles of 95℃ for 15 s (denaturation), 60℃ for 1 min (annealing and elongation), and 72℃ for 2 min (final extension). GAPDH was used as an internal control. The data were analyzed using the $2^{-\Delta\Delta Ct}$ method.

Statistical analysis

Statistical analysis and graphic presentation were carried out with GraphPad Prism 10.0 and SPSS version 20.0. A Student's t-test was used between two groups where the samples passed the normality test; otherwise, the non-parametric Mann-Whitney test was used to analyze the data. Spearman's method was used for correlation analysis. $P<0.05$ was considered to indicate statistically significant differences.

Results

Peripheral blood neutrophil counts are reduced and inversely correlate with disease activity in SLE

The neutrophil counts of patients with SLE were significantly reduced, with 29.4% of them showing neutropenia, defined as a peripheral neutrophil count less than 2×10^9/L (Fig. 1a). Further analysis revealed that

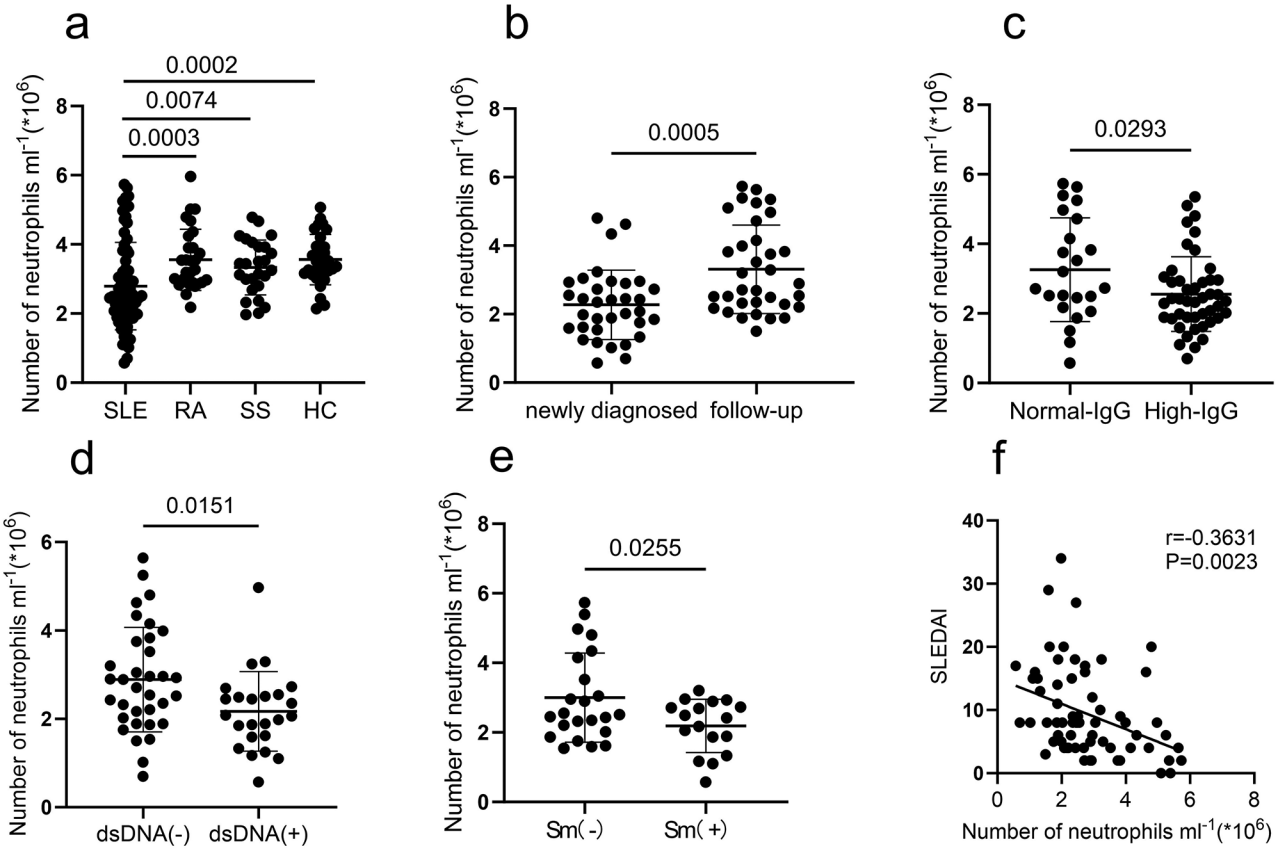

Fig. 1 Peripheral neutrophil counts are correlated with the disease activity of SLE patients. Peripheral neutrophil counts were determined and compared between patients with SLE, RA, SS, and HCs (**a**). Neutrophil counts were further analyzed between newly diagnosed and follow-up SLE patients (**b**), and between SLE patients with normal serum IgG and high serum IgG (**c**), positive and negative anti-dsDNA antibody (**d**), positive and negative anti-Sm antibody (**e**). Pearson's correlation analysis was used to analyze the correlations between the neutrophil counts and SLEDAI (**f**)

neutrophils were significantly fewer in newly diagnosed SLE patients than in follow-up patients (Fig. 1b). Interestingly, SLE patients with high serum IgG (>15.5 g/ml) had fewer neutrophils than those with normal serum IgG (7–15.5 g/ml) (Fig. 1c). Moreover, Neutrophils were significantly fewer in SLE patients with positive anti-Smith antibody (Sm+) than in those with negative anti-Smith antibody (Sm-) (Fig. 1d). Neutrophils were significantly fewer in SLE patients with positive anti-double-stranded DNA antibody (dsDNA+) than in dsDNA- SLE patients. Furthermore, peripheral neutrophil counts were inversely correlated with the disease activity of SLE patients, as indicated by the SLEDAI (Fig. 1f).

The neutrophil KLF2 levels in SLE are significantly decreased and correlate with cell apoptosis

The results showed that both the mRNA and protein levels of KLF2 were markedly decreased compared with HCs (Fig. 2a, b, c). It was shown that the apoptosis levels of neutrophils in SLE patients were significantly increased compared to HCs (Fig. 2d). The caspase-3 mRNA levels of neutrophils in SLE patients were markedly increased compared with HCs, as expected (Fig. 2e). Considering that KLF2 contributes to the maintenance of quiescence in a wide range of cell types, the relationship between the KLF2 levels and the apoptosis levels in neutrophils was analyzed. It was shown that the KLF2 mRNA levels in neutrophils were positively correlated with the neutrophil counts in SLE patients (r=0.5395, P=0.0209; Fig. 2f). The KLF2 mRNA levels in neutrophils were inversely correlated with the proportion of apoptotic cells in SLE patients (r=-0.5375, P=0.0214; Fig. 2h). Furthermore, the neutrophil counts were inversely correlated with the frequency of apoptotic cells (r=-0.7641, P=0.0002; Fig. 2j) and the caspase-3 mRNA levels (r=-0.5358, P=0.0219; Fig. 2k).

KLF2 controls the apoptosis of neutrophils

To determine the effect of KLF2 on neutrophil apoptosis, neutrophils isolated from HCs were administered with GGPP or GGTI-298, and then the KLF2 levels and the apoptosis levels were detected in neutrophils. The results showed that the mRNA levels of KLF2 were significantly decreased in the presence of GGPP while markedly

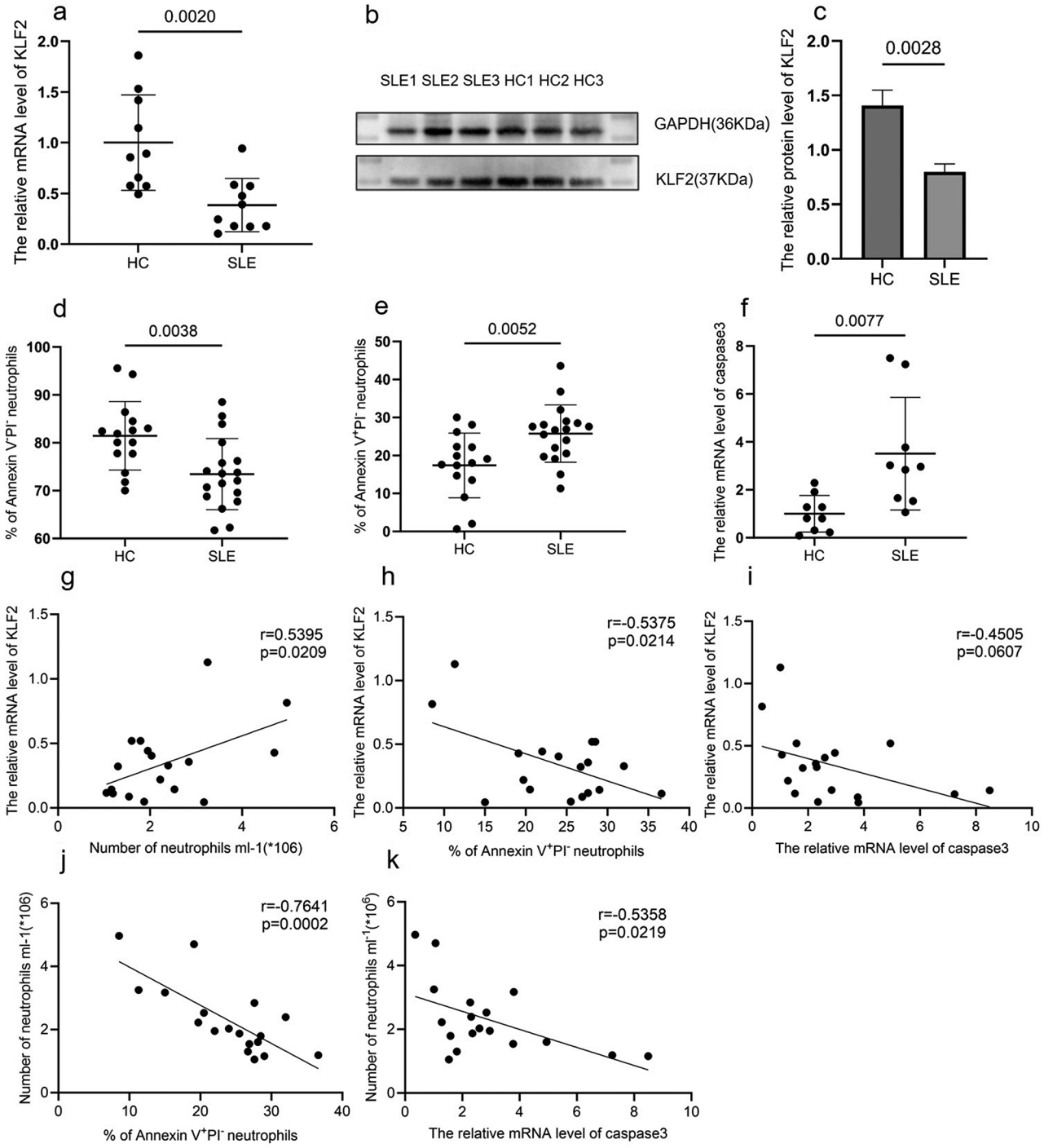

Fig. 2 The levels of KLF2 in peripheral neutrophils of SLE patients decreased and correlated with the apoptosis levels of neutrophils. The levels of KLF2 in peripheral neutrophils were detected by RT-PCR (**a**) and Western blotting (**b**). Fold change in each protein level normalized to GAPDH is shown numerically (**c**). The levels of Caspase-3 mRNA and cell apoptosis in peripheral neutrophils were analyzed by RT-PCR and flow cytometry, respectively (**d**, **e**, **f**). The correlations between the KLF2 levels and neutrophil count values (**g**), the KLF2 levels and neutrophil apoptosis levels (**h**), the KLF2 levels and caspase-3 levels (**i**), the neutrophil counts and the levels of neutrophil apoptosis (**j**), the neutrophil counts and the caspase-3 mRNA levels (**k**) were analyzed by Pearson's correlation analysis. Data are shown as mean ± SD of at least three independent experiments or specimen numbers

increased in the presence of GGTI-298 (Fig. 3a). Concurrently, treatment with GGPP resulted in a dramatic increase in neutrophil apoptosis, while GGTI-298 significantly suppressed the apoptosis of neutrophils (Fig. 3b, c, d).

SLE serum regulates KLF2 expression and induces apoptosis in neutrophils

To clarify whether serum from SLE patients affects neutrophil KLF2 expression and apoptosis, neutrophils from HCs were stimulated by serum from SLE patients, followed by the detection of KLF2 mRNA levels and apoptosis levels of neutrophils. Interestingly, the levels of KLF2 mRNA in neutrophils were significantly decreased when incubated with SLE serum, compared to autologous serum (Fig. 4a). In addition, the apoptosis levels of neutrophils were significantly increased after the incubation with SLE serum compared to autologous serum as expected (Fig. 4b, c).

KLF2 levels in neutrophils of SLE patients are associated with SLEDAI

The aforementioned results demonstrated that the expression of KLF2 in neutrophils was significantly suppressed in SLE patients, and the levels of KLF2 in neutrophils modulated the apoptosis of neutrophils. Because peripheral neutrophil counts were inversely correlated with the disease activity of SLE patients, we therefore want to explore whether the expression of KLF2 in neutrophils plays some roles in the disease activity of SLE patients. It was shown that KLF2 levels in neutrophils were significantly lower in newly diagnosed SLE patients than in follow-up patients (Fig. 5a). KLF2 levels were significantly lower in patients with higher serum IgG than their counterparts (Fig. 5b). KLF2 levels in neutrophils were decreased in SLE patients with positive Sm but not in patients with positive dsDNA (Fig. 5c, d). The KLF2 mRNA levels in neutrophils were inversely correlated with SLEDAI of SLE patients (r=-0.4768, P=0.0454; Fig. 5e). Further analysis found that the KLF2 mRNA

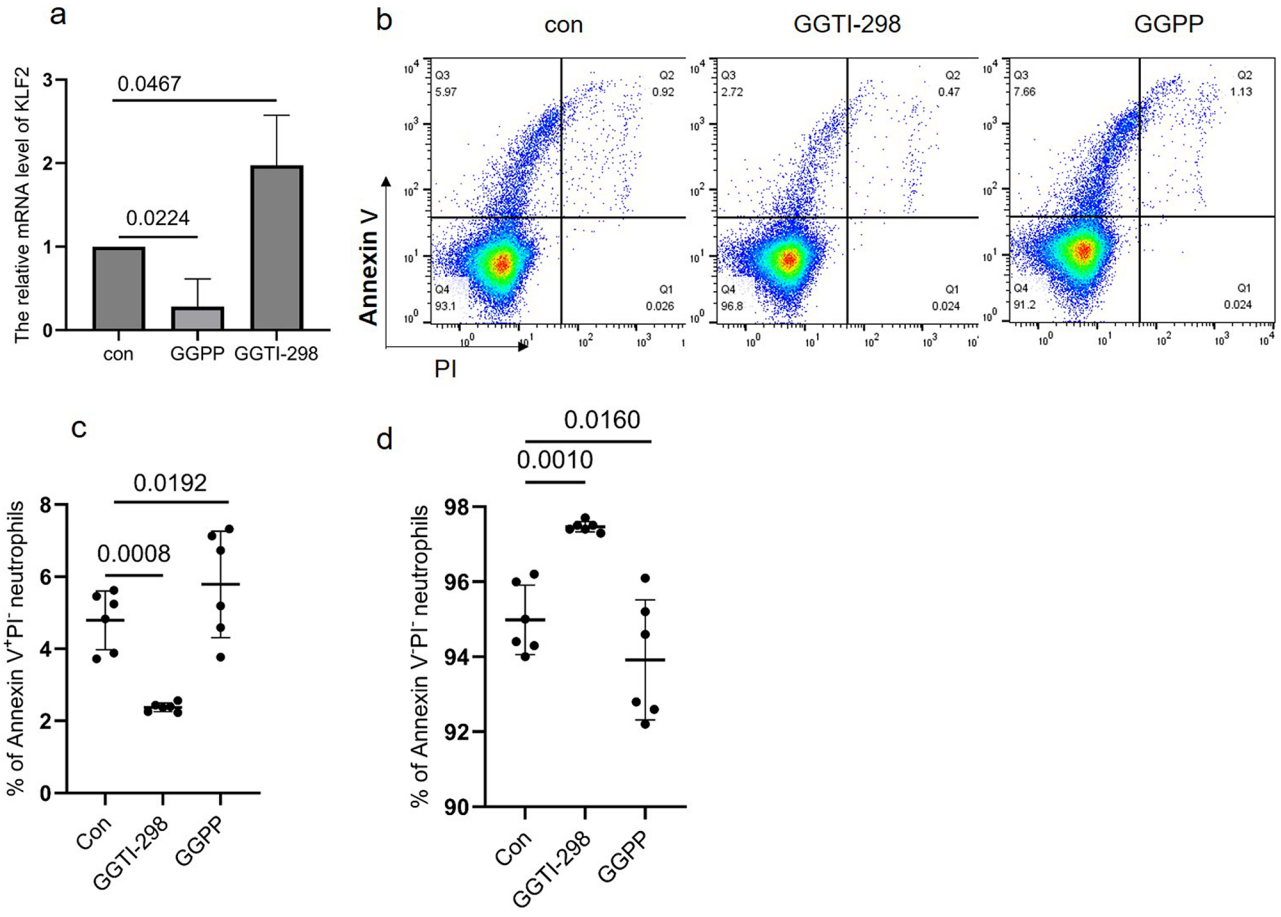

Fig. 3 Regulation of KLF2 expression affects neutrophil apoptosis. The levels of KLF2 mRNA of neutrophils from HCs were analyzed by RT-PCR after the treatment with the inhibitor (GGPP) or the inducer (GGTI-298) of KLF2 for 4 h (**a**). The proportion of neutrophil apoptosis and alive cells were detected utilizing the Annexin V-FITC/PI double-stained apoptosis assay kit by flow cytometry (**b**, **c**, **d**). Data are shown as mean ± SD of at least three independent experiments or specimen numbers

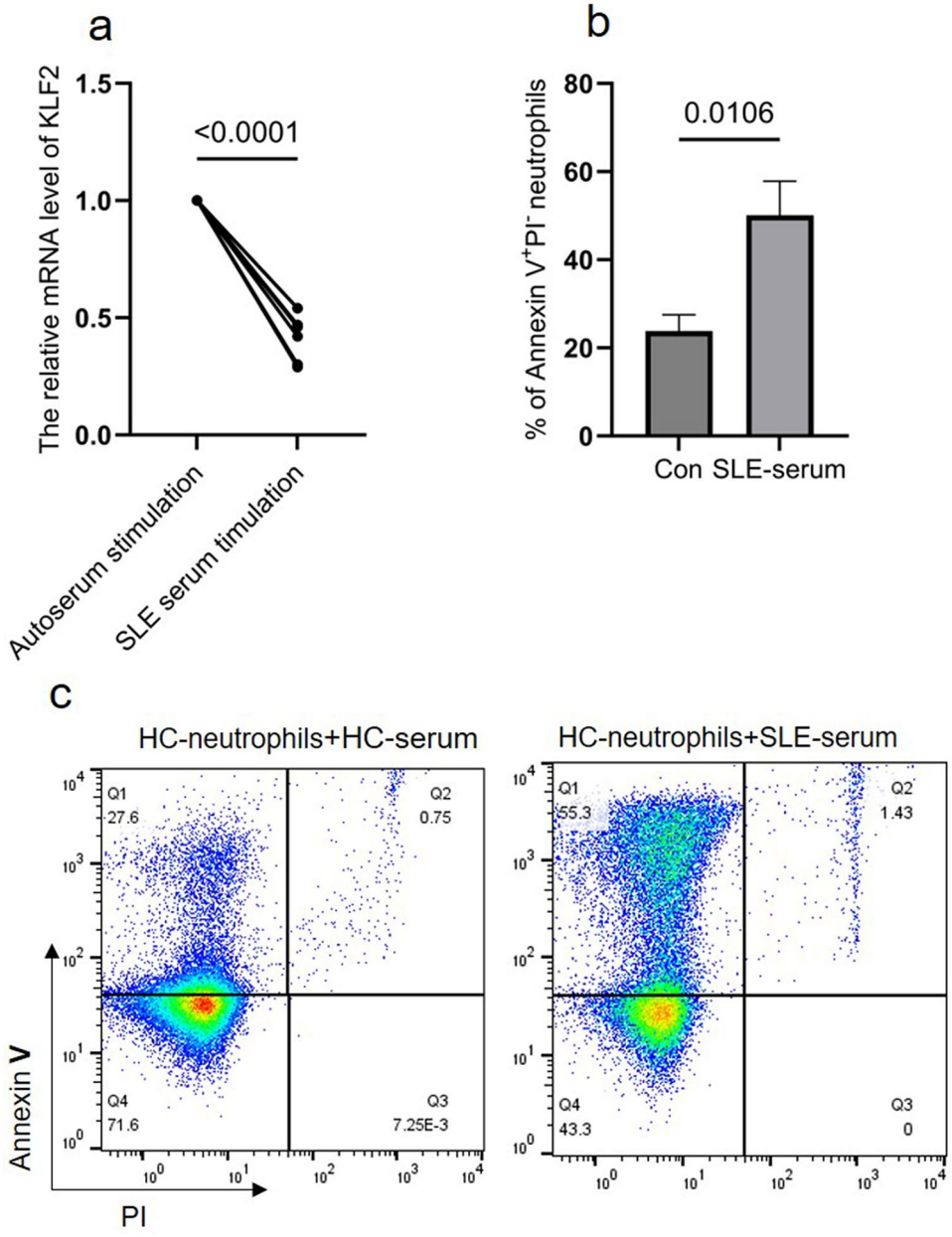

Fig. 4 Serum from SLE patients affects the expression of KLF2 and the apoptosis in neutrophils. The levels of KLF2 mRNA in neutrophils from HCs were analyzed by RT-PCR after incubation with serum from SLE patients for 4 h (**a**). The proportion of apoptotic neutrophils was detected utilizing the Annexin V-FITC/PI double-stained apoptosis assay kit by flow cytometry (**b**, **c**). Data are shown as mean ± SD of at least three independent experiments or specimen numbers

expression in neutrophils was positively correlated with serum complement 3 (C3) ($r=0.3665$, $P=0.0464$; Fig. 5f).

Discussion

Neutrophils, also known as polymorphonuclear leukocytes, are the most numerous white blood cells in the peripheral blood of the human body [7]. Recent studies showed that neutropenia is more common in SLE patients and is a major cause of life-threatening infections [5, 21]. In this study, we confirmed that the neutrophil counts of patients with SLE were significantly reduced. In addition, we found that this decline was more significant in newly diagnosed patients, patients with high serum IgG, Sm+patients, and dsDNA+patients. Furthermore, we found that the peripheral neutrophil counts were inversely correlated with the disease activity of SLE patients, as indicated by the SLEDAI. These results suggested that neutrophils may be closely related to the occurrence and development of SLE. Previous studies have found that the peripheral neutrophils in SLE

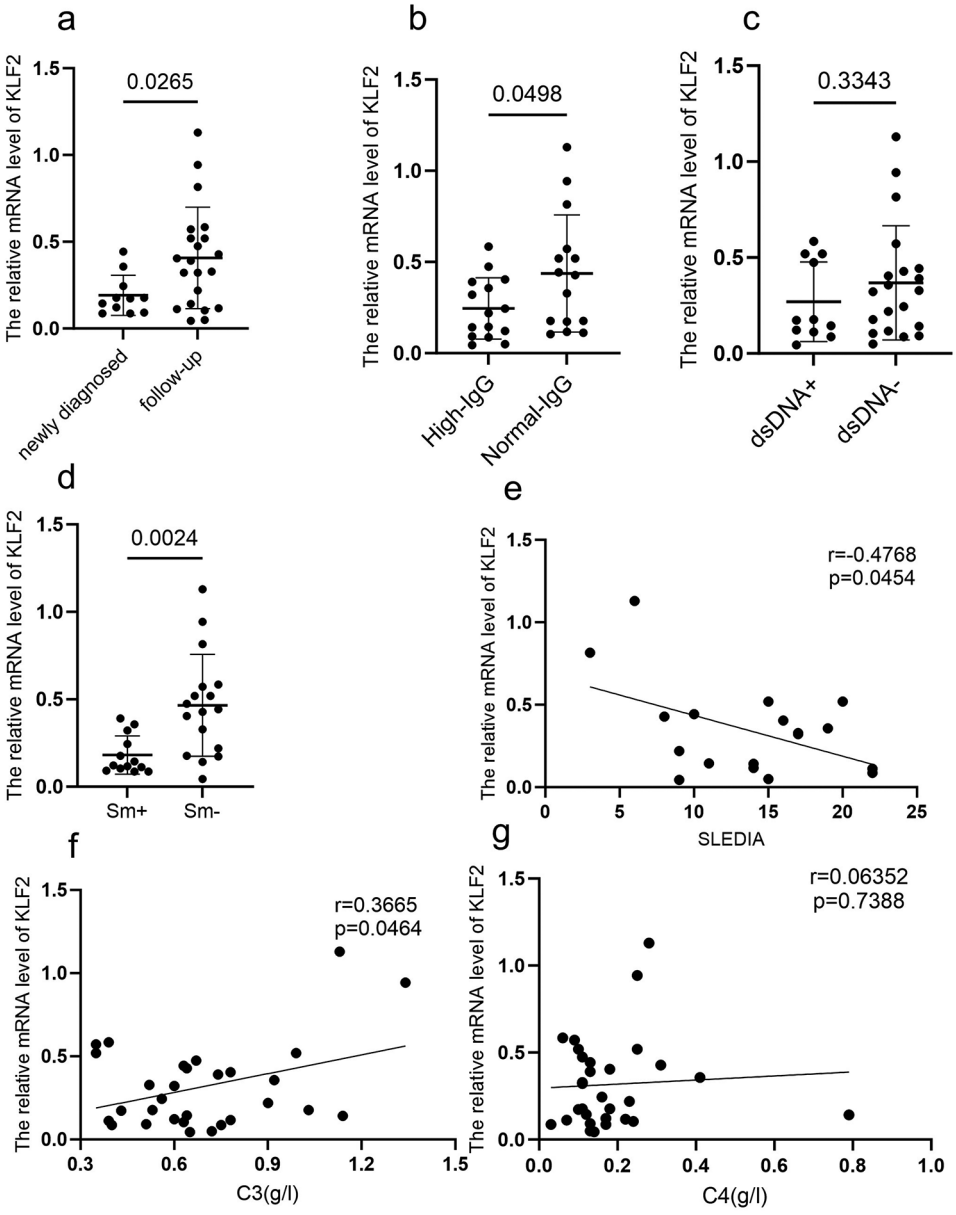

Fig. 5 The levels of KLF2 in neutrophils were negatively correlated with disease activity of SLE patients. The levels of KLF2 in peripheral neutrophils of SLE patients were detected by RT-PCR (**a**, **b**, **c**, **d**). The correlations between the levels of KLF2 mRNA and SLEDAI (**e**), and between the levels of KLF2 mRNA and serum complements levels (**f**, **g**) were analyzed using Pearson's correlation analysis

patients showed increased apoptosis [11, 22]. However, the relevant mechanism has not been fully elucidated.

KLF2 is one of eukaryotic transcription factors that is known to play a key role in regulating inflammation [13, 14]. Previous studies have shown that KLF2 mainly serves as a negative regulator of immune cell activation, maintaining the quiescent state of immune cells [13, 15]. Two recent studies have suggested that KLF2 may be associated with the occurrence and development of SLE, and is likely to be a potential therapeutic target for SLE [17, 18]. Many studies have demonstrated that KLF2 can inhibit NF-κB signaling in myeloid cells, thereby maintaining cellular quiescence [14, 23]. Previous study found that KLF2 deficiency increased neutrophil death [16]. However, the relationship between KLF2 and neutrophil apoptosis has not been reported yet. In this study, we have revealed for the first time that KLF2 can control the apoptosis of neutrophils. We found that the expression level of KLF2 in neutrophils was significantly decreased in SLE patients, regulating the expression of KLF2 could control the apoptosis level of neutrophils. Furthermore, we demonstrated that serum from SLE patients could decrease the expression of KLF2 and promote the apoptosis of neutrophils.

The correlation between KLF2 and neutrophil apoptosis suggests that the decline of KLF2 levels may be one of the important mechanisms for the neutropenia of SLE patients. Considering the fact that neutropenia in SLE patients often predicts a worse outcome [21]. We therefore want to know if the levels of KLF2 are related to the disease activity of SLE. Our results demonstrated that the levels of KLF2 in neutrophils were inversely correlated with SLEDAI of SLE patients. Although KLF2 has also been reported to be involved in the activation regulation of neutrophils and the release of neutrophil extracellular traps [24, 25], both of which are associated with the occurrence and tissue damage of SLE [26, 27], this study suggests that the upregulation of neutrophil apoptosis caused by the decrease in KLF2 levels is one of the important mechanisms leading to the occurrence and development of SLE.

There are some limitations in this study. First, we did not provide insights into the detailed mechanisms of how KLF2 regulates the apoptosis in neutrophils. Although we found that the neutrophil counts were inversely correlated with the caspase-3 mRNA levels in neutrophils, we did not reveal a significant correlation between KLF2 and caspase-3 probably due to the limitation of the number of study subjects. Considering that TNF-α is one of the potential suppressors of KLF2 expression and inducer of apoptosis in neutrophils [28, 29], and TNF-α mainly mediates apoptosis of neutrophils through activation of caspase-3 and caspase-8 [30, 31], We speculate that KLF2 may also be an inhibitory factor of caspase-3. In SLE patients, decreased KLF2 leads to the upregulation of caspase-3 expression and increased apoptosis of neutrophils. However, more studies are needed to support this speculation. Additionally, although we found an inverse correlation between the levels of KLF2 and SLEDAI of SLE patients, whether KLF2 downregulation in neutrophils is a causative factor for SLE development is unclear.

In summary, we revealed for the first time that KLF2 can regulate the apoptosis of neutrophils in this study. Moreover, our research showed that the KLF2 levels in neutrophils were closely related to the disease activity of SLE, which suggests that KLF2 in neutrophils may be involved in the occurrence and development of SLE. Our interesting findings may raise the possibilities for the involvement of KLF2 in autoimmunity and the potential to target it for therapeutic purposes.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13075-024-03461-z.

Supplementary Material 1

Acknowledgements
Not applicable.

Author contributions
JML, QL, and HSZ participated in designing the study, performing statistical analyses, and drafting the manuscript. ZCL and PMZ carried out flow cytometry analysis and drafted the manuscript. SMX and JYR performed data acquisition of disease activity and severity, performed statistical analyses, and drafted the manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
This study was approved by the Human Ethics Committee of The First Affiliated Hospital of Nanchang University (Ethical Review No. (2024) CDYFYYLK (06–028)). Informed consent was approved to be exempt.

Patient consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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