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Angiopoietin-like 4 (ANGPTL4) Suppression Ameliorates Lupus Nephritis in MRL/lpr Mice by Inactivating NLRP3 Inflammasome and Inhibiting Inflammatory Response

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ABSTRACT

Background: Lupus nephritis (LN) refers to the injury caused by systemic lupus erythematosus (SLE) involving the kidneys. A previous study identified angiopoietin-like protein 4 (ANGPTL4) as a novel urinary biomarker for tracking disease activity in LN.

Objective: To investigate the detailed role and regulatory mechanism of ANGPTL4 in experimental models of LN.

Methods: MRL/lpr mice 11-week-old were injected with adeno-associated virus (AAV)-mediated ANGPTL4 short hairpin RNA (shRNA). At 16 and 20 weeks of age, 24-h urine samples were harvested to measure proteinuria levels. After the mice were sacrificed, blood and kidney tissues were harvested to examine serum creatinine (cr) and blood urea nitrogen (BUN) levels, kidney histological changes, and pro-inflammatory cytokine production. Additionally, the levels of NLRP3 inflammasome-associated molecules in mouse renal tissues were detected to clarify the underlying mechanism.

Results: The AAV-sh-ANGPTL4 injection significantly reduced the proteinuria, cr, and BUN levels in MRL/lpr mice. ANGPTL4 silencing ameliorated glomerular, tubular, and interstitial damage in mice, mitigating the pathological alternations of LN. In addition, ANGPTL4 knockdown repressed pro-inflammatory cytokine production in the kidneys. Mechanically, ANGPTL4 suppression inhibited NLRP3 inflammasome expression in renal tissues of mice.

Conclusion: ANGPTL4 silencing inhibits the NLRP3 inflammasome-mediated inflammatory response, thereby ameliorating LN in MRL/lpr mice.

Keywords: ANGPTL4, Inflammation, Lupus Nephritis, NLRP3 Inflammasome, Systemic Lupus Erythematosus

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INTRODUCTION

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease featured by the formation of pathogenic autoantibodies and immune complexes that mediate tissue and organ damage (1). Lupus nephritis (LN) refers to renal injury caused by SLE involving the kidneys, one serious complication of the SLE (2). LN can develop into renal failure at the advanced stage and is an important cause contributing to the death of SLE patients (2). The pathogenesis of LN is multifactorial, in which immune complex formation and deposition are considered to be one of the main pathogenic mechanisms (3). The deposited immune complexes activate the complement system, resulting in inflammatory cell infiltration and inflammatory mediator release, ultimately causing kidney injury (4). There is no standard and unified treatment plan for LN, and different treatment plans should be selected according to the clinical manifestations and pathological types of patients (5). Currently, most patients can be treated with hormones and immunosuppressants (6). However, long-term use of immunosuppressive agents may weaken the immune system, making the body more susceptible to infection (7). Thus, there is an urgent need to find an alternative therapy that can effectively improve LN with few side effects.

Angiopoietin-like protein 4 (ANGPTL4) is a circulating glycoprotein belonging to the angiopoietin-like protein family (8). ANGPTL4 is mainly distributed in the liver, adipose tissue, cardiac muscle, bone muscle, and kidney (9). Since its discovery, the unique biological functions exhibited by ANGPTL4 have been extensively studied. ANGPTL4 participates in multiple physiopathological conditions including lipid metabolism, glucose metabolism, inflammation, angiogenesis, tumorigenesis, energy homeostasis, and wound healing (10). Recently, overexpressing ANGPTL4 in podocytes was suggested to induce a series of characteristic changes including diffuse foot process fusion of podocytes, irregular thickening and thinning of glomerular basement membrane (GBM), and massive selective proteinuria, which thereby result in the development of the nephritic syndrome (11). Clement et al. disclosed that ANGPTL4 was the missing link between hypertriglyceridemia to albuminuria in the nephrotic syndrome (12). In addition, patients with minimal change nephropathy (MCD) present upregulated levels of ANGPTL4 in urine, peripheral serum, and podocytes, and further research has discovered that the excessive secretion of ANGPTL4 in glomerular tissues is closely related to podocyte injury and proteinuria (13). ANGPTL4 was demonstrated to play a vital role in diabetic nephropathy by inhibiting lipoprotein lipase activity, which suggests ANGPTL4 as a biochemical marker to detect diabetic nephropathy in type 2 diabetes (T2D) patients (14). A new quantitative planar protein microarray was used in the previous study to perform unbiased planar array screening of proteins from the urine of SLE patients and identified ANGPTL4 as a new urinary biomarker for tracking disease activity in the LN (15). Nevertheless, the detailed function of ANGPTL4 in the LN has not been clarified so far.

Inflammasomes are large multimeric protein complexes found mainly in innate immune cells that attack pathogens, triggering an inflammatory response in response to exogenous pathogens or endogenous danger signals (16). Typical inflammasomes include NLRP1, NLRP3, NLRC4, and inflammation-related gene absent in melanoma 2 (AIM2) inflammasomes (17). The NLRP3 inflammasome is composed of the receptor protein NLRP3, the adaptor protein apoptosis-associated speck-like protein containing CARD (ASC), and the effector protein caspase-1 (18). It is known that ASC interacts with NLRP3 through the pyrin domain (PYD), ASC interacts with caspase-1 through the caspase recruitment domain (CARD), and NLRP3 interacts

with caspase-1 through ASC, forming a complex (19). Subsequently, NLRP3 activates IL-18 and IL-1β by recruiting and activating procaspase-1, thereby triggering inflammatory responses and mediating the pyroptosis (19). As a key component of innate immunity, the NLRP3 inflammasome plays a pivotal role in the pathogenesis of the LN (20). NLRP3 inflammasome was activated in podocytes from lupus-prone mice and LN patients, causing podocyte injury and proteinuria in the LN (21). In a study by Lu et al., NLRP3 inflammasome hyperactivation in bone marrow cells results in serious renal injury in lupus animal models (22). Hence, targeting the NLRP3 inflammasome may be a potential therapeutic strategy for LN.

In our research, we aimed to figure out the function and therapeutic mechanism of ANGPTL4 in the LN by using a typical mouse model of SLE. We hypothesized that ANGPTL4 might affect the LN progression in lupus-prone mice by regulating NLRP3 inflammasome-mediated inflammatory response. The results might provide a rationale for identifying a new therapeutic target for the LN.

MATERIALS AND METHODS

Animals

Thirty-two female lupus-prone MRL/lpr mice and sixteen age-matched C57BL/6 mice were supplied by the Jackson Laboratory (Bar Harbor, ME, USA). All the mice were maintained in a 12-h light/dark cycle with free access to water and food. The Animal Research Committee of Wuhan Third Hospital and Tongren Hospital of Wuhan University approved all experimental protocols.

Experimental Design and Vector Delivery

All the mice were acclimatized for 2 weeks before the experiments. To investigate the influence of ANGPTL4 suppression on lupus nephritis, 32 MRL/lpr mice were randomized into 2 groups: AAV-sh-ANGPTL4 (n=16) and the control AAV-sh-NC group (n=16). The MRL/lpr mice were administrated at 11 weeks old with 100 μL containing 1.5×10^{11} vector genome (vg) infective units of recombinant adeno-associated virus (AAV; GeneChem, Shanghai, China) via renal pelvis injection using a 29-gauge insulin syringe. The C57BL/6 mice received the same administration. At 16 and 20 weeks, the 24-h urine samples of 8 MRL/lpr mice in each group were collected. Then, MRL/lpr mice were weighed and sacrificed under anesthesia with 1% pentobarbital (50 mg/kg i.p.), and blood and kidney samples were harvested for further analysis. The experiment schedule is shown in Fig. 1A.

Physiological Parameters

The levels of 24-h proteinuria, blood urea nitrogen (BUN), and serum creatinine (cr) were detected, using commercial kits from the Nanjing Jiancheng Bioengineering Institute (Jiangsu, China) to evaluate the kidney functions of mice. Fresh urine samples were centrifuged at 1500 rpm for 10 min at 4°C. The concentrations of urinary proteins in the supernatants were quantified by an assay kit and scored as previously described (23). BUN and cr levels were examined by calculating the OD values at 520 nm and 546 nm, respectively. All the OD values were detected using an ELx800 microplate reader (BioTek, USA).

Enzyme-linked Immunosorbent Assay (ELISA)

The kidney tissue homogenates were centrifuged at 3,000 ×g at 4°C for 10 min. Then, tumor necrosis factor alpha (TNF-α) (#MTA00B), IL-17 (#M1700), monocyte chemoattractant protein-1 (MCP-1) (#DCP00), IL-6 (#M6000B), and IL-1β (#MLB00C) levels in the supernatants were determined using ELISA kits (R&D system, USA). The absorbance was measured at 450 nm using an ELx800 microplate reader.

Renal Histopathology Examination

Kidney tissues were fixed in 4%

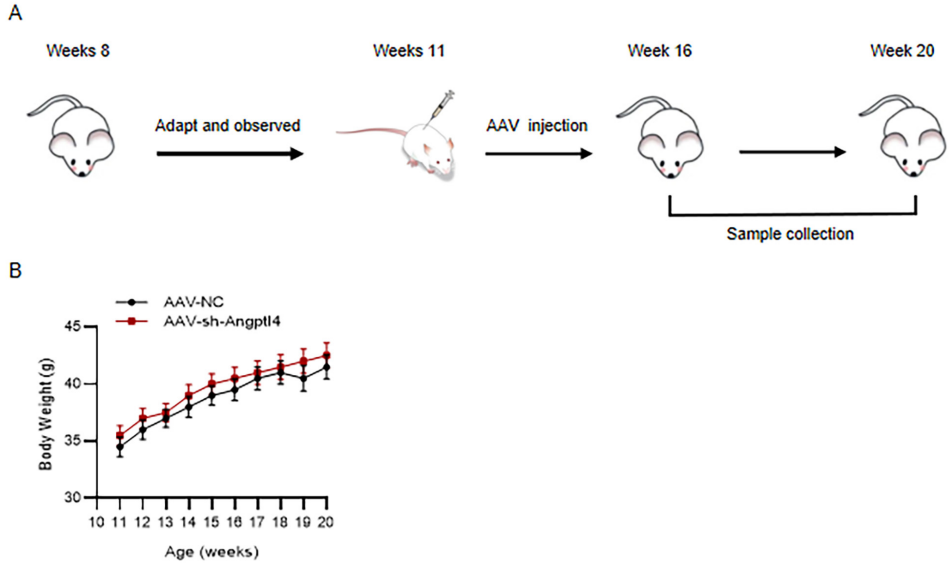

Fig. 1. AAV-sh-ANGPTL4 vector was injected into MRL/lpr mice to assess the influence of ANGPTL4 suppression on lupus nephritis. (A) The experiment schedule. (B) The body weight of MRL/lpr mice with AAV-sh-NC or AAV-sh-ANGPTL4 injection. N=8. Adeno-associated virus (AAV); Negative control (NC); Angiopoietin-like protein 4 (ANGPTL4)

paraformaldehyde, dehydrated by gradient ethanol, embedded in paraffin, serially sectioned to 2-µm, stained with hematoxylin and eosin (H&E), and Masson's trichrome. After dyeing, the slices were washed with water, dehydrated with graded ethanol, transparent with xylene, mounted with neutral gum, and placed under an Olympus BX43 microscope to observe the histopathological alternations. Mesangial proliferation, crescents, tubular atrophy/casts/dilatation, and interstitial congestion/fibrosis were scored as previously described (23).

Real-time Quantitative PCR

The total RNA was extracted from renal tissues using TRIzol reagent (Invitrogen) and reversely transcribed into cDNA using Super M-MLV Reverse Transcriptase (BioTeke, China). The qPCR reaction was conducted in triplicate on the ABI 7500HT Fast Real-Time PCR system (Applied Biosystems; Thermo Fisher Scientific) with the primers of ANGPTL4 (F: 5′-GACTTCAGATGGAGGCTGG-3′, R: 5′-CTTGTAGGCTTCCCAGGAC-3′) and GAPDH (F: 5′-CATCTTCTTGTG CAGTGCC-3′, R: 5′-CAAATCCGTTCA CACCGAC-3′). ANGPTL4 mRNA expression was calculated according to the formula of $2^{-\triangle\triangle ct}$ after normalization to GAPDH.

Western Blot

The kidney tissues were homogenized in RIPA buffer with protease inhibitors. Equal amounts of protein (20 mg/sample) were loaded on 12% SDS–PAGE and transferred to PVDF membranes. The membranes were then blocked with 3% skim milk and probed with primary antibodies, followed by thrice wash with TBST and further incubation with HRP-conjugated secondary antibodies (ab205718, 1/2000). The primary antibodies used were ANGPTL4 (ab196746, 1/2000), NLRP3 (ab270449, 1/1000), procaspase-1 (ab207802, 1/1000), IL-1β (ab254360, 1/1000) and β-actin (ab227387, 1/5000). All antibodies were bought from Abcam (Cambridge, UK). Protein bands were visualized by super ECL detection reagent (Proteintech, USA) and quantified by Image J software.

Statistical Analysis

SPSS 23.0 (IBM Corp., Armonk, N.Y., USA) software was used for the data analysis. The quantitative data were presented as the mean±SD from three independent experiments. Differences were assessed using

one-way ANOVA (among multiple groups) and unpaired Student's t-test (between two groups). $p<0.05$ indicated a statistically significant difference.

RESULTS

Knockdown of ANGPTL4 Inhibits ANGPTL4 Expression in Renal Tissues

First, we discovered that the administration of the AAV-sh-ANGPTL4 vector did not influence the weight of MRL/lpr mice (Fig. 1B). After the mice were sacrificed at 16 and 20 weeks of age respectively, the renal tissues were collected and ANGPTL4 expression was detected. As shown by western blot and RT-qPCR analyses, ANGPTL4 mRNA and protein levels in the 20-week-old group are higher than in the 16-week-old one (Figs. 2A-C). Nevertheless, the injection of AAV-sh-ANGPTL4 decreased ANGPTL4 mRNA and protein expression at 16 and in 20-week-old mice (Figs. 2A-C).

Knockdown of ANGPTL4 Reduces Proteinuria and Improves Renal Function

The urine and blood samples of MRL/lpr mice with 16 and 20-week-age were collected. After detection, we discovered that the proteinuria level in the 20-week-old group was considerably higher than in the 16-week-old one, whereas AAV-sh-ANGPTL4 injection significantly reduced the proteinuria levels in both groups of mice (Fig. 3A). Similarly, cr and BUN levels in mice injected with AAV-sh-NC also increased with time, while the injection of AAV-sh-ANGPTL4 markedly reduced their levels (Figs. 3B-C). These data suggested that ANGPTL4 downregulation protected renal function in MRL/lpr mice.

ANGPTL4 Knockdown Ameliorates Glomerular, Tubular, and Interstitial Damage

Next, kidney tissues of C57BL/6 mice and 16 and 20-week-age MRL/lpr mice were harvested and subjected to H&E and Masson staining to observe the pathological alterations of LN. Under light microscopy, we discovered that both 16 and 20-week-age MRL/lpr mice exhibited marked LN pathological alterations compared with the C57BL/6 mice. Furthermore, the control MRL/lpr mice presented more obvious pathological features than ANGPTL4-downregulated MRL/lpr mice, including endothelial and mesangial cell proliferation, inflammatory cell infiltration, renal interstitial fibrosis, basement membrane thickening, and even crescent formation (Figs. 4A-E). As shown by H&E staining, tubular epithelial cell necrosis or degeneration, tubular atrophy and dilation, tubular type formation, inflammatory cell

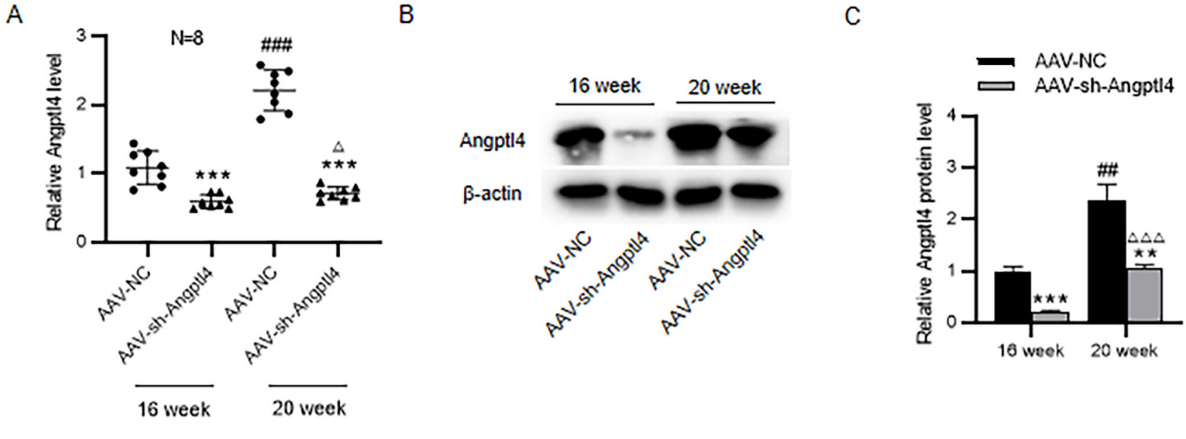

Fig. 2. Knockdown of ANGPTL4 inhibits ANGPTL4 expression in renal tissues. (A-C) Kidney tissues of mice were harvested to evaluate ANGPTL4 level by RT-qPCR and western blot analyses. N=8. **p<0.01, ***p<0.001 versus AAV-NC group at the same time; ##p<0.01, ###p<0.001 versus AAV-NC group at 16 weeks; △p<0.05, △△△p<0.001 versus AAV-sh-Angptl4 group at 16 weeks. Adeno-associated virus (AAV); Negative control (NC); Angiopoietin-like protein 4 (ANGPTL4)

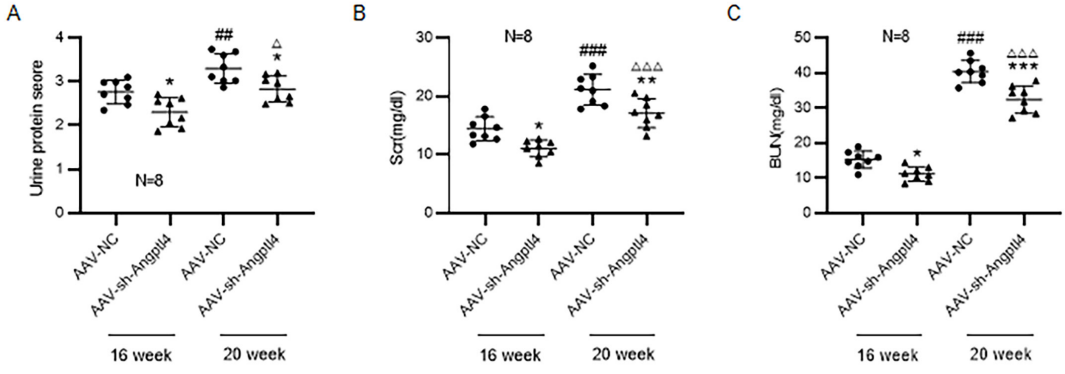

Fig. 3. Knockdown of ANGPTL4 reduces proteinuria and improves renal function. (A) Urine protein levels, (B) serum creatinine levels, and (C) blood urea nitrogen levels in mice. N=8. *p<0.05, **p<0.01, ***p<0.001 versus AAV-NC group at the same time; ##p<0.01, ###p<0.001 versus AAV-NC group at 16 weeks; Δp<0.05, ΔΔΔp<0.001 versus AAV-sh-Angptl4 group at 16 weeks. Adeno-associated virus (AAV); Negative control (NC); Angiopoietin-like protein 4 (ANGPTL4); Blood urea nitrogen (BUN); Serum creatinine (Scr)

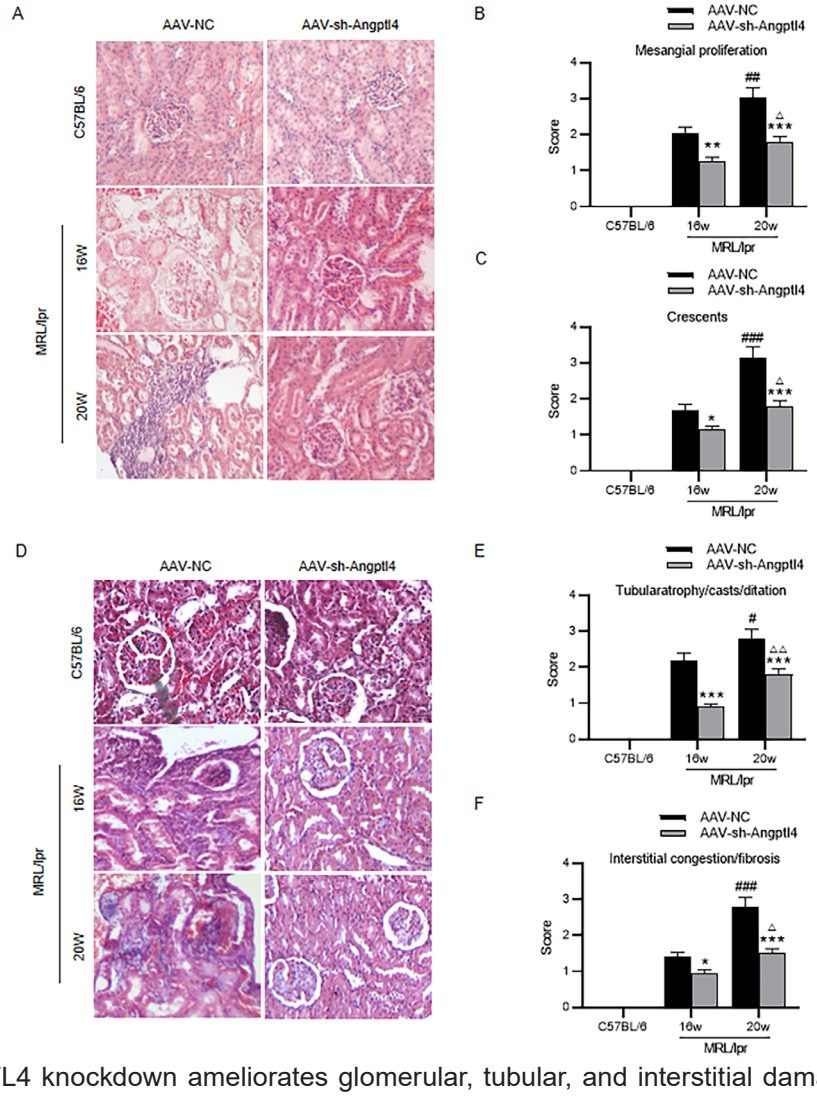

Fig. 4. ANGPTL4 knockdown ameliorates glomerular, tubular, and interstitial damage. (A) HE and (D) Masson staining showing histopathological phenotypes of the renal tissues in C57BL/6 and MRL/lpr mice. (B-C, E-F) Scoring results of histopathological features. N=8. *p<0.05, **p<0.01, ***p<0.001 versus AAV-NC group at the same time; #p<0.05, ##p<0.01, ###p<0.001 versus AAV-NC group at 16 weeks; Δp<0.05, ΔΔp<0.01 versus AAV-sh-Angptl4 group at 16 weeks.Adeno-associated virus (AAV); Negative control (NC); Angiopoietin-like protein 4 (ANGPTL4)

infiltration, renal interstitial congestion, and fibrosis can be observed in the control MRL/lpr mice. Masson staining showed obvious renal interstitial deposition of collagen fibers, basement membrane thickening, and the formation of fibrous crescents in glomeruli in the control MRL/lpr mice. Additionally, older MRL/lpr mice had more severe kidney damage than the younger MRL/lpr mice, as the pathological changes were more pronounced in the 20-week-old group than in the 16-week-old group. Nevertheless, AAV-sh-ANGPTL4 injection remarkably mitigated the above-mentioned pathological phenotypes. Therefore, the inhibition of ANGPTL4 ameliorated glomerular, tubular, and interstitial damage in LN murine models.

ANGPTL4 knockdown Suppresses Pro-inflammatory Cytokine Production in the Kidney

To determine the influence of ANGPTL4 suppression on kidney inflammation, pro-inflammatory cytokine levels were measured in mouse kidney tissues. As shown by ELISA, ANGPTL4-interfered mice had notably lower renal levels of TNF-α, IL-17, MCP-1, IL-6, and IL-1β than the control mice, whereas these levels increased with age (Figs. 5A-E). These findings suggested that interfering ANGPTL4 alleviated renal inflammation by reducing the release of pro-inflammatory cytokines.

ANGPTL4 Suppression Inhibits the NLRP3 Inflammasome

Since the occurrence of inflammation is closely linked with the NLRP3 inflammasome, we evaluated the impacts of ANGPTL4 depletion on the NLRP3 inflammasome in MRL/lpr mice. Western blot manifested that the control MRL/lpr mice displayed much higher protein levels of NLRP3, procaspase-1, and IL-1β compared

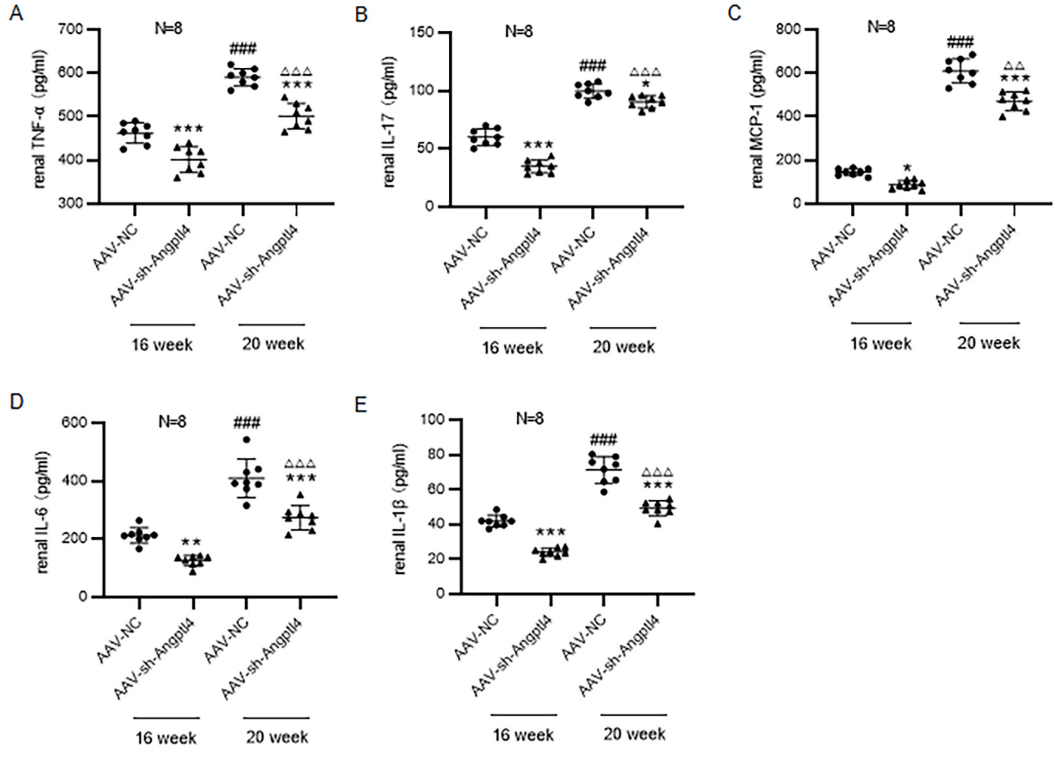

Fig. 5. ANGPTL4 knockdown suppresses pro-inflammatory cytokine production in the kidney. (A-E) The measurement of pro-inflammatory cytokine levels in renal tissues by using ELISA kits. N=8. *p<0.05, **p<0.01, ***p<0.001 versus AAV-NC group at the same time; ###p<0.001 versus AAV-NC group at 16 weeks; ΔΔp<0.01, ΔΔΔp<0.001 versus AAV-sh-Angptl4 group at 16 weeks. Adeno-associated virus (AAV); Negative control (NC); Angiopoietin-like protein 4 (ANGPTL4); Tumor necrosis factor alpha (TNF-α); Monocyte chemoattractant protein-1 (MCP-1); Interleukin-1 beta (IL-1β); Interleukin-6 (IL-6); Interleukin-17 (IL-17)

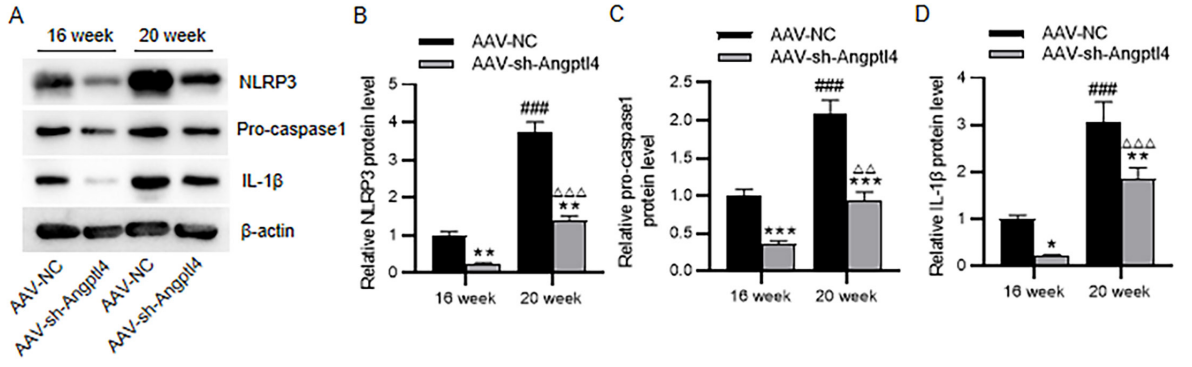

Fig. 6. ANGPTL4 suppression inhibits the NLRP3 inflammasome. (A-D) The detection of NLRP3 inflammasome-associated protein (NLRP3, procaspase-1, and IL-1β) levels by western blot analysis. N=8. *p<0.05, **p<0.01, ***p<0.001 versus AAV-NC group at the same time; ###p<0.001 versus AAV-NC group at 16 weeks; ΔΔp<0.01, ΔΔΔp<0.001 versus AAV-sh-Angptl4 group at 16 weeks. Adeno-associated virus (AAV); Negative control (NC); Angiopoietin-like protein 4 (ANGPTL4); Interleukin-1 beta (IL-1β); NOD-like receptor thermal protein domain associated protein 3 (NLRP3)

with the ANGPTL4-interferred MRL/lpr mice, and the levels of these NLRP3 inflammasome-associated proteins increased with time (Figs. 6A-D). Hence, the activation of NLRP3 inflammasome in MRL/lpr mice was inhibited after interfering with the ANGPTL4.

DISCUSSION

Since patients are often refractory to conventional treatments, SLE remains a formidable challenge for clinicians despite considerable progress in treatment modalities, highlighting the urgency to develop more effective therapies. In the current study, we adopted MRL/lpr mice, the well-established mouse model of SLE, to assess the potential of ANGPTL4 as a therapeutic target for LN. Our findings revealed that the knockdown of ANGPTL4 reduced proteinuria, improved renal functions, ameliorated renal histological change, and suppressed pro-inflammatory cytokine production in MRL/lpr mice. Mechanistically, such protective effects of ANGPTL4 silencing against renal damage and inflammation were achieved by the inhibition of NLRP3 inflammasome. Therefore, inhibiting ANGPTL4 expression is expected as a feasible therapeutic method for human LN.

MRL/lpr mice, a representative animal model to study lupus pathogenesis, have been extensively employed to evaluate the effectiveness of the lupus treatment (24). MRL/Lpr mice spontaneously develop systemic autoimmune diseases consistent with human SLE pathology at 16 weeks of age, such as lymphadenopathy, T-cell dysplasia, arthritis, and immune-complex glomerulonephritis (25). Furthermore, lupus-like alternations in MRL/lpr mice are linked with age (26). Herein, MRL/lpr mice in our experiments presented proteinuria, BUN, and creatinine levels that increased with age, indicating the occurrence of kidney damage. Additionally, Vanarsa et al. discovered that urinary ANGPTL4 markedly increased in active renal SLE patients compared with the healthy subjects. Urinary ANGPTL4 was remarkably upregulated in active renal SLE patients compared with the active non-renal SLE patients, suggesting that ANGPTL4 is indicative of LN in SLE patients (15). Nevertheless, the possible mechanism by which ANGPTL4 is implicated in LN development has not been elucidated yet. Previously, the involvement of ANGPTL4 in the development of kidney diseases has been investigated. ANGPTL4 expression significantly elevated in adipose, liver, and kidney tissues of cisplatin-treated mice, and peroxisome proliferator–activated receptor α (PPARα) ligand alleviates cisplatin-induced

acute kidney injury in mice by decreasing ANGPTL4 expression in proximal tubules (27). Epigallocatechin-3-gallate ameliorates Adriamycin-induced focal and segmental glomerular sclerosis in C57BL/6 mice by suppressing oxidative stress and apoptosis through the inhibition of ANGPTL4 (28). Thus, in our study, we silenced the expression of ANGPTL4 in MRL/lpr mice by utilizing AAV-sh-ANGPTL4 injection. We discovered that AAV-sh-ANGPTL4 injection considerably reduced proteinuria, cr, and BUN levels in MRL/lpr mice. Besides, ANGPTL4 silencing attenuated the pathological alterations in mouse renal tissues. These data suggested the protective role of ANGPTL4 silencing against renal injury. The immune inflammatory response is a crucial feature of the SLE occurrence and development (29). As mentioned earlier, the kidney inflammatory responses severely impaired renal functions, accelerating the progression of LN (30). In the LN kidney tissues, the increased secretion of various cytokines and the disturbance of the balance of cytokines with different biological effects are the molecular biological basis of immune inflammatory response (31). Many studies have shown that TNF-α, IFN-γ, MCP-1, IL-1, IL-10, IL-17, and other cytokines participate in regulating SLE development and determine the damage degrees of involved organs (31). Particularly, MCP-1 is a pro-inflammatory chemokine that plays a critical role in the physiopathological process of the immune response of the body, mainly mediating the migration and infiltration of macrophages and monocytes into the inflammatory sites (32). Large amounts of immune complexes deposited in the glomerular basement membrane can stimulate the secretion and expression of MCP-1 in the intrinsic renal cells and inflammatory cells, further promoting monocyte/macrophage infiltration and mediating LN disease activity (33). The anti-inflammatory role of ANGPTL4 silencing has been demonstrated in multiple human diseases. ANGPTL4 knockdown reduces pro-inflammatory cytokine expression in TNF-α-treated chondrocytes as well as inhibiting inflammation in mouse models of osteoarthritis (34). ANGPTL4 expression obviously elevated in both the imiquimod-induced human keratinocytes cells and the mice with imiquimod-induced psoriasiform dermatitis, and ANGPTL4 silencing effectively repress massive keratinocyte proliferation and inflammatory leukocyte infiltration (35). ANGPTL4 expression increased both in lipopolysaccharide-treated human alveolar epithelial A549 cells and the murine model of acute lung injury, and the silencing of ANGPTL4 greatly reduces TNF-α and IL-6 expression, suppresses neutrophil infiltration in lung tissues and improves lung inflammation in vivo as well as decreasing TNF-α and IL-6 expression and apoptosis rate in vitro (36). In our study, we discovered that TNF-α, IL-17, MCP-1, IL-6, and IL-1β levels in kidney tissues of MRL/lpr mice notably reduced after AAV-sh-ANGPTL4 injection, indicating the anti-inflammatory role of ANGPTL4 knockdown in LN.

The NLRP3 inflammasome, a group of protein complexes, is known as the core of the inflammatory reactions, closely associated with the occurrence of inflammation and various autoimmune diseases (37). The NLRP3 inflammasome is gaining increasing attention as an important contributor to LN in mouse models (38). Numerous studies have also disclosed that the progression of LN can be alleviated by the inhibition of NLRP3 inflammasome activation. For example, piperine treatment inhibits NLRP3 inflammasome and reduces serum level of IL-1β in BALB/c mice with pristane-induced LN (39). The administration of icariin in MRL/lpr mice reduces immune complex deposition, restrains macrophage infiltration, and suppresses NLRP3 inflammasome activation, attenuating LN progression (40). In our paper, we observed upregulated NLRP3, procaspase-1, and IL-1β levels in 20-week-old versus 16-week-old MRL/lpr mice, suggesting the activation of NLRP3 inflammasome in LN murine models. However, the injection of AAV-

sh-ANGPTL4 reduced NLRP3, procaspase-1, and IL-1β in MRL/lpr mice, indicating that ANGPTL4 silencing inhibits the inflammation in LN through the suppression of NLRP3 inflammasome.

MRL/lpr mice produce SLE symptoms similar to those in humans, and their study sheds light on many questions in human SLE pathogenesis, with important implications for the current development of new therapies. However, due to the multifactorial and complex nature of human SLE pathogenesis, mouse models alone cannot fully reveal the causes and pathological mechanisms of human pathogenesis. The pathogenesis cannot include all factors that affect humans, such as multiple genetic factors, estrogen, major histocompatibility complex genes, and environmental factors. Therefore, other lupus model mice or animals should be used in combination to elucidate the complexity of human pathogenesis and pathological changes.

Overall, our research affirms that the therapeutic effects of ANGPTL4 silencing in experimental LN is related to the inhibition of the NLRP3 inflammasome. This finding identifies a novel therapeutic target for the LN treatment and uncovers mechanistic insights that may aid in the future development of novel therapeutics with greater specificity and safety.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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