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Wnt pathway regulates IL-34 level in lupus nephritis

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Abstract. – OBJECTIVE: The aim of this study was to investigate the role of the Wnt pathway in regulating the IL-34 level of lupus nephritis (LN) patients, and to explore the underlying mechanism.

MATERIALS AND METHODS: Human mesangial cells (HMCs) of LN patients were selected. The expression level of IL-34 was detected by quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) and Western blot, respectively. Subsequently, HMCs were treated with the Wnt pathway antagonist, DDK1. Meanwhile, the IL-34 level in DDK1 transfected HMCs was then detected. In addition, the viability of HMCs treated with DDK1 was detected by cell counting kit-8 (CCK-8) and colony formation assay, respectively.

RESULTS: Both the mRNA and protein levels of IL-34 were significantly upregulated in HMCs of LN patients. Higher expression of β-catenin was observed in HMCs of LN patients than those of controls, which was reduced after DDK1 treatment. Meanwhile, IL-34 level in HMCs of LN patients was significantly downregulated after DDK1 treatment. In addition, DDK1 treatment remarkably increased the proliferative ability and colony formation ability of HMCs in LN patients.

CONCLUSIONS: IL-34 is highly expressed in HMCs of LN patients and is negatively regulated by the Wnt pathway. Furthermore, HMCs viability is remarkably enhanced after blocking the Wnt pathway.

Key Words:
Lupus nephritis, Wnt pathway, IL-34.

Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease caused by multiple factors related to heredity, estrogen, infection, injury and environment. These factors lead to continuous activation of a variety of inflammatory genes, resulting in pathological inflammation that mediates tissue damage, repair and proliferation in the body[1,2]. SLE involves multiple organ tissue damage, including kidneys, blood vessels, skin, joints and hematopoietic systems. Among the affected organs in SLE, the kidney is the most serious one. Kidney injury is almost observed in every SLE patient from pathological biopsy[3]. Some studies[4-7] have shown that about 60% of SLE patients suffer from lupus nephritis (LN). LN is one of the most common comorbidities of SLE. Meanwhile, it is also the leading cause of SLE-related deaths. Although the pathogenesis of SLE has been widely studied, further in-depth researches are still needed to elucidate the occurrence and progression of LN.

The pathogenesis of LN is very complicated, and its exact mechanism is still unclear. It is generally believed that abnormal activation of the autoimmune system in SLE resulted from genetic, endocrine and environmental factors (infection, ultraviolet radiation, etc.) further leads to the occurrence of LN. In addition, inflammation is also considered a vital factor in SLE-induced kidney injury[8]. Currently, LN belongs to a type of nephritis caused by the deposition of immune complexes. Typical symptoms of LN include hematuria, proteinuria, renal dysfunction and others. It is estimated that 5-10% of LN patients may deteriorate within 10 years, eventually developing into end-stage renal disease and renal failure[2,8]. Previous researches on the pathogenesis of LN have indicated that patients with immune-regulatory dysfunction are easily invaded by exogenous antigens or endogenous antigens. Subsequently, abnormal proliferation of B lymphocytes allows the production of multiple autoantibodies. This may eventually lead to the formation of immune complexes after binding to corresponding antigens. Furthermore, these immune complexes finally deposit in different parts of the glomerulus, leading to LN[9].

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The pathological development of LN has been widely investigated. Some studies have shown that stem cell maintenance, multiple embryonic developments, and immune-related signaling networks all participate in the pathogenesis of LN. Abnormalities of the Wnt pathway have been observed in many autoimmune diseases, including renal diseases. Wnt exerts a crucial role in a series of physiological processes, such as cell proliferation, differentiation, apoptosis, survival, necrosis and migration[10,11]. In the cascade reaction of the classical Wnt signaling pathway dependent on β-catenin, extracellular Wnt blocks the degradation of complexes by binding to Frizzled and LDH receptor-related proteins. This finally leads to the accumulation of cytoplasmic β-catenin. When a certain amount is reached, β-catenin translocates into the cell nucleus and activates the transcription as well as the expression of downstream genes. Accumulating evidence has proved the specific function of the Wnt/β-catenin pathway in autoimmune diseases, embryonic development and tumorigenesis[12,13]. Previous researches[14,15] of secondary nephritis mainly focused on LN and diabetic nephritis. However, the potential role of the Wnt pathway in LN remains unclear.

IL-34 is a homo-dimeric secreted protein belonging to the novel interleukin, which is one of the major ligands of colony-stimulating factor CSF-1R. It can directly bind to CSF-1R, and serve similar but independent biological functions of CSF-1[16]. IL-34 shows a similar biological role to CSF-1. It mainly regulates the proliferation, activation, survival, chemotaxis and secretion of corresponding cytokines in mononuclear/macrophages, such as IL-6 and IL-8[17-19]. Macrophage-mediated inflammatory damage is greatly involved in the pathogenesis of LN[20,21]. Some studies[22-24] have shown that activated macrophages are observed in proliferative and fibrotic renal tissues. Moreover, the biological effects of IL-34 on macrophages and cellular inflammatory damage suggest its underlying important role in the pathogenesis of LN.

Materials and Methods

Cell Culture

Human mesangial cells (HMCs) were cultured in Hyclone Roswell Park Memorial Institute (RPMI) Medium Modified-1640 (HyClone, South Logan, UT, USA) containing 10% fetal bovine serum (FBS) (Gibco, Grand Island, NY, USA) in a 37°C, 5% CO_2 incubator.

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

Total RNA was extracted from HMCs according to the instructions of TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Extracted RNA was reversely transcribed into complementary deoxyribose nucleic acid (cDNA) in strict accordance with Primescript RT Reagent (TaKaRa, Otsu, Shiga, Japan). QRT-PCR was performed using SYBR® Premix Ex TaqTM (TaKaRa, Otsu, Shiga, Japan) and StepOne Plus Real-time PCR System (Applied Biosystems, Foster City, CA, USA). Specific qRT-PCR reaction conditions were: 94°C for 30 s, 55°C for 30 s and 72°C for 90 s, for a total of 40 cycles. The relative expression level of the target gene was calculated by the $2^{-\Delta\Delta Ct}$ method. β-actin was used as an internal reference in the quantitative analysis of IL-34 and β-catenin expression. Primers used in this study were as follows: IL-34, F: 5'- 0CTTTGGGAAACGAGAATTTGGAGA-3', R: 5'-GCAATCCTGTAGTTGATGGGGAAG-3'; β-catenin, F: 5'-TTCACTCTAGGAATGAAGGTGTGG-3', R: 5'-CGTTTCTTGTAATCTTGTGGCTTG-3'; β-actin: F: 5'-CCTGGCACCCAGCACAAT-3', R: 5'-GCTGATCCACATCTGCTGGAA-3'.

Western Blot

Total protein was extracted using cell lysate (RIPA; Beyotime, Shanghai, China). Protein samples were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). After blocking in the blocking solution for 1 h, the membranes were incubated with primary antibodies at room temperature for 2 h. After washing with Tris-Buffered Saline and Tween 20 (TBST), the membranes were incubated with the corresponding secondary antibody for 2 h at room temperature. Immunoreactive bands were visualized by enhanced chemiluminescence (ECL) and analyzed using Image J software.

Cell Counting Kit-8 (CCK-8) Assay

Cells (100 μL) were seeded into 96-well plates and maintained in a 5% CO_2 incubator at 37°C. Briefly, 10 μL CCK-8 (Dojindo Laboratories, Kumamoto, Japan) reagent was added in each well, followed by incubation for 1-4 hours in the dark. Optical density (OD) value at the wavelength of 450 nm was measured using a microplate reader.

Colony Formation Assay

After transfection for 24-48 h, the cells were seeded into 6-well plates at a density of 500 cells per well, followed by culture in complete medium for 2 weeks. Subsequently, the cells were washed with phosphate-buffered saline (PBS) twice and fixed with 2 mL methanol for 20 min. Then the cells were washed with PBS and stained with 0.1% crystal violet staining solution for 20 min. Finally, formed colonies were observed and captured using a microscope.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 22.0 software (IBM, Armonk, NY, USA) was used for all statistical analysis. GraphPad Prism5.0 (La Jolla, CA, USA) was used for figure editing. Experimental data were expressed by $\bar{x}\pm s$. Categorical data were analyzed by chi-square test. $p<0.05$ was considered statistically significant (*$p<0.05$, **$p<0.01$).

Results

IL-34 Was Upregulated in HMCs of LN Patients

We first detected the expression level of IL-34 in HMCs of LN patients by qRT-PCR and Western blot, respectively. Results showed that compared with those of controls, both the mRNA (Figure 1A) and protein (Figure 1B) levels of IL-34 in HMCs of LN patients were significantly higher ($p<0.05$).

DDK1 Blocked Wnt Pathway by Inhibiting β-Catenin Expression

β-catenin is a key molecule in the Wnt pathway. In our study, we detected the expression level of β-catenin in HMCs. Results found that the expression of β-catenin in HMCs of LN patients was significantly higher than that of controls, suggesting the activation of the Wnt pathway. Subsequently, HMCs were treated with DDK1, the antagonist of the Wnt pathway. DDK1 treatment remarkably downregulated β-catenin expression in HMCs of LN patients, suggesting that the Wnt pathway was blocked ($p<0.01$, Figure 2).

IL-34 was Downregulated in HMCs of LN Patients after Blocking Wnt Pathway

To elucidate the effect of the Wnt pathway on IL-34 level, we detected the expression level of IL-34 in HMCs of LN patients after DDK1 treatment. Results indicated that after DDK1 treatment, the IL-34 level was markedly downregulated in HMCs of LN patients ($p<0.01$, Figure 3).

Enhanced Proliferation in HMCs of LN Patients After Blocking Wnt Pathway

The viability of HMCs in LN patients was determined by CCK-8 and colony formation assay, respectively. As expected, the proliferative ability (Figure 4A) and colony formation ability (Figure 4B) in HMCs of LN patients after DDK1 treatment were significantly promoted ($p<0.05$).

Discussion

SLE is an autoimmune disease; meanwhile, LN is a common complication of SLE. Pathologically, LN is mainly an immune complex-mediated glomerular disease[22-24].

In 2004, Renal Pathology Society proposed that renal glomerular diseases were classified into 6 types, including class I disease (minimal me-

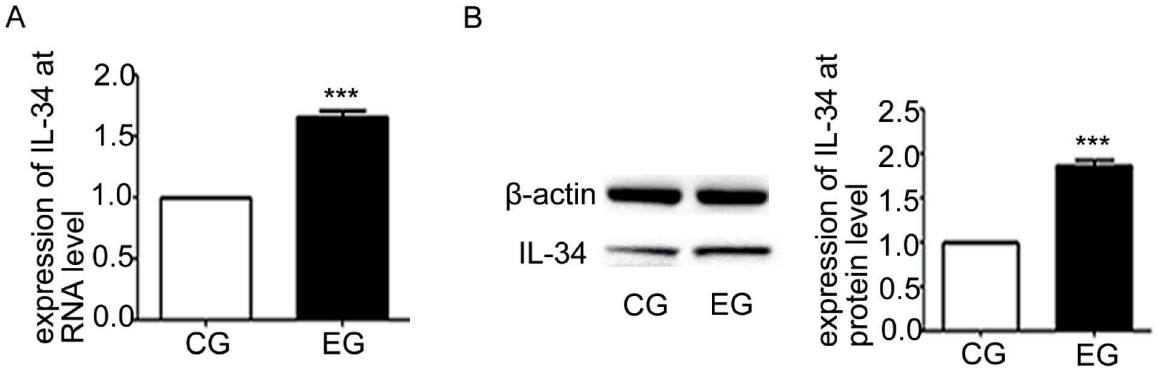

Figure 1. Upregulated mRNA (***A***) and protein (***B***) levels of IL-34 in HMCs of LN patients ($p<0.05$).

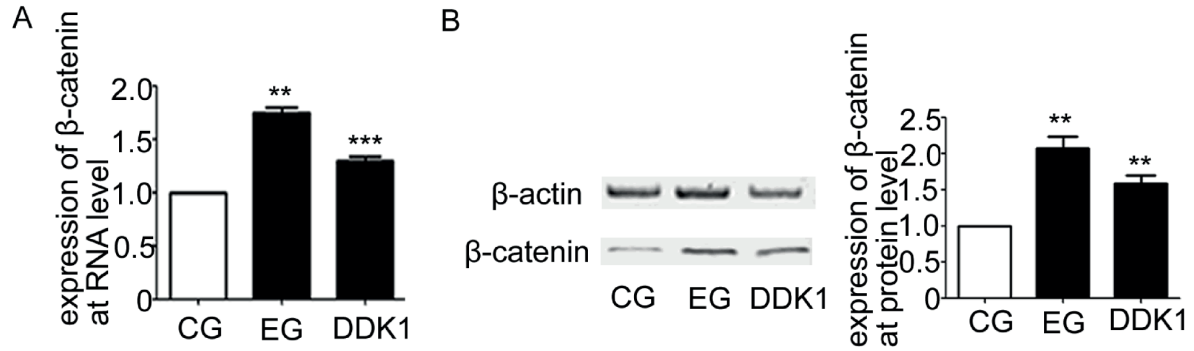

Figure 2. Expression level of β-catenin was upregulated in HMCs of LN patients, which was remarkably downregulated after DDK1 treatment ($p<0.01$).

sangial glomerulonephritis), class II disease (mesangial proliferative glomerulonephritis), class III disease (focal glomerulonephritis), class IV disease (diffuse proliferative nephritis), class V disease (membranous glomerulonephritis) and class VI (advanced sclerosing lupus nephritis). Among them, class IV and V are the most serious, which exert the strongest activity. In addition to glomerular lesions, LN also presents the symptoms of tubulointerstitial nephritis, intrarenal vascular disease and immunological necrotic glomerulonephritis[25,26]. A variety of autoantibodies have been found in LN patients. Meanwhile, immune complexes composed of anti-DNA antibodies are deposited in different parts of the glomerulus. Chemotactic compounds C3a and C5a are subsequently produced by activated complements, eventually leading to an influx of neutrophils and monocytes. These changes are histologically characterized by mesangial foci and diffuse proliferative glomerulonephritis[27].

As the main ligand of colony-stimulating factor CSF-1R, IL-34 is capable of activating macrophages, as well as promoting the proliferation, differentiation, aggregation and secretion of the corresponding cell active factors[17,28]. Studies have shown that IL-34 and CSF-1 have different effects on macrophages. IL-34 strongly activates CSF-1R tyrosine site, FAK and MAPK, instead of CSF-1[29]. Meanwhile, compared with CSF-1, IL-34 plays different roles in macrophage function. IL-34 can stimulate the production of MCP-1 released by macrophages. However, this effect of IL-34 is remarkably weaker than that of CSF-1. Compared with CSF-1, IL-34 exerts stronger effects on promoting the expressions of complement 3a receptor (C3aR1) and cell membrane chemokine receptor (CCR2)[29,30]. An animal experiment has pointed out that the mRNA level of IL-34 in LN mice is remarkably higher[31]. In the present work, we found that IL-34 level in HMC cells of LN patients was significantly higher than that of controls, indicating high expression of IL-34 in the serum of LN patients. Moreover, we believed that the inflammatory injury response occurred in the pathological process of LN.

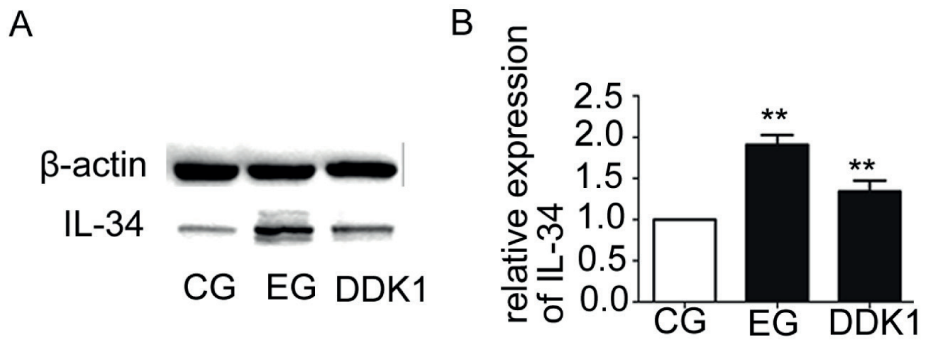

Figure 3. IL-34 level in HMCs of LN patients was downregulated after DDK1 treatment ($p<0.01$).

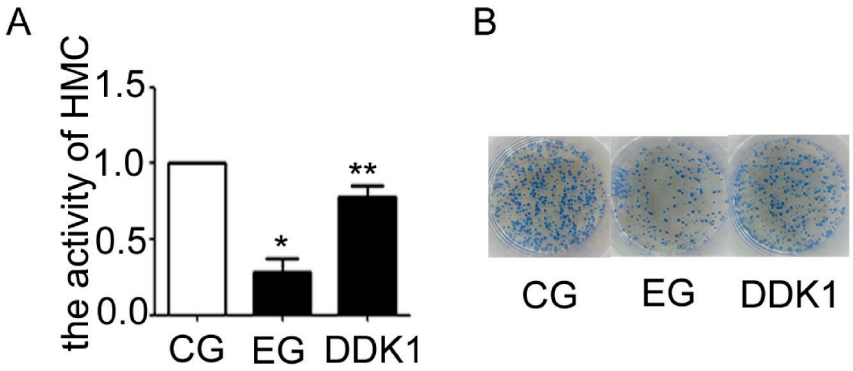

Figure 4. Higher proliferative ability (***A***) and colony formation ability (***B***) were observed in HMCs of LN patients after DDK1 treatment ($p<0.05$).

A growing number of studies have shown that disordered Wnt/β-catenin pathway is involved in the development of various diseases, including autoimmune diseases. Several key molecules in the Wnt/β-catenin pathway have been used as diagnostic and prognostic markers. Investigations have indicated that Wnt is of great significance in skeletal development and homeostasis in adolescents. Meanwhile, its dysregulation is associated with bone pathology[31]. Our findings revealed that the IL-34 level was negatively regulated by the Wnt pathway. Moreover, the blockage of the Wnt pathway significantly improved the proliferation of HMCs in LN patients.

Conclusions

We demonstrated that IL-34 was highly expressed in HMCs of LN patients and was negatively regulated by the Wnt pathway. The viability of HMCs was remarkably enhanced after blocking the Wnt pathway. Our study might provide novel therapeutic targets for the treatment of LN.

Conflict of Interest

The Authors declare that they have no conflict of interest.

References

1) Tsokos GC. Systemic lupus erythematosus. A disease with a complex pathogenesis. Lancet 2001; 358 Suppl: S65.
2) Bell JK, Botos I, Hall PR, Askins J, Shiloach J, Segal DM, Davies DR. The molecular structure of the Toll-like receptor 3 ligand-binding domain. Proc Natl Acad Sci U S A 2005; 102: 10976-10980.
3) Rubin RL, Joslin FG, Tan EM. Specificity of anti-histone antibodies in systemic lupus erythematosus. Arthritis Rheum 1982; 25: 779-782.
4) Singh RP, Waldron RT, Hahn BH. Genes, tolerance and systemic autoimmunity. Autoimmun Rev 2012; 11: 664-669.
5) Sprangers B, Monahan M, Appel GB. Diagnosis and treatment of lupus nephritis flares--an update. Nat Rev Nephrol 2012; 8: 709-717.
6) Yamada A. [Systemic lupus erythematosus]. Ryoikibetsu Shokogun Shirizu 1997: 419-422.
7) Bajaj S, Albert L, Gladman DD, Urowitz MB, Hallett DC, Ritchie S. Serial renal biopsy in systemic lupus erythematosus. J Rheumatol 2000; 27: 2822-2826.
8) Faurschou M, Dreyer L, Kamper AL, Starklint H, Jacobsen S. Long-term mortality and renal outcome in a cohort of 100 patients with lupus nephritis. Arthritis Care Res (Hoboken) 2010; 62: 873-880.
9) Lech M, Anders HJ. The pathogenesis of lupus nephritis. J Am Soc Nephrol 2013; 24: 1357-1366.
10) Ireland SJ, Monson NL, Davis LS. Seeking balance: potentiation and inhibition of multiple sclerosis autoimmune responses by IL-6 and IL-10. Cytokine 2015; 73: 236-244.
11) Long L, Liu Y, Wang S, Zhao Y, Guo J, Yu P, Li Z. Dickkopf-1 as potential biomarker to evaluate bone erosion in systemic lupus erythematosus. J Clin Immunol 2010; 30: 669-675.
12) Tveita A, Rekvig OP, Zykova SN. Glomerular matrix metalloproteinases and their regulators in the pathogenesis of lupus nephritis. Arthritis Res Ther 2008; 10: 229.
13) Guo YZ, Xie XL, Fu J, Xing GL. SOX9 regulated proliferation and apoptosis of human lung carcinoma cells by the Wnt/beta-catenin signaling pathway. Eur Rev Med Pharmacol Sci 2018; 22: 4898-4907.
14) Tveita AA, Rekvig OP. Alterations in Wnt pathway activity in mouse serum and kidneys during lupus development. Arthritis Rheum 2011; 63: 513-522.
15) He W, Dai C, Li Y, Zeng G, Monga SP, Liu Y. Wnt/beta-catenin signaling promotes renal interstitial fibrosis. J Am Soc Nephrol 2009; 20: 765-776.

16) Lee JG, Lee SH, Park DW, Lee SH, Yoon HS, Chin BR, Kim JH, Kim JR, Baek SH. Toll-like receptor 9-stimulated monocyte chemoattractant protein-1 is mediated via JNK-cytosolic phospholipase A2-ROS signaling. Cell Signal 2008; 20: 105-111.

17) Foucher ED, Blanchard S, Preisser L, Garo E, Ifrah N, Guardiola P, Delneste Y, Jeannin P. IL-34 induces the differentiation of human monocytes into immunosuppressive macrophages antagonistic effects of GM-CSF and IFNgamma. PLoS One 2013; 8: e56045.

18) Menke J, Iwata Y, Rabacal WA, Basu R, Stanley ER, Kelley VR. Distinct roles of CSF-1 isoforms in lupus nephritis. J Am Soc Nephrol 2011; 22: 1821-1833.

19) Lin H, Lee E, Hestir K, Leo C, Huang M, Bosch E, Halenbeck R, Wu G, Zhou A, Behrens D, Hollenbaugh D, Linnemann T, Qin M, Wong J, Chu K, Doberstein SK, Williams LT. Discovery of a cytokine and its receptor by functional screening of the extracellular proteome. Science 2008; 320: 807-811.

20) Lech M, Anders HJ. The pathogenesis of lupus nephritis. J Am Soc Nephrol 2013; 24: 1357-1366.

21) Chalmers SA, Chitu V, Ramanujam M, Putterman C. Therapeutic targeting of macrophages in lupus nephritis. Discov Med 2015; 20: 43-49.

22) Wang YF, Xu YX, Tan Y, Yu F, Zhao MH. Clinicopathological characteristics and outcomes of male lupus nephritis in China. Lupus 2012; 21: 1472-1481.

23) Gonzalo E, Toldos O, Martinez-Vidal MP, Ordonez MC, Santiago B, Fernandez-Nebro A, Loza E, Garcia I, Leon M, Pablos JL, Galindo M. Clinicopathologic correlations of renal microthrombosis and inflammatory markers in proliferative lupus nephritis. Arthritis Res Ther 2012; 14: R126.

24) Hill GS, Delahousse M, Nochy D, Remy P, Mignon F, Mery JP, Bariety J. Predictive power of the second renal biopsy in lupus nephritis: significance of macrophages. Kidney Int 2001; 59: 304-316.

25) Vlahakos DV, Foster MH, Adams S, Katz M, Ucci AA, Barrett KJ, Datta SK, Madaio MP. Anti-DNA antibodies form immune deposits at distinct glomerular and vascular sites. Kidney Int 1992; 41: 1690-1700.

26) Rosen A, Casciola-Rosen L, Ahearn J. Novel packages of viral and self-antigens are generated during apoptosis. J Exp Med 1995; 181: 1557-1561.

27) Gaipl US, Munoz LE, Grossmayer G, Lauber K, Franz S, Sarter K, Voll RE, Winkler T, Kuhn A, Kalden J, Kern P, Herrmann M. Clearance deficiency and systemic lupus erythematosus (SLE). J Autoimmun 2007; 28: 114-121.

28) Lin H, Lee E, Hestir K, Leo C, Huang M, Bosch E, Halenbeck R, Wu G, Zhou A, Behrens D, Hollenbaugh D, Linnemann T, Qin M, Wong J, Chu K, Doberstein SK, Williams LT. Discovery of a cytokine and its receptor by functional screening of the extracellular proteome. Science 2008; 320: 807-811.

29) Baek JH, Zeng R, Weinmann-Menke J, Valerius MT, Wada Y, Ajay AK, Colonna M, Kelley VR. IL-34 mediates acute kidney injury and worsens subsequent chronic kidney disease. J Clin Invest 2015; 125: 3198-3214.

30) Barve RA, Zack MD, Weiss D, Song RH, Beidler D, Head RD. Transcriptional profiling and pathway analysis of CSF-1 and IL-34 effects on human monocyte differentiation. Cytokine 2013; 63: 10-17.

31) Bethunaickan R, Berthier CC, Zhang W, Kretzler M, Davidson A. Comparative transcriptional profiling of 3 murine models of SLE nephritis reveals both unique and shared regulatory networks. PLoS One 2013; 8: e77489.