Clinical Rheumatology
https://doi.org/10.1007/s10067-019-04594-2

ORIGINAL ARTICLE

Vitamin D receptor expression in peripheral blood mononuclear cells is inversely associated with disease activity and inflammation in lupus patients

Jian Sun[1] · Chunyan Liu[2] · Shuang Zhang[1] · Bin Yi[1] · Ming Gui[1] · Wei Zhang[1] · Yan Chun Li[3] · Hao Zhang[1]

Received: 15 November 2018 / Revised: 23 April 2019 / Accepted: 6 May 2019

Abstract

Objective Systemic lupus erythematosus (SLE) is characterized by uncontrolled production of pro-inflammatory cytokines. Vitamin D receptor (VDR) has potent anti-inflammatory activities. The aim of this study was to examine the correlation between VDR expression and inflammation and disease activity in patients with SLE.

Methods Ninety-five SLE patients were recruited and divided into two groups, active and inactive, according to their SLE disease activity index (SLEDAI)-2 K, and 40 healthy individuals served as controls. The expression of VDR and NF-κB p65 in peripheral blood mononuclear cells (PBMCs) was determined by quantitative RT-PCR and Western blotting. VDR expression was correlated with inflammatory and diseases parameters in SLE patients. VDR regulation was also studied in THP-1 and Jurkat cell lines.

Results PBMC VDR expression was downregulated in SLE patients, especially in the active SLE group. VDR mRNA levels were negatively correlated with SLEDAI-2 K ($r = -0.348$, $P = 0.001$), Systemic Lupus International Collaborating Clinics (SLICC) renal activity scores ($r = -0.346$, $P = 0.014$), and proteinuria ($r = -0.309$, $P = 0.002$) and positively associated with serum complement C3 levels ($r = 0.316$, $P = 0.002$). Multiple stepwise regression analysis indicated that PBMC VDR downregulation was an independent risk factor for SLEDAI-2 K. VDR levels were also negatively correlated with NF-κB p65 ($r = -0.339$, $P = 0.001$), TNF-α ($r = -0.268$, $P = 0.009$), and IL-6 ($r = -0.313$, $P = 0.002$) levels. In monocyte and T lymphocyte cell lines, TNF-α suppressed VDR expression, whereas 1,25-dihydroxyvitamin D blocked TNF-α-induced VDR downregulation.

Conclusion PBMC VDR expression is inversely associated with disease activity and inflammation in SLE patients, and VDR downregulation is likely driven by inflammation.

Keywords Inflammation · Systemic lupus erythematosus · Vitamin D receptor

Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease that often results in multiple organ damages [1], among which renal failure and cardiovascular disease are the leading causes of death. Previous studies have shown that both innate and adaptive immunities are involved in the pathogenesis of SLE, and uncontrolled inflammation is a key feature of SLE [2, 3]. Vitamin D deficiency is common in patients with SLE and has been shown to be associated with high disease activity [4, 5], but whether vitamin D is a pathogenic factor causing SLE remains unclear [6]. It is well known that vitamin D receptor (VDR), a multifunctional nuclear hormone receptor, has potent anti-inflammatory activities by blocking NF-κB activation and inhibiting the production of pro-inflammatory cytokines [7–10]. VDR is activated by the vitamin D hormone, 1,25-dihydroxyvitamin D ($1,25(OH)_2D_3$), or its active analogs [11]. Once activated, liganded VDR is translocated to the nuclei to act as a transcription factor to regulate target gene expression [12]. Numerous studies have suggested that vitamin D-VDR signaling is protective in many

✉ Hao Zhang
zhanghaoliaoqing@163.com

[1] Department of Nephrology and Rheumatology, The Third Xiangya Hospital, Central South University, 138 Tongzipo Road, Changsha 410013, Hunan Province, China

[2] Department of Pathology, Hunan University of Chinese Medicine, Changsha 410208, Hunan, China

[3] Department of Medicine, Division of Biological Sciences, The University of Chicago, Chicago, IL 60637, USA

Published online: 18 May 2019

autoimmune diseases, including type 1 diabetes, rheumatoid arthritis, multiple sclerosis, and SLE [13–16]. It is reported that the reduction of VDR levels is associated with Crohn's disease and ulcerative colitis [17] and colon cancer [18]. Other studies suggest that VDR gene polymorphisms are correlated with autoimmune diseases such as Graves' diseases [19] and ankylosing spondylitis [20]. Recently, the immune regulatory effect of VDR in SLE has drawn some attention, but many researches focused on the gene polymorphism of VDR. For example, a Japanese study showed that, in a cohort of 58 SLE patients, the B/B (BsmI) allele frequencies in the VDR gene were much higher compared with the healthy controls, whereas in patients with nephrotic syndrome showed a higher frequency of b/b allele genotype among these subjects [21]. In another study with 337 SLE patients in Chinese Han population, the VDR BsmI B allele frequency was reported to be significantly higher than the control group, and the frequency of B allele was positively correlated with incidence of both lupus nephritis and positive antinuclear antibodies [22]. These studies suggest that the VDR BsmI B allele is a risk factor for SLE, but the underlying mechanism is unknown. It is conceivable that this polymorphism may influence VDR expression that consequently affects SLE pathogenesis, yet the expression status of VDR in SLE as well as its correlation with disease activity and renal impairment has not been addressed. Therefore, in the present study, we determined the expression of VDR in SLE patients and analyzed its correlation with disease activity and renal involvement of SLE. Our data show that VDR downregulation in peripheral blood mononuclear cells (PBMCs) is associated with disease activity and inflammation in SLE patients and suggest that VDR downregulation is a useful biomarker for SLE development.

Materials and methods

Patients All of the patients were recruited from the outpatient department and ward in the Third Xiangya Hospital affiliated with Central South University between April 2014 to December 2016 who were over 18-year old and fulfilled the American College of Rheumatology (ACR) classification criteria of SLE [23]. Patients obtained standard-of-care evaluation and management. The clinical management was determined based on disease activity at each visit. In general, patients with renal disease were treated with glucocorticoid, together with cyclophosphamide or mycophenolate, and also with calcium and vitamin D supplements, according to published protocols [24]. In this cohort, 89% of patients were treated with glucocorticoids (median daily dose 10 mg), 54% were treated with 1,25-dihydroxyvitamin D_3 (daily dose 0.25 μg), 31% were treated with mycophenolate, and 29% were treated with cyclophosphamide. SLE disease activity index (SLEDAI)-2 K was used for assessing the activity of global disease [25]. Active SLE was defined according to SLEDAI-2 K > 4 [26]. Of the 95 patients, 50 were assigned in the active group and 45 in inactive group based on their SLEDAI-2 K values. A non-zero score was used to define active renal disease in any of the renal components of the SLEDAI-2 K. Individuals suffered from other autoimmune or rheumatic disorders, renal diseases other than lupus nephritis, infections, any other comorbidities or malignant tumors were eliminated from this work. We also recruited 40 healthy volunteers as controls. The healthy cohort was matched consistently for gender and age. This cross-sectional study was approved by the institution of the ethical committee of the Third Xiangya Hospital and carried out at the Section of Nephrology and Rheumatology of the Third Xiangya Hospital. All research subjects signed an informed consent.

Sample collection and clinical data Peripheral venous blood samples were collected from all subjects after they underwent overnight fasting (at least 8 h). PBMCs were isolated with Percoll density gradient centrifugation as described [27]. Serum biochemical indices were measured by automatic biochemical analyzers (Hitachi 7600, Japan). Patient records were reviewed. Serologic data included complement factor 3 (C3), C4, anti-dsDNA, and antinuclear antibodies. The Systemic Lupus International Collaborating Clinics (SLICC) renal activity score was utilized to quantify renal disease activity [28]. It was calculated based on the following points: proteinuria > 3 g/day (11 points), proteinuria 1 to 3 g/day (5 points), proteinuria 0.5 to 1 g/day (3 points), urine white blood cells ≥ 5/high power field (hpf; 1 points), and urine red blood cells ≥ 5/hpf (3 point). Renal activity scores ranged from 0 (inactive renal disease) to a maximum of 16 [29].

Cell culture studies THP-1 cells, considered as a human leukemia monocytic cell line, and Jurkat T lymphocytic cells were cultured in RPMI 1640 media with fetal bovine serum (10%). Cells were stimulated by tumor necrosis factor α (TNF-α) with different concentrations (10 ng/mL, 50 ng/mL and 100 ng/mL) respectively for 24 h, followed by the isolation of total RNAs or protein. In some experiments, cells were under the 24-h co-treatment of TNF-α (50 ng/ml) and $1,25(OH)_2D_3$ at 10^{-9} mmol/L, 10^{-8} mmol/L, and 10^{-7} mmol/L before harvesting total RNAs and cell lysates, which were used for qRT-PCR and Western blot analyses.

Quantitative RT-PCR Total RNAs were extracted from PBMCs, THP-1, or Jurkat cells using the TRIzol reagent (Invitrogen). First strand cDNA synthesis was carried out using a reverse transcription kit ReverTra Ace qPCR RT Kit (Toyobo, Japan) according to the manufacturer's instruction. Quantitative real-time PCR was conducted using the SYBR Green PCR Master Mix (Toyobo, Japan) on the 7300 Real Time PCR System (Eppendorf, Germany). PCR reactions

were performed using the following cycle conditions: predenaturation at 95 °C for 2 min, followed by denaturation at 95 °C for 10 s, annealing at 58 °C for 20 s, and extension at 72 °C for 30 s, with 40 cycles in total, followed by extension at 72 °C for 5 min. The melting curve was used to confirm the specificity of the amplification products. Relative amounts of transcripts were calculated in term of the $2^{-\Delta\Delta Ct}$ formula. GAPDH or b-actin was used as the internal control for normalization. The PCR primer sequences are as follows: VDR: 5'AGTGCAGAGGAAGCGGGAGATG3' (forward) and 5'CTGGCAGAAGTCGGAGTAGGTG3' (reverse); NF-κBp65: 5'ATCCCATCTTTGACAATCGTGC3' (forward), and 5'CTGGTCCCGTGAAATACACCTC3' (reverse); TNF-α: 5'AGCTCCAGTGGCTGAACCG3' (forward), 5'TGGTAGGAGACGGCGATGC3'(reverse); IL-6: 5'TGAGGCTACGGCGCTGTCA3' (forward), and 5'GGCATTCTTCACCTGCTCCAC3' (reverse); GAPDH: 5'GCACCGTCAAGGCTGAGAAC3' (forward), and 5'TGGTGAAGACGCCAGTGGA3' (reverse); and β-actin: 5'CATCCTGCGTCTGGACCTGG3' (forward), and 5' TAATGTCACGCACGATTTCC3' (reverse).

Western blot PBMC nuclear protein levels were analyzed in 45 randomly selected study subjects, including 15 subjects from each of the inactive SLE, active SLE groups, and control group. All subjects were matched for age, sex, and disease duration. Nuclear proteins were extracted using a Nucleoprotein kit (Merck, USA) according to the manufacturer's instructions, and protein concentrations were measured using BCA protein assay kit (Pierce, USA). Nuclear proteins (30 μg) from each sample were separated by SDS-PAGE. Lysates from THP-1 and Jurkat cells (50 μg) were also separated by SDS-PAGE and analyzed for VDR expression. Proteins separated were transferred onto PVDF membranes and incubated overnight by the primary antibody against VDR (sc-13,133, Santa Cruz Biotechnology), NF-κB p65 (sc-8008, Santa Cruz Biotechnology), PCNA (sc-25,280, Santa Cruz Biotechnology), and β-actin (sc-47,778, Santa Cruz Biotechnology) at 4 °C. After three-time washes with TBST buffer, the PVDF membranes were treated with HRP (horseradish peroxidase-conjugated) secondary antibody for 2 h at room temperature. Then, the membranes were exposed with an enhanced chemiluminescence advanced machine (GE Healthcare, UK) and images captured on X-ray film. The densitometry of immunoreactive bands was quantified by Image J software (NIH, USA).

Statistical analyses Data values were presented as mean ± SD. Statistical analyses were carried out by SPSS17.0 software. A post hoc test in one-way ANOVA was used to determine differences among the SLE and the control groups. Student's *t* test was carried out to analyze the differences between active group and inactive group. Spearman correlation and stepwise multiple linear regression analysis were carried out for determining the correlations between clinical and biochemical data. $P < 0.05$ was considered to be statistically significant.

Results

Demographic and baseline clinical data SLE patients and healthy control volunteers were matched for age and sex. There were no statistically significant differences between patients with active and inactive disease regarding age and sex composition. The demographic characteristics of the control, active, and inactive SLE groups are given in Table 1. Consistent with early reports [4, 5], the vitamin D status (i.e., serum 25(OH)D_3 levels) was lower in the inactive SLE and active SLE groups compared with the control group ($P < 0.01$), with the active SLE group showing the lowest serum 25(OH)D_3 levels ($P < 0.01$ compared with the inactive SLE group).

VDR levels in PMBCs We first assessed VDR mRNA and protein levels in PBMCs by quantitative RT-PCR and Western blotting. As shown in Fig. 1, the levels of VDR mRNA (Fig. 1a) and protein (Fig. 1b and c) in the inactive SLE and active SLE groups were lower than that in the control group ($P < 0.01$), and the active SLE group had even lower levels of VDR mRNA and protein compared with the inactive SLE group ($P < 0.01$).

VDR and SLE disease activity We then correlated PBMC VDR mRNA levels with a number of clinical parameters in the SLE patients. VDR mRNA levels were inversely associated with patients' SLEDAI-2 K values ($r = -0.348$, $P = 0.001$) (Fig. 2a), and positively correlated with serum C3 concentration ($r = 0.316$, $P = 0.002$) (Fig. 2b). However, there was no correlation between VDR mRNA and erythrocyte sedimentation rate (ESR) ($r = -0.009$, $P = 0.960$), dsDNA antibody ($r = -0.233$, $P = 0.054$), or C4 ($r = -0.027$, $P = 0.843$). After adjusting for potential confounding variables (albumin, WBC, Hb, PLT), stepwise multiple regression analysis indicated that VDR mRNA is an independent variable of SLEDAI-2 K ($\beta = -0.260$, $P = 0.036$).

VDR and SLE with renal disease We further correlated VDR mRNA levels with renal disease. The relative expression of VDR mRNA was negatively correlated with 24-h total urinary protein ($r = -0.309$, $P = 0.002$) (Fig. 3a). In the 50 patients with active renal disease in the active SLE group, there was an inverse correlation between VDR mRNA and SLICC renal active score ($r = -0.346$, $P = 0.014$) (Fig. 3b); however, no correlation was seen in the inactive SLE group.

Table 1 Demographic and baseline clinical data of the patients

Parameters	Controls	Active SLE	Inactive SLE
N	40	50	45
Age (years)	31.65 ± 9.01	31.54 ± 11.52	31.20 ± 12.97
Sex (male/female)	4/36	6/44	5/40
WBC (10^9/L)	5.53 ± 1.03	6.62 ± 4.79	6.08 ± 2.14
Hb (g/L)	127.45 ± 12.52	85.16 ± 16.87**$^{\#\#}$	118.04 ± 16.31**
PLT (10^9/L)	211.82 ± 51.47	134.56 ± 62.31**$^{\#\#}$	185.07 ± 62.86*
Serum albumin (g/L)	41.72 ± 3.41	26.56 ± 6.31**$^{\#\#}$	38.51 ± 2.84**
Estimated GFR (ml/min)	108.08 ± 11.90	90.89 ± 22.16**$^{\#\#}$	102.15 ± 11.93
Proteinuria (mg/day)	71.22. ± 12.91.	2323.00 ± 1569.74**$^{\#\#}$	152.58 ± 106.87
C3 (g/L)	0.82 ± 0.17	0.42 ± 0.19**$^{\#\#}$	0.75 ± 0.19
C4 (g/L)	0.24 ± 0.09	0.15 ± 0.14**	0.19 ± 0.11
dsDNA positive (%)	–	70%	49%
ESR (mm/h)	10.25 ± 4.09	58.10 ± 30.06**$^{\#\#}$	32.53 ± 22.55**
25-OH-D3 (ng/L)	32.49 ± 6.28	20.81 ± 5.74**$^{\#\#}$	24.42 ± 6.54**

Values are expressed as mean ± SD. Data are compared by chi-square test or one-way ANOVA

*$P < 0.05$, **$P < 0.01$ vs. controls; $^{\#\#}P < 0.01$ vs. inactive SLE group

SLE systemic lupus erythematosus; *GFR* glomerular filtration rate; *C* complement factor; *ESR* erythrocyte sedimentation rate; *WBC*: white blood cell; *PLT*: platelet; (−) no data

VDR expression and pro-inflammatory cytokines To assess the association between PBMC VDR and pro-inflammatory cytokines in SLE patients, we measured TNF-α and IL-6 mRNAs in the PBMCs. Consistent with previous studies [30], compared with control group, the levels of TNF-α and IL-6 were significantly increased in two SLE groups, with active SLE group showing the highest increase ($P < 0.01$) (Fig. 4a and b). Spearman correlation analyses showed the VDR mRNA was negatively correlated with TNF-α mRNA ($r = -0.268$, $P = 0.009$) or IL-6 mRNA levels ($r = -0.313$, $P = 0.002$) (Fig. 4c and d). These observations suggest that PBMC VDR downregulation increases the production of pro-inflammatory cytokines in SLE patients.

VDR expression and NF-κB VDR signaling is known to intrinsically suppress NF-κB, a key signaling pathway for pro-inflammatory cytokine production [7, 8, 10]. Therefore, we measured the mRNA and nuclear levels of NF-κB p65 in

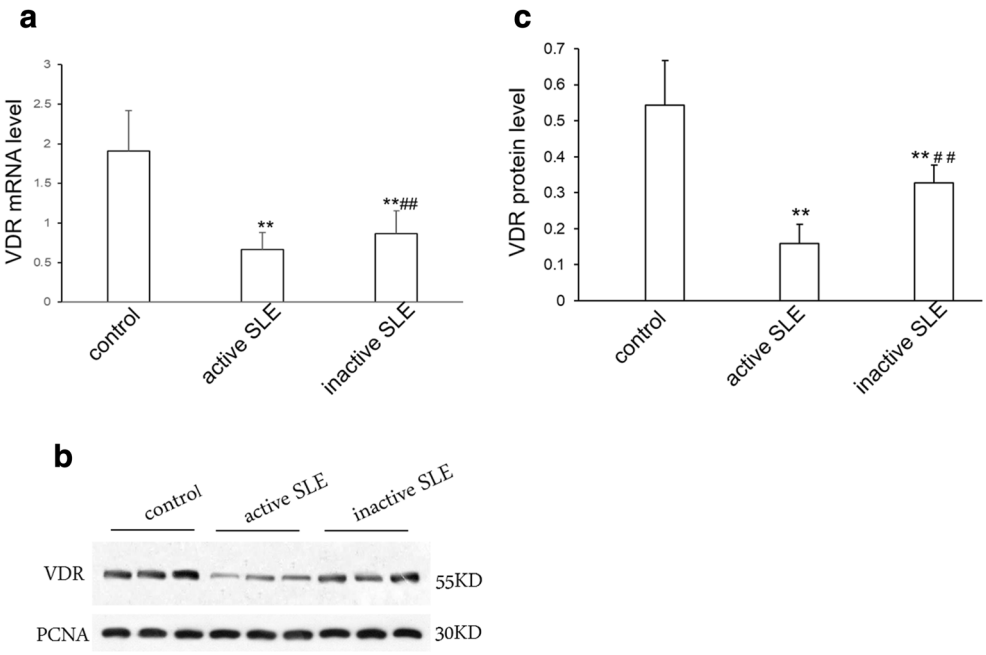

Fig. 1 VDR expression in PBMCs from SLE patients. **a** VDR mRNA levels in healthy control ($n = 40$), active SLE group ($n = 50$), and inactive SLE group ($n = 45$), determine by real-time RT-PCR. **b** and **c** VDR protein levels in these groups. VDR protein was measured by **b** Western blotting and **c** quantified by densitometry. Dates are presented as the mean ± SD. **$P < 0.01$ vs. control group; ##$P < 0.01$ vs. active SLE group

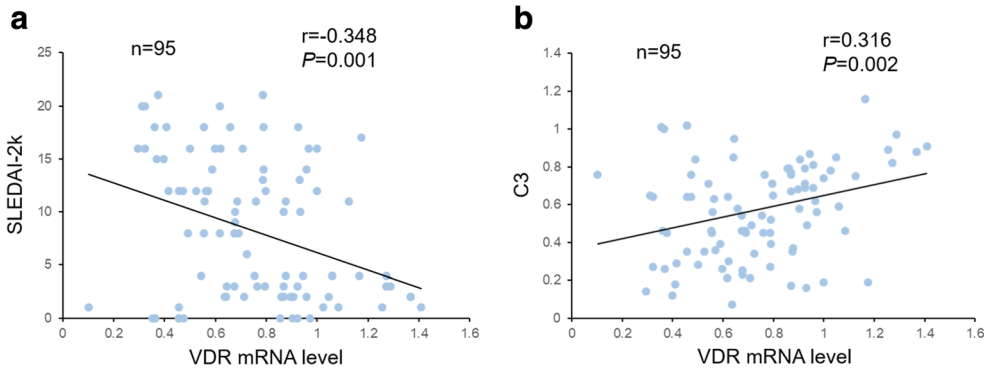

Fig. 2 Correlation between PBMC VDR and SLE disease activity. **a** Scatter plot showing an inverse relationship between VDR mRNA and SLEDAI-2 K ($r = -0.348$, $P = 0.001$), and **b** positively correlated with serum C3 levels ($r = 0.316$, $P = 0.002$)

PBMCs. As shown in Fig. 5, NF-κB p65 mRNA and protein levels in the inactive SLE and active SLE groups were higher than that in the control group ($P < 0.01$), and the active SLE group had the highest increase ($P < 0.01$) (Fig. 5a, b). Spearman correlation analysis showed that the VDR mRNA was negatively correlated with NF-κB p65mRNA ($r = -0.339$, $P = 0.001$) (Fig. 5c), and VDR protein was negatively correlated with NF-κB p65 protein ($r = -0.588$, $P = 0.001$) (Fig. 5d). Given the key role of NF-κB in inflammatory response, these results together suggest that VDR downregulation increases the production of pro-inflammatory cytokines in SLE patients due to NF-κB activation.

TNF-α and VDR downregulation Previous studies showed that pro-inflammatory cytokine TNF-α suppresses VDR expression in human colon cancer cells [31]. To address whether VDR downregulation is caused by inflammation, we studied the effect of TNF-α on VDR expression in THP-1 cells, a human leukemia monocytic cell line, and Jurkat cells, a human leukemia T lymphocyte line. As shown in Fig. 6, VDR mRNA and protein were suppressed by TNF-α in a dose-dependent manner in THP-1 cells, and the downregulation was significant in the dose of 50 ng/ml TNF-α or higher (Fig. 6a–c). When THP-1 cells were cotreated with TNF-α and $1,25(OH)_2D_3$, VDR downregulation was attenuated by $1,25(OH)_2D_3$ in a dose-dependent manner (Fig. 6d–f). Similar observations were seen in Jurkat cells with regard to the effects of TNF-α and $1,25(OH)_2D_3$ on VDR expression, although the suppression of VDR by TNF-α appeared to be less dramatic than in THP-1 cells (Fig. 7a–f). These results confirmed the inhibitory effect of inflammation on VDR expression in monocytes and T lymphocytes.

Discussion

In this study, we showed that VDR expression in SLE patients' PBMCs is markedly reduced, and VDR downregulation worsens in active SLE patients. Accompanying VDR reduction is an increased production of pro-inflammatory cytokines in these patients. Further analyses revealed that PBMC VDR expression is inversely correlated with SLEDAI-2 K, a clinical index for assessment of SLE disease activity, SLICC renal active score, and urinary albumin levels in the SLE patients. Overall, our data show that PBMC VDR expression is negatively correlated with SLE disease activity, suggesting that VDR downregulation might potentially serve as a biomarker for lupus activity in clinical practice. These correlations stand true after adjustments for the use of glucocorticoid or $1,25(OH)_2D_3$ in these patients. To our knowledge, this is the first time that PBMC VDR status is linked to the development of SLE.

Dysregulation of immune activities is considered to be a crucial step in the development of SLE [32]. Abnormalities of

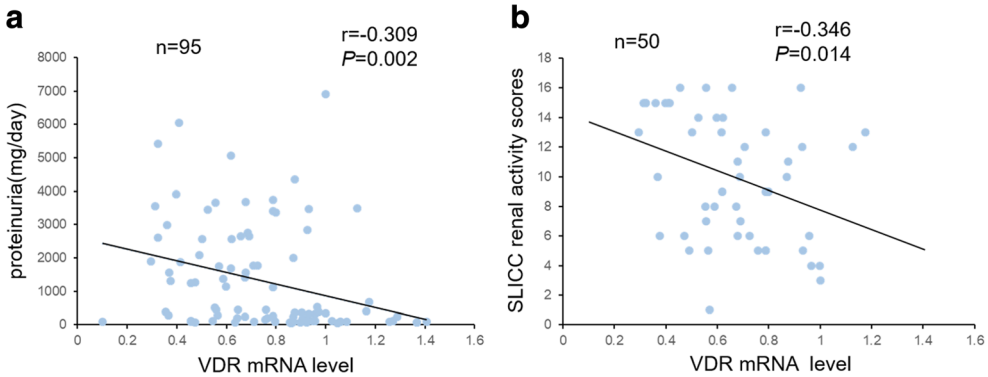

Fig. 3 Correlation between PBMC VDR and SLE with renal disease. **a** Scatter plot showing an inverse relationship between PBMC VDR mRNA and proteinuria ($r = -0.309$, $P = 0.002$). **b** Scatter plot showing an inverse relationship between PBMC VDR mRNA and SLICC active renal scores ($r = -0.346$, $P = 0.014$)

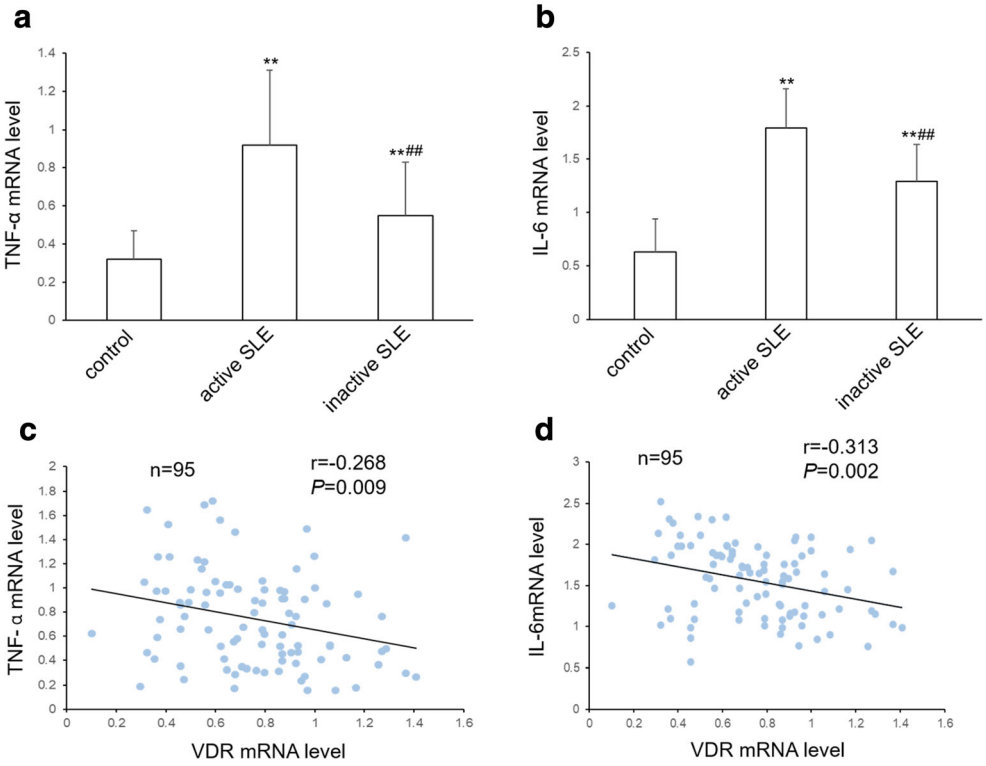

Fig. 4 The expression of TNF-α and IL-6 in patients with SLE. **a** PBMC TNF-α and IL-6 mRNA levels in healthy control ($n = 40$), active SLE group ($n = 50$), and inactive SLE group ($n = 45$). **a** and **b** The mRNA levels were quantified by real-time RT-PCR. Dates are presented as the mean ± SD. $^{**}P < 0.01$ vs. control group; ##: $P < 0.01$ vs. active SLE group. **c** Scatter plot showing an inverse relationship between PBMC VDR mRNA and TNF-α mRNA ($r = -0.268$, $P = 0.009$). **d** Scatter plot showing an inverse relationship between PBMC VDR mRNA and IL-6 mRNA ($r = -0.313$, $P = 0.002$). Both scatter plots cover both active and inactive SLE groups

the cytokine networks have been described in patients suffering from SLE as well as in murine lupus models. Pro-inflammatory cytokines TNF-α and IL-6 are believed to play an important role in the pathogenesis of SLE [30, 33, 34], and upregulated NF-κB signaling is regarded as one of the most important factors in SLE pathogenesis [35]. Vitamin D is well known to have strong immune-modulatory effects, particularly anti-inflammatory activity. Indeed, previous studies have well documented that VDR signaling inhibits inflammation via targeting NF-κB pathway [7, 36–40]. The baseline

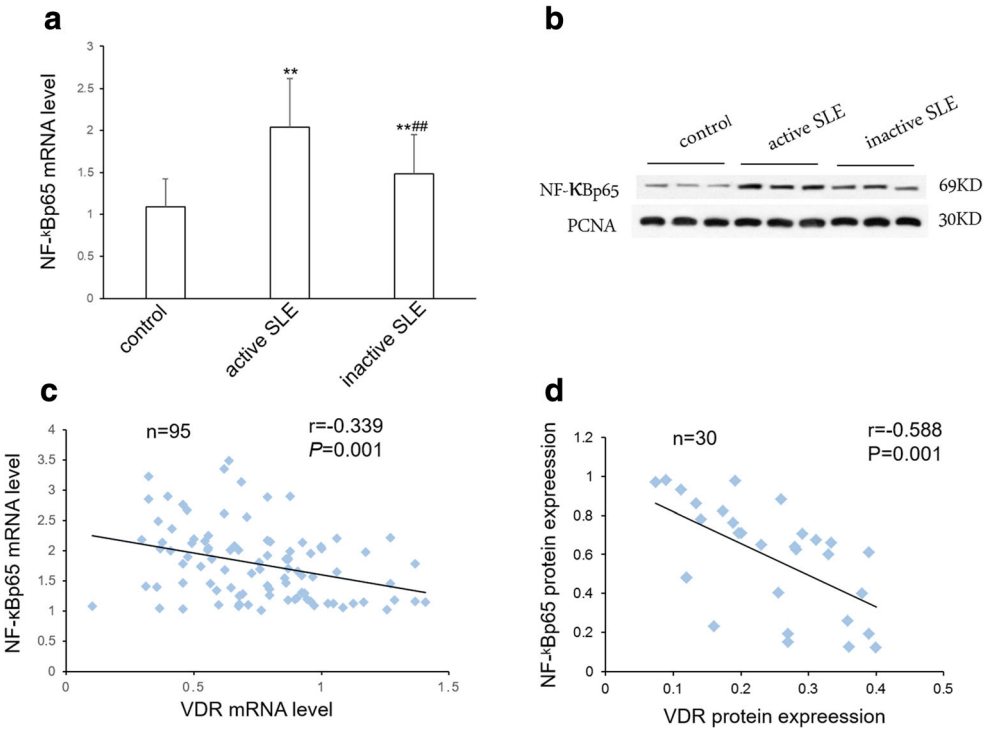

Fig. 5 NF-κBp65 levels in PBMCs from SLE patients. **a** NF-κBp65 mRNA in healthy controls ($n = 40$), active SLE group ($n = 50$), and inactive SLE group ($n = 45$). The mRNA levels were quantified by real-time RT-PCR. **b** NF-κB p65 protein levels were measured by Western blotting. The data are presented as the mean ± SD. $^{**}P < 0.01$ vs. control group; $^{\#\#}P < 0.01$ vs. active SLE group. **c** Scatter plot showing an inverse relationship between PBMC VDR mRNA and NF-κB p65 mRNA ($r = -0.339$, $P = 0.001$). **d** Scatter plot showing an inverse relationship between PBMC VDR protein and NF-κB p65 protein ($r = -0.588$, $P = 0.001$). Both scatter plots cover both active and inactive SLE groups

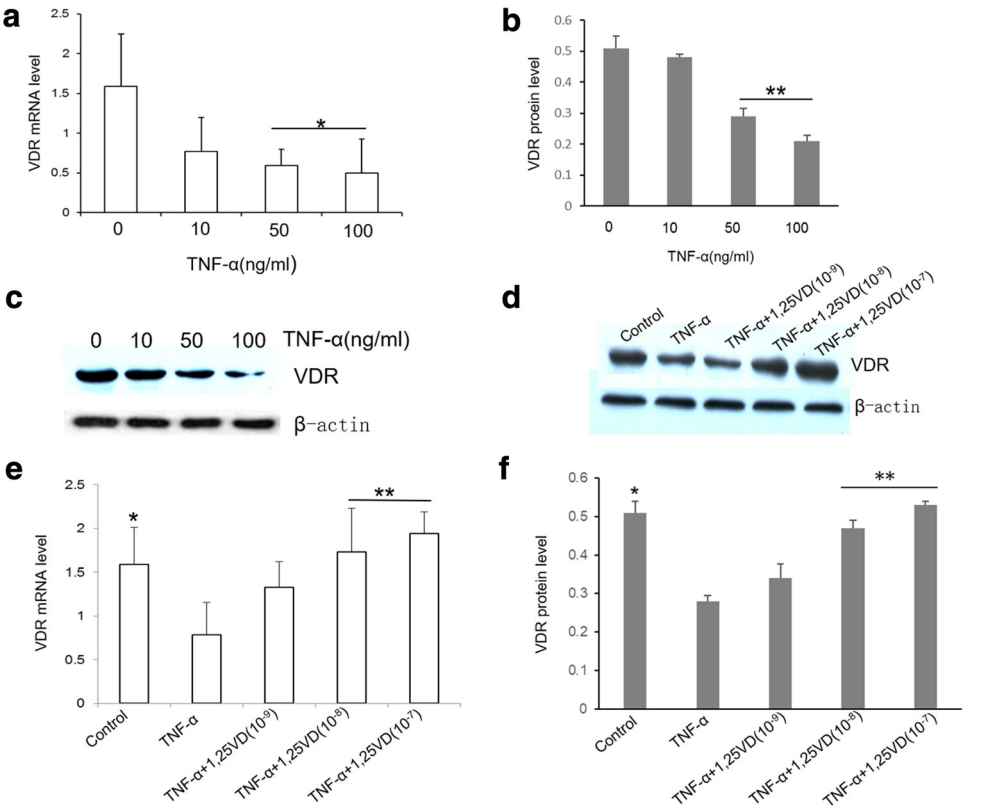

Fig. 6 TNF-α downregulates VDR expression in human monocytic cell line. **a**-**c**. THP-1 cells were treated with increasing doses of TNF-α for 24 h. VDR expression was measured by **a** real-time RT-PCR and **b** and **c** Western blotting. $^{*}P < 0.05$ $^{**}P < 0.01$ vs. 0. **d**-**f** THP-1 cells cotreated with TNF-α (50 ng/ml) and increasing doses of $1,25(OH)_2D_3$ (10^{-9} mmol/L, 10^{-8} mmol/L, 10^{-7} mmol/L). VDR expression was measured by **e** real-time RT-PCR and **d** and **f** Western blotting. β-actin was used as an internal control. $^{*}P < 0.05$ $^{**}P < 0.01$ vs. TNF-α. Experiments were done at least three times

NF-κB activity is elevated in the case of genetic VDR deletion, suggesting that VDR signaling intrinsically suppresses NF-κB activation [8]. VDR blocks NF-κB activation by directly interacting with IKKβ and thus increasing IκB concentration in the cytoplasm [7]. Here we found that PBMC VDR expression is inversely correlated with the NF-κB p65, IL-6, and TNF-α levels. These observations suggest that VDR downregulation may contribute to the increased production of pro-inflammatory cytokines in SLE patients via upregulation of NF-κB, the major pathway regulating pro-inflammatory cytokine production. It is speculated that this mechanism may be part of the events promoting SLE disease development. However, the PBMC preparation in our study contains many cell types involved in SLE pathogenesis, such as monocytes and lymphocytes. It is unclear at this point in what cell type(s) the VDR downregulation observed here occurs that contributes to inflammation in SLE. Future studies should be designed to address this important issue.

The cause for VDR downregulation in SLE is unclear. We cannot exclude the possibility that the therapy received by the SLE patients, particularly glucocorticoid, might suppress VDR expression; however, glucocorticoid has been reported to suppress as well as induce VDR expression [41, 42]. Our previous study demonstrated that inflammation, especially TNF-α, suppresses VDR expression in intestinal epithelial cells via miRNA-346 [31]. Here, we confirmed that TNF-α is able to decrease the expression of VDR in THP-1 cells and Jurkat cells, which are monocyte-like and T lymphocyte-like leukemia cell lines, respectively. TNF-α is thought to be a critical cytokine involved in pathogenesis of SLE [43]. Although anti-TNF therapy is not a standard treatment for SLE, there have been reports of anti-TNF therapy in SLE management [44]. In our cohort of SLE patients, serum TNF-α level is clearly elevated. Therefore, it is speculated that elevated TNF-α may in turn suppress VDR expression in PBMCs in SLE patients. We also showed that $1,25(OH)_2D_3$ treatment is able to reverse TNF-α induced downregulation of VDR in both monocyte and T lymphocyte cell lines. It is well known that the vitamin D hormone can induce VDR expression [45], and suppress TNF-α production via VDR activation [31]. We have shown previously that TNF-α down-regulates VDR expression by stimulation of miR-346 via NF-κB [31], and $1,25(OH)_2D_3$ blocks NF-κB through stimulating VDR interaction with IKKα [7]. Thus, the VDR expression level in these cells is likely a combined net effect of $1,25(OH)_2D_3$ direct stimulation of VDR transcription at the genomic level and $1,25(OH)_2D_3$ inhibition of TNF-α signaling by blocking NF-κB. Because activated VDR suppresses inflammation and inflammation can suppress VDR expression, these reciprocal regulations may create a vicious cycle that drives VDR downregulation and

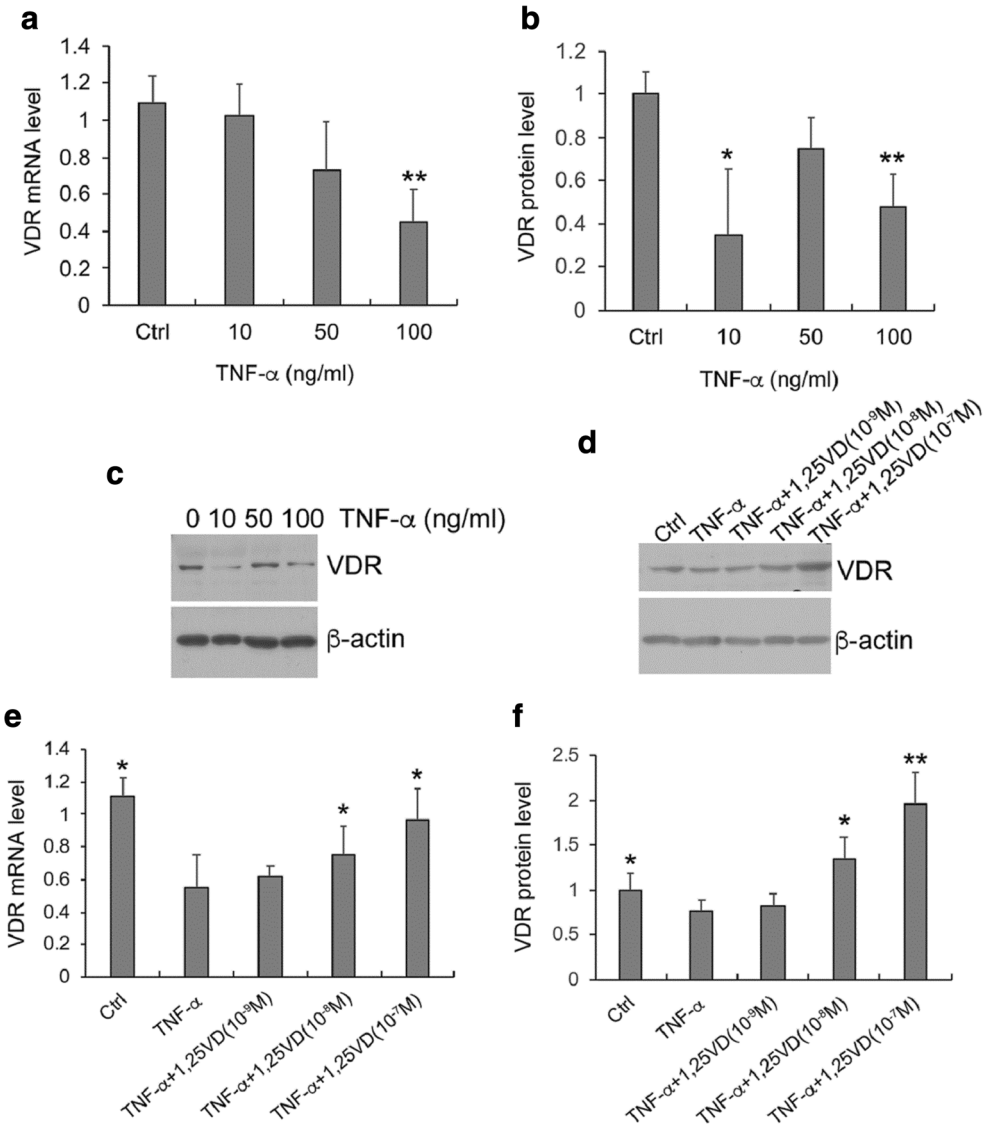

Fig. 7 TNF-α downregulates VDR expression in human lymphocytic cell line. **a**-**c** Jurkat T cells were treated with increasing doses of TNF-α for 24 h. VDR expression was measured by **a** real-time RT-PCR and **b** and **c** Western blotting. $*P < 0.05$ $**P < 0.01$ vs.Control. **d**-**f** Jurkat T cells cotreated with TNF-α (50 ng/ml) and increasing doses of $1,25(OH)_2D_3$ (10^{-9} mmol/L, 10^{-8} mmol/L, 10^{-7} mmol/L). VDR expression was measured by **e** real-time RT-PCR and **d** and **f** Western blotting. β-actin was used as an internal control. $*P < 0.05$ $**P < 0.01$ vs. TNF-α. Experiments were done three times.

inflammation in the presence of vitamin D deficiency. In fact, any event that triggers inflammatory response could drive this vicious cycle leading to VDR downregulation and more inflammation, which could in part contribute to SLE disease progression. Therefore, vitamin D therapy, by increasing 1,25-dihydroxyvitamin D concentration and activating VDR, could break this vicious cycle and shift the balance to favor inhibition of inflammation in the management of SLE. Further studies are warranted to test this hypothesis in preclinical and clinical settings.

Although this is the first study, to our knowledge, linking PBMC VDR status to SLE disease severity, there are a number of limitations. The sample size in this study was relatively small; larger multi-center studies may need to confirm our findings. The correlations established in this study do not provide insights into the disease mechanism of SLE, and whether PBMC VDR deficiency is a causative factor for SLE development is unclear. More studies are needed to explore the pathological role of the vitamin D-VDR signaling in SLE in the future. Moreover, the exact mechanism of VDR regulation in PBMCs remains not completely understood. The principle established based on the cell line data needs to be validated in primary cells, as cell lines are not exactly the same as primary cells. Finally, given the difference seen in the suppressive effect of TNF-α on VDR in THP-1 and Jurkat cells, it is conceivable that the relative contribution of vitamin D induction and vitamin D blocking of TNF-α suppression to VDR expression could be different in different PBMC subsets. This issue also needs further exploration.

Acknowledgments We thank all of the patients for their participation in this study.

Funding sources This study was funded by the National Natural Science Foundation of China (No. 81470961 and 2019JJ40463) and the Natural Science Foundation of Hunan Province (NO. 14JJ2037 and 2019JJ40463).

Compliance with ethical standards

Disclosures None.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

References

1. D'Cruz DP, Khamashta MA, Hughes GR (2007) Systemic lupus erythematosus. Lancet (London, England) 369(9561):587–596
2. Qu B, Cao J, Zhang F, Cui H, Teng J, Li J, Liu Z, Morehouse C, Jallal B, Tang Y, Guo Q, Yao Y, Shen N (2015) Type I interferon inhibition of microRNA-146a maturation through up-regulation of monocyte chemotactic protein-induced protein 1 in systemic lupus erythematosus. Arthritis Rheumatol 67(12):3209–3218
3. Ronnblom L, Pascual V (2008) The innate immune system in SLE: type I interferons and dendritic cells. Lupus 17(5):394–399
4. Schoindre Y, Jallouli M, Tanguy ML, Ghillani P, Galicier L, Aumaitre O, Frances C, Le Guern V, Liote F, Smail A, Limal N, Perard L, Desmurs-Clavel H, Le Thi Huong D, Asli B, Kahn JE, Sailler L, Ackermann F, Papo T, Sacre K, Fain O, Stirnemann J, Cacoub P, Leroux G, Cohen-Bittan J, Hulot JS, Lechat P, Musset L, Piette JC, Amoura Z, Souberbielle JC, Costedoat-Chalumeau N, Group P (2014) Lower vitamin D levels are associated with higher systemic lupus erythematosus activity, but not predictive of disease flare-up. Lupus Sci Med 1(1):e000027. https://doi.org/10.1136/lupus-2014-000027
5. Yap KS, Northcott M, Hoi AB, Morand EF, Nikpour M (2015) Association of low vitamin D with high disease activity in an Australian systemic lupus erythematosus cohort. Lupus Sci Med 2(1):e000064. https://doi.org/10.1136/lupus-2014-000064
6. Dall'Ara F, Cutolo M, Andreoli L, Tincani A, Paolino S (2018) Vitamin D and systemic lupus erythematous: a review of immunological and clinical aspects. Clin Exp Rheumatol 36(1):153–162
7. Chen Y, Zhang J, Ge X, Du J, Deb DK, Li YC (2013) Vitamin D receptor inhibits nuclear factor kappaB activation by interacting with IkappaB kinase beta protein. J Biol Chem 288(27):19450–19458. https://doi.org/10.1074/jbc.M113.467670
8. Xu S, Chen YH, Tan ZX, Xie DD, Zhang C, Zhang ZH, Wang H, Zhao H, Yu DX, Xu DX (2015) Vitamin D3 pretreatment regulates renal inflammatory responses during lipopolysaccharide-induced acute kidney injury. Sci Rep 5:18687. https://doi.org/10.1038/srep18687
9. Sun J, Mustafi R, Cerda S, Chumsangsri A, Xia YR, Li YC, Bissonnette M (2008) Lithocholic acid down-regulation of NF-kappaB activity through vitamin D receptor in colonic cancer cells. J Steroid Biochem Mol Biol 111(1–2):37–40. https://doi.org/10.1016/j.jsbmb.2008.01.003
10. Wu S, Liao AP, Xia Y, Li YC, Li JD, Sartor RB, Sun J (2010) Vitamin D receptor negatively regulates bacterial-stimulated NF-kappaB activity in intestine. Am J Pathol 177(2):686–697
11. Haussler MR, Whitfield GK, Kaneko I, Haussler CA, Hsieh D, Hsieh JC, Jurutka PW (2013) Molecular mechanisms of vitamin D action. Calcif Tissue Int 92(2):77–98. https://doi.org/10.1007/s00223-012-9619-0
12. Bouillon R, Carmeliet G, Verlinden L, van Etten E, Verstuyf A, Luderer HF, Lieben L, Mathieu C, Demay M (2008) Vitamin D and human health: lessons from vitamin D receptor null mice. Endocr Rev 29(6):726–776
13. Levi M (2011) Nuclear receptors in renal disease. Biochim Biophys Acta 1812(8):1061–1067
14. Navarro-Gonzalez JF, Mora-Fernandez C, Muros DFM, Garcia-Perez J (2011) Inflammatory molecules and pathways in the pathogenesis of diabetic nephropathy. Nat Rev Nephrol 7(6):327–340
15. Lin Z, Li W (2016) The roles of vitamin D and its analogs in inflammatory diseases. Curr Top Med Chem 16(11):1242–1261
16. Zhang Z, Sun L, Wang Y, Ning G, Minto AW, Kong J, Quigg RJ, Li YC (2008) Renoprotective role of the vitamin D receptor in diabetic nephropathy. Kidney Int 73(2):163–171
17. Liu W, Chen Y, Golan MA, Annunziata ML, Du J, Dougherty U, Kong J, Musch M, Huang Y, Pekow J, Zheng C, Bissonnette M, Hanauer SB, Li YC (2013) Intestinal epithelial vitamin D receptor signaling inhibits experimental colitis. J Clin Invest 123(9):3983–3996. https://doi.org/10.1172/JCI65842
18. Zhu Y, Wang PP, Zhai G, Bapat B, Savas S, Woodrow JR, Sharma I, Li Y, Zhou X, Yang N, Campbell PT, Dicks E, Parfrey PS, McLaughlin JR (2017) Vitamin D receptor and calcium-sensing receptor polymorphisms and colorectal cancer survival in the Newfoundland population. Br J Cancer 117(6):898–906. https://doi.org/10.1038/bjc.2017.242
19. Zhou H, Xu C, Gu M (2009) Vitamin D receptor (VDR) gene polymorphisms and Graves' disease: a meta-analysis. Clin Endocrinol 70(6):938–945
20. Cai G, Zhang X, Xin L, Wang L, Wang M, Yang X, Li X, Xia Q, Xu S, Ding C, Pan F (2016) Associations between vitamin D receptor gene polymorphisms and ankylosing spondylitis in Chinese Han population: a case-control study. Osteoporos Int 27(7):2327–2333. https://doi.org/10.1007/s00198-016-3500-3
21. Ozaki Y, Nomura S, Nagahama M, Yoshimura C, Kagawa H, Fukuhara S (2000) Vitamin-D receptor genotype and renal disorder in Japanese patients with systemic lupus erythematosus. Nephron 85(1):86–91
22. Luo XY, Yang MH, Wu FX, Wu LJ, Chen L, Tang Z, Liu NT, Zeng XF, Guan JL, Yuan GH (2012) Vitamin D receptor gene BsmI polymorphism B allele, but not BB genotype, is associated with systemic lupus erythematosus in a Han Chinese population. Lupus 21(1):53–59
23. Tiao J, Feng R, Carr K, Okawa J, Werth VP (2016) Using the American College of Rheumatology (ACR) and Systemic Lupus International Collaborating Clinics (SLICC) criteria to determine the diagnosis of systemic lupus erythematosus (SLE) in patients with subacute cutaneous lupus erythematosus (SCLE). J Am Acad Dermatol 74(5):862–869. https://doi.org/10.1016/j.jaad.2015.12.029
24. Chan TM, Li FK, Tang CS, Wong RW, Fang GX, Ji YL, Lau CS, Wong AK, Tong MK, Chan KW, Lai KN (2000) Efficacy of mycophenolate mofetil in patients with diffuse proliferative lupus nephritis. Hong Kong-Guangzhou Nephrology Study Group N Engl J Med 343(16):1156–1162. https://doi.org/10.1056/nejm200010193431604
25. Gladman DD, Ibanez D, Urowitz MB (2002) Systemic lupus erythematosus disease activity index 2000. J Rheumatol 29(2):288–291
26. Abdel GSM, Ezzeldin N, El-Boshy ME (2015) The role of serum IL-17 and IL-6 as biomarkers of disease activity and predictors of remission in patients with lupus nephritis. Cytokine 76(2):280–287
27. Ulmer AJ, Scholz W, Ernst M, Brandt E, Flad HD (1984) Isolation and subfractionation of human peripheral blood mononuclear cells (PBMC) by density gradient centrifugation on Percoll. Immunobiology 166(3):238–250
28. Petri M, Kasitanon N, Lee SS, Link K, Magder L, Bae SC, Hanly JG, Isenberg DA, Nived O, Sturfelt G, van Vollenhoven R, Wallace DJ, Alarcon GS, Adu D, Avila-Casado C, Bernatsky SR, Bruce IN,

Clarke AE, Contreras G, Fine DM, Gladman DD, Gordon C, Kalunian KC, Madaio MP, Rovin BH, Sanchez-Guerrero J, Steinsson K, Aranow C, Balow JE, Buyon JP, Ginzler EM, Khamashta MA, Urowitz MB, Dooley MA, Merrill JT, Ramsey-Goldman R, Font J, Tumlin J, Stoll T, Zoma A (2008) Systemic lupus international collaborating clinics renal activity/response exercise: development of a renal activity score and renal response index. Arthritis Rheum 58(6):1784–1788

29. Pitashny M, Schwartz N, Qing X, Hojaili B, Aranow C, Mackay M, Putterman C (2007) Urinary lipocalin-2 is associated with renal disease activity in human lupus nephritis. Arthritis Rheum 56(6): 1894–1903
30. Sabry A, Sheashaa H, El-Husseini A, Mahmoud K, Eldahshan KF, George SK, Abdel-Khalek E, El-Shafey EM, Abo-Zenah H (2006) Proinflammatory cytokines (TNF-alpha and IL-6) in Egyptian patients with SLE: its correlation with disease activity. Cytokine 35(3–4):148–153
31. Chen Y, Du J, Zhang Z, Liu T, Shi Y, Ge X, Li YC (2014) MicroRNA-346 mediates tumor necrosis factor alpha-induced downregulation of gut epithelial vitamin D receptor in inflammatory bowel diseases. Inflamm Bowel Dis 20(11):1910–1918. https://doi.org/10.1097/MIB.0000000000000158
32. Waldner H (2009) The role of innate immune responses in autoimmune disease development. Autoimmun Rev 8(5):400–404. https://doi.org/10.1016/j.autrev.2008.12.019
33. Ball EM, Gibson DS, Bell AL, Rooney MR (2014) Plasma IL-6 levels correlate with clinical and ultrasound measures of arthritis in patients with systemic lupus erythematosus. Lupus 23(1):46–56. https://doi.org/10.1177/0961203313512882
34. Solus JF, Chung CP, Oeser A, Li C, Rho YH, Bradley KM, Kawai VK, Smith JR, Stein CM (2015) Genetics of serum concentration of IL-6 and TNFalpha in systemic lupus erythematosus and rheumatoid arthritis: a candidate gene analysis. Clin Rheumatol 34(8): 1375–1382. https://doi.org/10.1007/s10067-015-2881-6
35. Cen H, Zhou M, Leng RX, Wang W, Feng CC, Li BZ, Zhu Y, Yang XK, Yang M, Zhai Y, Zhang M, Hu LF, Li R, Chen GM, Chen H, Pan HF, Li XP, Ye DQ (2013) Genetic interaction between genes involved in NF-kappaB signaling pathway in systemic lupus erythematosus. Mol Immunol 56(4):643–648
36. Chen Y, Kong J, Sun T, Li G, Szeto FL, Liu W, Deb DK, Wang Y, Zhao Q, Thadhani R, Li YC (2011) 1,25-Dihydroxyvitamin D(3) suppresses inflammation-induced expression of plasminogen activator inhibitor-1 by blocking nuclear factor-kappaB activation. Arch Biochem Biophys 507(2):241–247
37. D'Ambrosio D, Cippitelli M, Cocciolo MG, Mazzeo D, Di Lucia P, Lang R, Sinigaglia F, Panina-Bordignon P (1998) Inhibition of IL-12 production by 1,25-dihydroxyvitamin D3. Involvement of NF-kappaB downregulation in transcriptional repression of the p40 gene. J Clin Invest 101(1):252–262
38. Deb DK, Chen Y, Zhang Z, Zhang Y, Szeto FL, Wong KE, Kong J, Li YC (2009) 1,25-Dihydroxyvitamin D3 suppresses high glucose-induced angiotensinogen expression in kidney cells by blocking the NF-{kappa}B pathway. Am J Physiol Renal Physiol 296(5): F1212–F1218
39. Chung J, Koyama T, Ohsawa M, Shibamiya A, Hoshi A, Hirosawa S (2007) 1,25(OH)(2)D(3) blocks TNF-induced monocytic tissue factor expression by inhibition of transcription factors AP-1 and NF-kappaB. Lab Investig 87(6):540–547. https://doi.org/10.1038/labinvest.3700550
40. Song Y, Hong J, Liu D, Lin Q, Lai GJSJoI (2013) 1,25-dihydroxyvitamin D3 inhibits nuclear factor kappa B activation by stabilizing inhibitor IκBα via mRNA stability and reduced phosphorylation in passively sensitized human airway smooth muscle cells. 77(2):109–116
41. Conceicao EP, Moura EG, Manhaes AC, Carvalho JC, Nobre JL, Oliveira E, Lisboa PC (2016) Calcium reduces vitamin D and glucocorticoid receptors in the visceral fat of obese male rats. J Endocrinol 230(2):263–274. https://doi.org/10.1530/joe-16-0041
42. Hidalgo AA, Trump DL, Johnson CS (2010) Glucocorticoid regulation of the vitamin D receptor. J Steroid Biochem Mol Biol 121(1–2):372–375. https://doi.org/10.1016/j.jsbmb.2010.03.081
43. Mosca M, Tani C, Filice ME, Carli L, Delle SA, Vagnani S, Della RA, Baldini C, Bombardieri S (2015) TNF-alpha inhibitors in systemic lupus erythematosus. A case report and a systematic literature review. Mod Rheumatol 25(4):642–645
44. Robinson ES, Werth VP (2015) The role of cytokines in the pathogenesis of cutaneous lupus erythematosus. Cytokine 73(2):326–334
45. Zella LA, Meyer MB, Nerenz RD, Lee SM, Martowicz ML, Pike JW (2010) Multifunctional enhancers regulate mouse and human vitamin D receptor gene transcription. Mol Endocrinol 24(1):128–147. https://doi.org/10.1210/me.2009-0140