Inflammation, Vol. 42, No. 4, August 2019 (© 2019)
DOI: 10.1007/s10753-019-00993-4

ORIGINAL ARTICLE

Amelioration of Lipopolysaccharide-Induced Nephrotic Proteinuria by NFAT5 Depletion Involves Suppressed NF-κB Activity

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Abstract— Idiopathic nephrotic syndrome (INS) is characterized by proteinuria, in which podocyte dysfunction associated with NF-κB-mediated inflammation plays an important role. The nuclear factor of activated T cells 5 (NFAT5) has been shown to enhance NF-κB activity. However, whether NFAT5 is associated with proteinuria remains uncharacterized. NFAT5 is upregulated in the glomeruli in lipopolysaccharide (LPS)-induced mouse nephrotic proteinuria, as well as in LPS-treated podocytes *in vitro*. In addition, NFAT5 depletion improves filtration barrier function of LPS-treated podocytes *in vitro*. Mechanistically, NFAT5 depletion suppresses NF-κB activation and downstream proinflammatory reaction in LPS-treated podocytes, and moreover, NF-κB inhibition improves filtration barrier function of LPS-treated podocytes, suggesting that the suppressed NF-κB activity at least partly accounts for NFAT5 depletion-improved filtration barrier function. Furthermore, *in vivo*, depletion of NFAT5 suppresses NF-κB activity and ameliorates nephrotic proteinuria in LPS-treated mice. These findings suggest a protective role of NFAT5 depletion against LPS-induced nephrotic proteinuria and relate it to the suppression of NF-κB activity.

KEY WORDS: idiopathic nephrotic syndrome; NFAT5; lipopolysaccharide; nephrotic proteinuria; NF-κB.

INTRODUCTION

Proteinuria is commonly caused by the dysfunction of kidney glomerulus, a highly organized structure with specialized function that selectively controls the ultrafiltration of plasma so as to prevent the leakage of essential proteins from the blood [1]. Proteinuria is a major risk factor for both renal and extrarenal diseases, including idiopathic nephrotic syndrome (INS), a disorder predominantly occurring in children and imposing significant clinical challenges due to frequent relapse [2]. Podocytes are specialized epithelial cells lining the glomerulus that play a crucial role in the maintenance of the glomerular filtration barrier (GFB) in the kidney, and accordingly, injury to these podocytes inevitably leads to the dysfunction of GFB and occurrence of proteinuria in human and experimental animal models [3, 4].

Transcription factors of nuclear factor-κB (NF-κB) family are central regulators of inflammatory responses in almost all mammalian cells [5]. Under unstimulated condition, NF-κB resides in the cytosol in the form of an inactive dimer which binds to inhibitory protein IκB, whereas, upon stimulation such as lipopolysaccharide (LPS) treatment, IκB is

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phosphorylated and then degraded in a proteasomal-dependent manner, whereby releasing NF-κB from IκB constraint and permitting its translocation into the nucleus for initiating transcription of target genes, like cytokines, chemokines, and adhesion molecules [6]. Previous studies have shown that NF-κB is activated in podocytes of patients with INS and rats with passive Heymann nephritis [7, 8]. Furthermore, systemic administration of NF-κB inhibitors was found to ameliorate proteinuria in animal models, which suggests that targeting the activated NF-κB in podocytes could be a potential therapeutic option for treating proteinuria [9, 10]. The nuclear factor of activated T cells 5 (NFAT5), belonging to the NFAT/Rel family of transcription factors, is related to NF-κB and activated by osmotic stress [11]. Recently, NFAT5 was reported to enhance NF-κB transcriptional activity through associating with p65-containing complexes [12]. The association between NFAT5 and p65 was also observed in HLE-B3 cells upon exposure to ultraviolet B [13]. In addition, NFAT5 silencing reduces NF-κB activation in NR8383 alveolar macrophage cells [14]. These studies suggest that NFAT5 could serve as a positive regulator of NF-κB activity.

LPS is composed of lipid A and oligosaccharide from the cell wall of gram-negative bacteria, and its treatment is known to induce podocyte injury *in vitro* and transient proteinuria in animal model [15–17]. In the current study, by utilizing models of LPS-induced injury in the immortalized mouse podocytes and proteinuria in mice, we investigated the possible role of NFAT5 involved in podocyte function and proteinuria.

METHODS

Mice and LPS-Induced Nephrotic Proteinuria

Male BALB/c mice were used for establishing experimental nephrotic proteinuria. Eight-week-old mice were injected intraperitoneally with 300 μg LPS (Sigma, diluted in 200 μl volume) for inducing proteinuria. Mice in parallel group were injected with equivalent volume of vehicle. Each group includes seven mice. After 36 h of injection, mice were sacrificed and the kidneys of each mouse were collected. All animal experiments were performed in accordance with the protocols approved by Institutional Animal Care and Use Committees of The First Hospital of Jilin University.

Isolation of Glomeruli

The glomeruli were isolated as described previously [18]. In brief, after sacrifice, the mouse kidneys were removed and decapsulated. Then, the cortex was isolated, minced, and pushed through a 150-μm mesh, followed by pipetted through a 106-μm sieve. The obtained homogenates were pipetted onto a 75-μm sieve, and then the filtrates were pushed through a 53-μm sieve. The glomeruli were rinsed from both the 75-μm and 53-μm sieves. The isolated glomeruli were used for further biochemical analyses.

Cell Culture, Treatment, and Transfection

The proliferative maintenance and differentiation induction of immortalized mouse podocyte clone JR07 were performed as described previously [19]. Briefly, podocytes were maintained at 33 °C for expansion in complete RPMI 1640 media containing 10% FBS, 100 U/ml penicillin/streptomycin, and 10 U/ml murine interferon-γ. The differentiation of podocytes was induced by removing murine interferon-γ and cultured for 10 days at 37 °C. For treatment, different concentrations of LPS (Sigma-Aldrich, L2630, dissolved in sterile saline) were added into the media and podocytes were cultured for 24 h with or without 10 μM Bay 11-7082. siRNA-mediated NFAT5 depletion was conducted 2 days before LPS treatment. The transfection was performed with Lipofectamine RNAimax Reagent (ThermoFisher Scientific) according to the manufacturer's instructions. siRNAs were synthesized by GenePharma (Shanghai, China). The final concentration of 20 nM siRNAs was used. The sequences of siRNAs are listed as follows: siNFAT5 SS sequence 5′-CAGUUACUGUUACAAGUUACA-3′; siNFAT5 AS sequence 5′-sequence UAACUUGUAACAGUAACUGGG-3′; siCtrl SS sequence 5′-CUUACGCUGAGUACUUCGA-3′; and siCtrl AS sequence 5′-sequence UCGAAGUACUCAGCGUAAG-3′.

qRT-PCR Analysis

The total RNA was extracted from the isolated glomeruli and podocytes using TRIzol Reagent Kit (ThermoFisher Scientific), and then the complimentary cDNA was prepared from 2 μg total RNA using SuperScrip IV First-Strand Synthesis System (ThermoFisher Scientific) according to the manufacturer's instructions. The determination of mRNA levels of targets was performed using SYBR Green qPCR Master Mix (MedChemExpress) and a 7700 PCR

Detection Instrument (Applied Biosystems). The cycling condition was set as the following: 95 °C for 10 min, 40 cycles of 95 °C for 15 s, and 60 °C for 60 s, followed by a hold at 4 °C. Actb was used as an endogenous control. The primers are listed below: Nfat5 forward, 5′-TCAACCCATGTCACCCCTAC-3′, reverse, 5′-TACAAAGGCTCTGTCGCTGA-3′; *Il1b* forward, 5′-TCAGGCAGGCAGTATCACTC-3′, reverse, 5′-AGCTCATATGGGTCCGACAG-3′; *Tnf* forward, 5′-AAGTCAACCTCCTCTCTGCC-3′, reverse, 5′-TGGATGAACACCCATTCCCT-3′; and *Act*b forward, 5′-TCTTTGCAGCTCCTTCGTTG-3′, reverse, 5′-TCCTTCTGACCCATTCCCAC-3′. Results were analyzed by an automatic cycle threshold (Ct) setting for assigning baseline and threshold for Ct determination.

Western Blotting Analysis

Protein samples were obtained by lysing the isolated glomeruli and podocytes in RIPA lysis buffer (Solarbio, R0010). The preparation of nuclei and cytoplasmic extracts from podocytes was conducted with the Nuclear/Cytosol Fractionation Kit (Biovision, K266-25) according to the manufacturer's instructions. A mount of 30 μg proteins in each sample were separated by SDS-PAGE and then transferred onto PVDF membrane. Membrane was blocked with 5% skim milk diluted in TBST (0.05 M Tris, 0.138 M NaCl, 2.7 μM KCl, 0.2% Tween 20, pH 8.0) prior to incubation overnight with primary antibodies at 4 °C. The primary antibodies were obtained from the following sources: anti-NFAT5 (abcam, ab3446), β-Actin (Santa Cruz, sc-47778), p-IκBα (Cell Signaling, 2859), IκBα (Cell Signaling, 4814), NF-κB p65 (Cell Signaling, 8242), and histone 3 (Cell Signaling, 14269). After rinse with TBST, membrane was incubated with goat anti-mouse IgG-HRP (Santa Cruz, sc-2031) or goat anti-rabbit IgG-HRP (Santa Cruz, sc-2030) secondary antibodies for 1 h at room temperature. Membrane was washed with TBST, and the protein bands were visualized with ECL substrate (Pierce) according to the manufacturer's instructions. The protein band was analyzed by ImageJ.

Transwell Permeability Assay

Transwell permeability assay for albumin influx was performed as previously described [20]. In brief, podocytes were plated on the transwell filter (3 μm size pore, Corning) of the top chamber. When podocyte monolayer reached complete confluency, podocytes were serum starved overnight and treated with different concentrations of LPS for 24 h in the presence or absence of 10 μM Bay 11-7082. After treatment, the media was removed and washed two times with 1 mmol/L $MgCl_2$ and 1 mmol/L $CaCl_2$. Fresh normal culture media were replenished in the top chamber, whereas the culture media supplemented with 40 mg/ml bovine serum albumin were added into the bottom chamber. Podocytes were cultured at 37 °C for 4 h, and then the media in the top chamber was collected for quantifying albumin concentration with the Bicinchoninic Acid Kit (Sigma) according to the manufacturer's instructions.

Proteinuria and Creatinine Quantification

After treatment, all mice were maintained in metabolic cages with free access to chow and water. The urine was collected for 24 h. The level of urine protein and creatinine was quantified individually by using a Detergent Compatible Protein Kit (Bio-Rad) and Creatinine Assay Kit (Cayman Chemical) according to manufacturer's instructions. The final result of proteinuria was normalized to creatinine excretion. The serum creatinine level from the blood sample of mice was also measured using the Creatinine Assay Kit.

Statistics

Data are presented as mean ± standard deviation (S.D.). The statistical significance between two groups was calculated by the Student *t* test. One-way ANOVA followed by the Tukey post hoc test was used to compare data among more than two groups. *P* value less than 0.05 is considered to be significant.

RESULTS

NFAT5 Expression Is Upregulated in the Glomeruli of Mice and Podocytes Treated with LPS

To explore whether NFAT5 is involved in proteinuria and podocyte function, we first monitored its expression in the glomeruli of a mouse model with nephrotic proteinuria that was induced by LPS injection, which has been demonstrated to be a valuable tool to study this sort of subject [21]. qRT-PCR analysis showed that compared with vehicle control, the mRNA level of NFAT5 was induced in the glomeruli of LPS-treated mice (Fig. 1a). Likewise, the

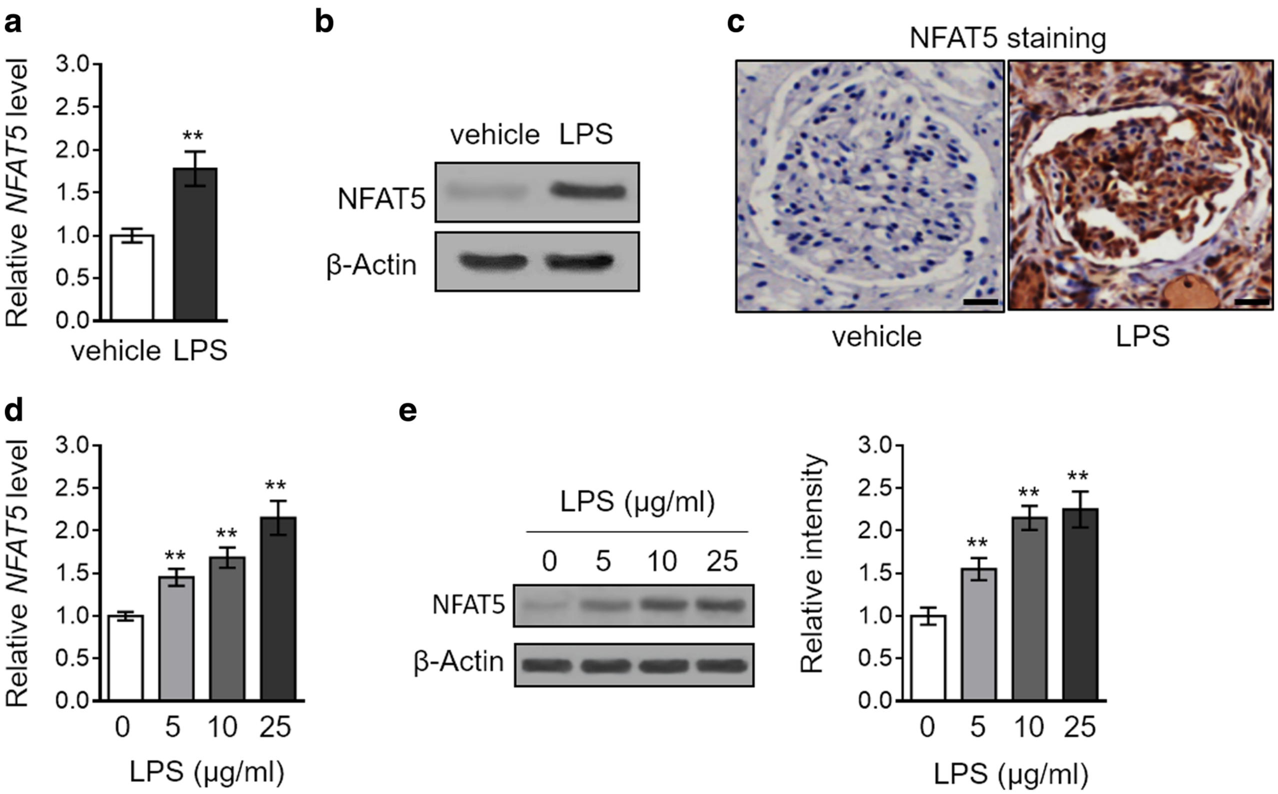

Fig. 1. NFAT5 expression is upregulated in the glomeruli of LPS-treated mice and LPS-treated podocytes. **a**–**c** Male BALB/c mice were injected intraperitoneally with 300 μg LPS for inducing proteinuria. Equal volume of vehicle was injected as negative control ($n = 7$). After 36 h of injection, the mRNA level of *NFAT5* (**a**) in the glomeruli was determined by qRT-PCR analysis. The expression level was normalized to *β-Actin* and expressed as relative to vehicle control. **b** The NFAT5 protein level in the glomeruli was determined by western blotting. The representative images are depicted. **c** The expression of NFAT5 was detected on renal specimens by immunohistochemistry. The representative images are shown. Scale bar, 50 μm. **d**, **e** Immortalized mouse podocyte clone JR07 was cultured *in vitro* and treated with increasing concentrations of LPS as indicated for 24 h. The mRNA level (**d**) and protein level (**e**) of *NFAT5* was determined. The expression level was normalized to *β-Actin* and expressed as relative to vehicle control (sterile saline, referred as 0 μg/ml LPS). The representative images (left) and quantification of band intensity (right) are shown. Data are mean ± S.D. Student *t* test (**a**); one-way ANOVA followed by Tukey post-hoc test (**d**, **e**). $^{**}P < 0.01$.

protein expression of NFAT5 was also upregulated (Fig. 1b). Moreover, this tendency of NFAT5 expression change was further confirmed by immunohistochemistry analysis, showing enhanced NFAT5 staining on mouse renal specimens and podocytes in the LPS-treated group (Fig. 1c). To verify whether NFAT5 is upregulated in podocytes when treated with LPS, a mouse podocyte clone JR07 was cultured *in vitro* [19] and then treated with increasing concentrations of LPS. Indeed, LPS treatment dose-dependently caused an elevated expression of NFAT5 in podocytes in both mRNA (Fig. 1d) and protein levels (Fig. 1e), as compared with the vehicle treatment. Taken together, these results show that NFAT5 expression is induced in mouse glomeruli and podocytes under the treatment of LPS.

NFAT5 Depletion Improves Filtration Barrier Function of LPS-Treated Podocytes

The upregulation of NFAT5 in the glomeruli of mice with LPS-induced nephrotic proteinuria suggests that it may play a role in disease pathogenesis. The maintenance of glomerular filter function of podocytes is crucial for preventing nephrotic proteinuria [22]. To test whether NFAT5 affects filtration barrier function of podocytes, we performed a well-established paracellular albumin permeability experiment [23] (Fig. 2a) and depleted NFAT5 expression in LPS-treated podocytes *via* siRNA transfection (Fig. 2b). Consistent with previous studies [24, 25], LPS treatment increased albumin permeability across podocyte monolayer; however, this effect was substantially

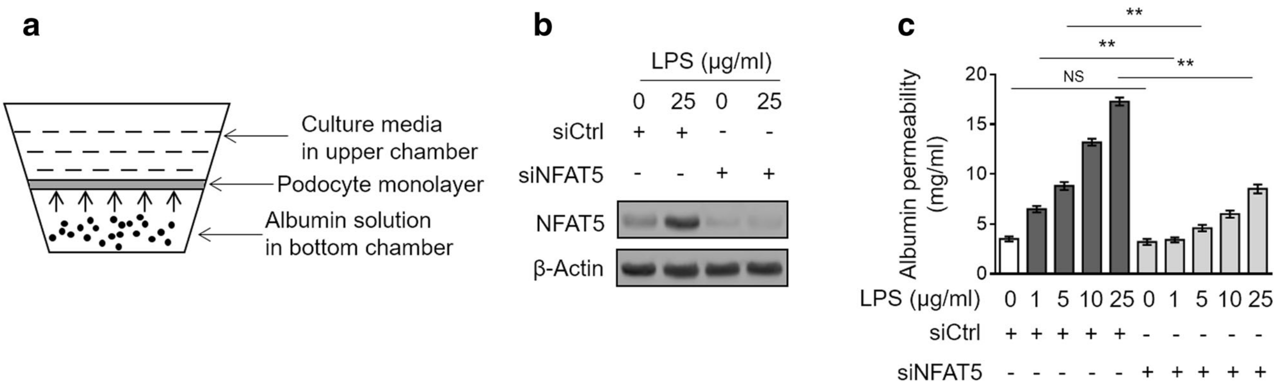

Fig. 2. Depletion of NFAT5 improves filtration barrier function of LPS-treated podocytes. **a** The schema of the paracellular albumin permeability experiment. Podocyte monolayer was plated onto the collagen-coated transwell filters (3 μm pore) and cultured with media in the upper chamber. The albumin solution was in the bottom chamber, and the albumin was allowed to transfer to the upper chamber. The amount of the albumin present in the upper chamber was then quantified. **b** The podocytes were transfected with siRNA targeting control (siCtrl) or NFAT5 (siNFAT5). After 48 h of transfection, podocytes were treated with 0 or 25 μg/ml LPS for another 24 h. The protein level of NFAT5 was determined by western blotting. **c** The podocytes transfected with siCtrl or siNFAT5 were further treated with increasing concentrations of LPS as indicated for 24 h. The results of albumin influx across the podocyte monolayer (mg/ml) are shown. Each column represents the mean value from five replicates. Data as mean ± S.D. Student *t* test. $^{**}P < 0.01$; NS, not significant.

attenuated in NFAT5-depleted podocytes (Fig. 2c), suggesting an ameliorating effect of NFAT5 knockdown on filtration barrier function of LPS-treated podocytes.

NFAT5 Depletion Suppresses NF-κB Activity, and NF-κB Inhibition Improves Filtration Barrier Function of LPS-Treated Podocytes

To gain insight into the molecular mechanism by which NFAT5 influences podocyte function, we focused on NF-κB pathway, a proinflammatory pathway that can be activated by LPS and its activation has connection with proteinuria progression [26, 27]. Besides, one study has also shown that NFAT5 modulates NF-κB activity in mCCD$_{c11}$ and HepG2 cells [12]. We found that NFAT5 depletion in podocytes suppressed the NF-κB activity induced by LPS treatment, as evidenced by decreased cytoplasmic p-IκBα and nuclear translocation of p65, as well as recovered level of cytoplasmic IκBα (Fig. 3a). In accordance with this result, the expression of downstream targets of NF-κB, including IL-1β (Fig. 3b) and TNF-α (Fig. 3c), was significantly minimized when NFAT5 was depleted. Collectively, these data suggest that NFAT5 knockdown suppresses NF-κB activity and downstream proinflammatory reaction in LPS-treated podocytes.

To establish a causal link between suppressed NF-κB activity and improved filtration barrier function of LPS-treated podocytes by NFAT5 depletion, we inhibited NF-κB activity in LPS-treated podocytes by a pharmacologic NF-κB inhibitor, Bay 11-7082 [28]. Expectedly, Bay 11-7082 treatment efficiently caused an inhibition of NF-κB activity in the presence of LPS stimulation, as shown by the abrogated induction of expression of IL-1β (Fig. 3d) and TNF-α (Fig. 3e). Keeping in line with the suppressed NF-κB activity, Bay 11-7082 treatment starkly recovered the increased albumin permeability across podocyte monolayer induced by LPS stimulation (Fig. 3f), indicating an improved filtration barrier function of podocytes when NF-κB activity is inhibited. This result suggests that the suppressed NF-κB activity by NFAT5 depletion at least in part accounts for its ameliorating effect on filtration barrier function of LPS-treated podocytes.

NFAT5 Depletion Suppresses NF-κB Activity and Ameliorates Nephrotic Proteinuria in LPS-Treated Mice

To broaden our findings into *in vivo* setting, we performed NFAT5 depletion in mice by transfecting NFAT5 siRNA through the internal jugular vein. Western blotting analysis showed an effective NFAT5 depletion in mouse glomeruli (Fig. 4a). Consistent with *in vitro* results of Fig. 3, LPS-induced NF-κB activity in mouse glomeruli was significantly attenuated when NFAT5 was depleted, as shown by decreased expression of IL-1β (Fig. 4b) and TNF-α (Fig. 4c). More importantly, compared with the siRNA control group injected with LPS, NFAT5 depletion remarkably decreased LPS-induced proteinuria (Fig. 4d), illustrating that NFAT5 depletion ameliorates nephrotic

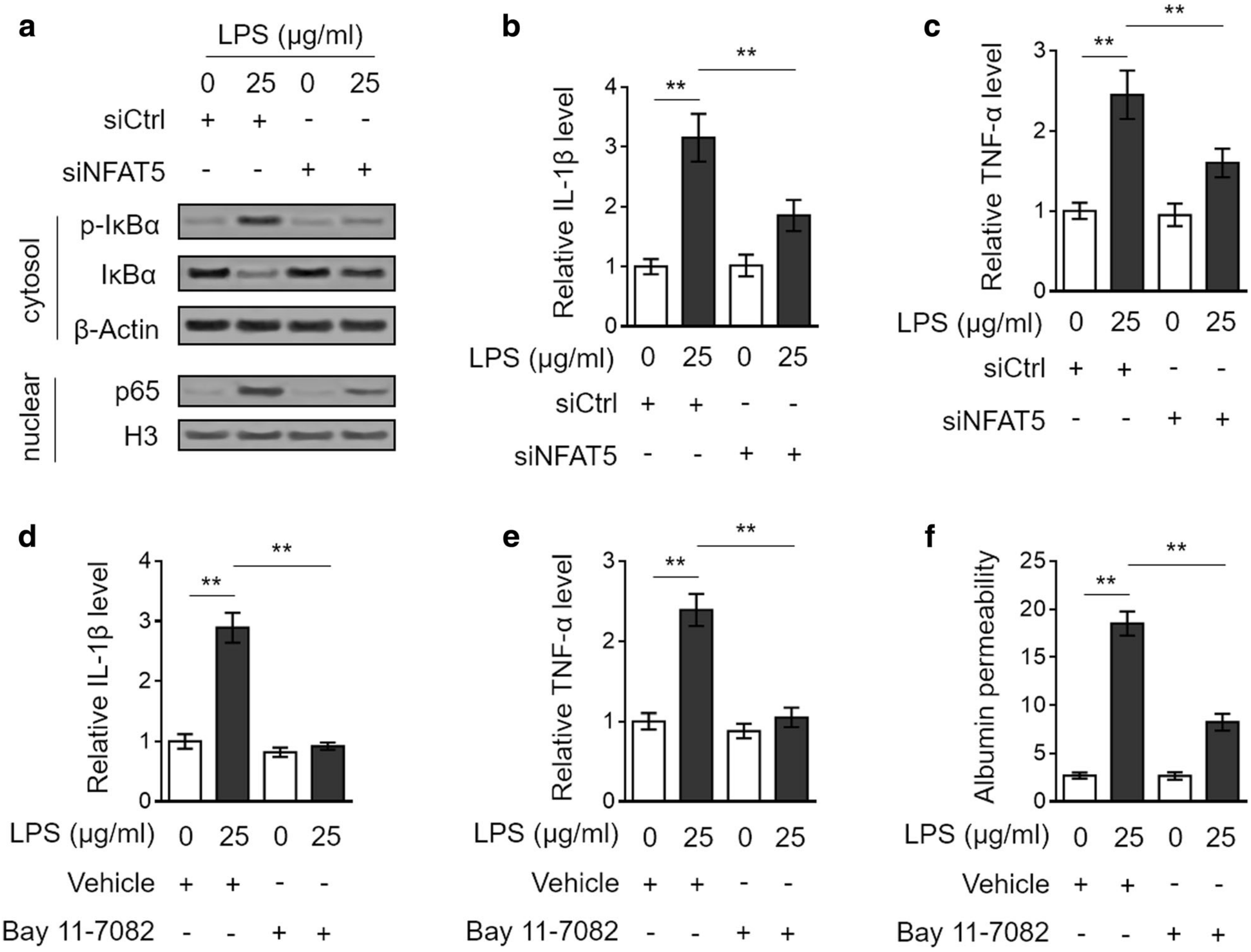

Fig. 3. Depletion of NFAT5 suppresses NF-κB activity and NF-κB inhibition improves filtration barrier function of LPS-treated podocytes. **a**–**c** The podocytes were transfected with siCtrl or siNFAT5. After 48 h of transfection, podocytes were treated with 0 or 25 μg/ml LPS for another 24 h. **a** The protein level of p-IκBα, IκBα in the cytosol, and p65 in the nuclear was determined by western blotting. β-Actin and H3 were used as loading controls, respectively. **b**, **c** The mRNA level of IL-1β (**b**) and TNF-α (**c**) was determined by qRT-PCR analysis. The expression level was normalized to *β-Actin*. (**d**–**f**) The podocytes were treated with 0 or 25 μg/ml LPS for 24 h in the presence or absence of 10 μM Bay 11-7082. The mRNA level of IL-1β (**d**) and TNF-α (**e**) was determined by qRT-PCR analysis. The expression level was normalized to *β-Actin*. The results are expressed as relative to control. **f** The result of albumin influx across the podocyte monolayer (mg/ml) is shown. Each column represents the mean value from five replicates. Data as mean ± S.D. Student *t* test. $^{**}P < 0.01$.

proteinuria in LPS-treated mice, which may relate to the suppressed NF-κB activity.

DISCUSSION

Due to the central role involved in the proteinuric kidney diseases, the injury to podocytes has attracted much attention in recent researches, such as foot process effacement, cytoplasmic vacuoles, blebs, disruption of organelles, plasma membrane, and cell death that can be caused by various survival stresses and pathological stimuli [29]. But to date, the molecular mechanisms underlying podocyte injury remain largely unclear, which may impede the development of efficient therapeutic treatments. A growing body of evidence has pointed out that NF-κB activity is activated during human and experimental kidney injury and exerts a vital role in orchestrating inflammatory response by controlling the expression of numerous target genes [27]. Moreover, inhibition NF-κB activity has been shown to ameliorate renal injury and proteinuria in animal models [10, 30]. These findings suggest that the

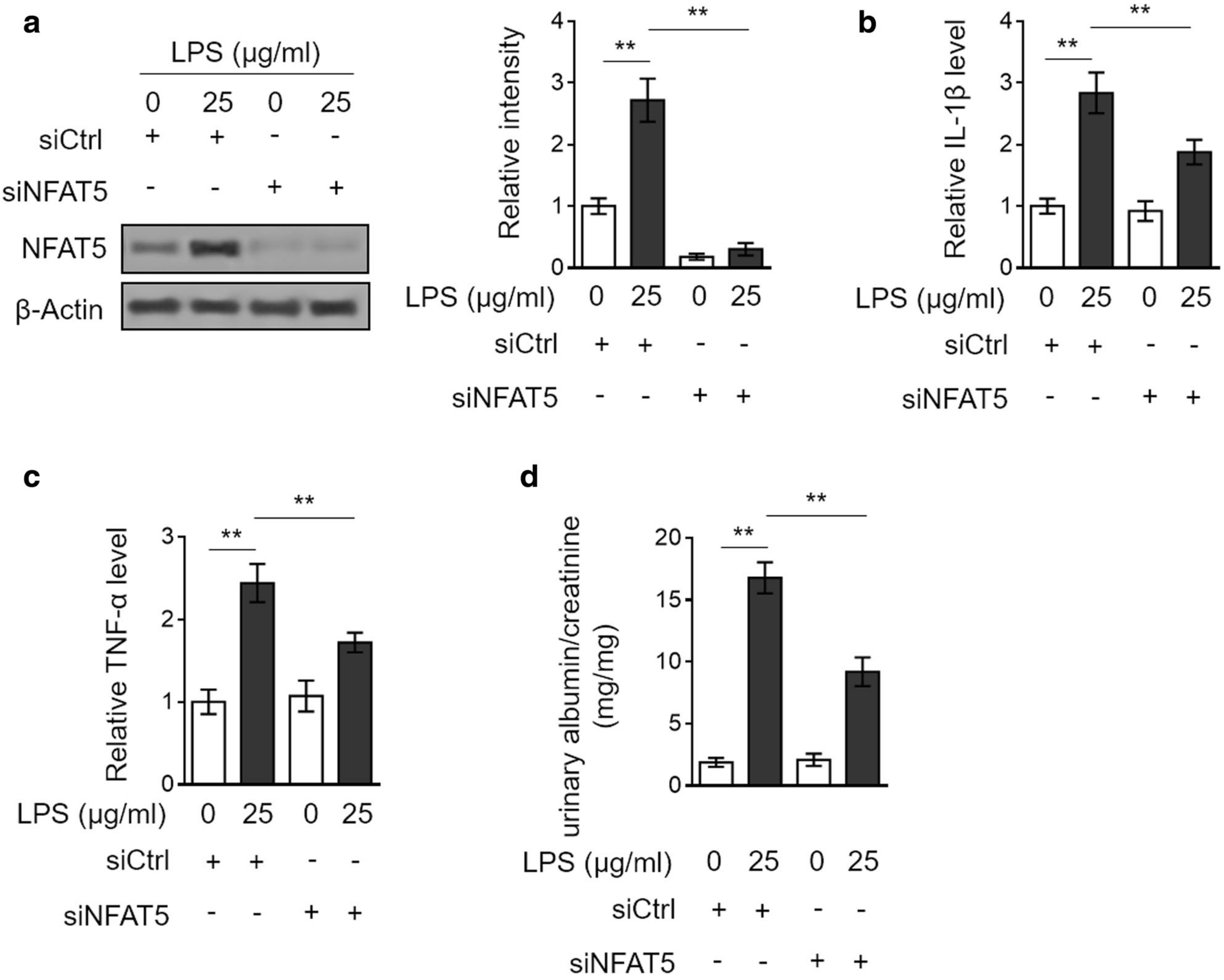

Fig. 4. Depletion of NFAT5 suppresses the activity of NF-κB and ameliorates nephrotic proteinuria in LPS-treated mice. **a**–**d** Male BALB/c mice were transfected with siCtrl or siNFAT5 (10 mg/kg) into the internal jugular vein using Invivofectamine (*n* = 5). After 1 h after transfection, mice were injected intraperitoneally with 300 μg LPS for inducing proteinuria. Equal volume of vehicle was injected as negative control. Glomeruli were harvested 36 h after injection, and NFAT5 protein level (**a**) was determined by western blotting. The representative images (left) and quantification of band intensity (right) are shown. β-Actin was used a loading control. **b**, **c** The mRNA level of IL-1β (**b**) and TNF-α (**c**) in the glomeruli was determined by qRT-PCR analysis. The expression level was normalized to *β-Actin*, and the results are expressed as relative to control. Data are mean ± S.D. Student *t* test. $^{**}P < 0.01$. **d** Urine was collected for 24 h. The proteinuria was quantified by urinary albumin/creatinine (mg/mg). Data are mean ± S.D. Student *t* test. $^{**}P < 0.01$.

manipulation of NF-κB activity may hold the potentiality to interfere the disease progression of proteinuric kidney diseases. Therefore, elucidating the regulatory pathway of NF-κB activation in the context of podocyte injury is a task of clinical significance. In the present study, we discovered that NFAT5 was upregulated in the glomeruli of LPS-treated mice and in LPS-treated podocytes, and functionally, NFAT5 depletion improved filtration barrier function of LPS-treated podocytes. We further found that NFAT5 depletion suppressed NF-κB activity and that NF-κB inhibition improved filtration barrier function of LPS-treated podocytes. At last, in the animal model with LPS-induced nephrotic proteinuria, NFAT5 depletion was shown to suppress the activity of NF-κB and ameliorate disease symptom. Thus, these results connect the beneficial role of NFAT5 knockdown in filtration barrier function of podocytes and ameliorated proteinuria to the inhibited NF-κB activation, underscoring the potentiality of targeting NFAT5 and NF-κB activity in interfering the disease progression of proteinuria.

We first noticed that NFAT5 expression was upregulated in the glomeruli of LPS-treated mice and in LPS-treated podocytes at both transcript level and protein levels. These results suggest that the transcription of NFAT5 in the podocytes is induced upon the treatment of LPS. One previous study has reported that NFAT5 is expressed in unstimulated macrophages and its expression is further induced in an IKKβ-NF-κB-dependent manner upon toll-like receptor (TLR) stimulation by LPS treatment [31]. When activated, NFAT5 acts as a transcriptional factor to regulate a plethora of genes encoding cytokines, chemokines, proteins that regulate nitric oxide production, cell cycle, proliferation, and inflammation, for protecting cells from osmotic stress or pathogen infection [31, 32]. It is possible that NFAT5 may also be upregulated through IKKβ-NF-κB-dependent manner under our experimental conditions; however, solid evidence from future studies is needed to demonstrate whether this is the case. If it is true, how NF-κB regulates the transcription of NFAT5 is another intriguing subject deserving further investigation. Furthermore, the expression pattern of NFAT5 in clinical nephrotic proteinuria samples is still unknown, and clarifying this issue may help us to understand whether NFAT5 is associated with nephrotic proteinuria in clinical practice.

NFAT5 is conventionally considered to be regulated by extracellular tonicity and plays an osmoprotective role by regulating the expression of osmotic response element/TonE elements of genes that participate in the activities against the deleterious effects of cellular shrinkage [33]. Except for this canonic function, enhancing NF-κB activity has also been shown to contribute to the protective role of NFAT5 against cell hypertonicity [12]. This finding seems to be in agreement with our following results showing that NFAT5 knockdown suppresses NF-κB activity in LPS-treated podocytes, as evidenced by decreased levels of p-IκB, nuclear translocation of p65, and production of target genes, including IL-1β, TNF-α, and IκBα. In LPS-treated mice, NFAT5 knockdown in the glomeruli also inhibits NF-κB activity, suggesting that the mechanism of the positive regulation of NFAT5 on NF-κB activity may be applied for both *in vitro* and *in vivo* conditions. As far as we know, the role of NF-κB in the filtration barrier function of podocytes has not yet been well characterized.

CONCLUSIONS

We in the first time show that the inhibition of NF-κB by Bay 11-7082 improves filtration barrier function of podocytes treated with LPS *in vitro*, implying that the pro-inflammatory signaling eliciting by LPS through NF-κB is detrimental for the maintenance of filtration barrier function of podocytes. This notion may be supported by the evidence that the proinflammatory activity of NF-κB in podocytes aggravates injury to podocytes and promotes proteinuria in an experimental mouse glomerulonephritis [34]. Because NFAT5 deficiency causes embryonic lethality in mice due to impaired cardiac development and function [35], animal models specifically depleted of podocyte NFAT5 may be a more useful tool to provide direct clues to reveal the role of NFAT5 involved in podocyte injury and proteinuria progression.

AUTHORS' CONTRIBUTIONS

YYH carried out the conception and design of the research, participated in the acquisition of data, and drafted the manuscript. SBZ carried out the analysis and interpretation of data. MNL and BCS participated in the statistical analysis. All authors read and approved the final manuscript.

FUNDING INFORMATION

This study was supported by Jilin Province Natural Science Foundation (D5111223428) and Jilin Province Health Department (2012Z103).

COMPLIANCE WITH ETHICAL STANDARDS

Ethics Approval and Consent to Participate. This study was approved by the Ethics Committee of The First Hospital of Jilin University.

Competing Interests. The authors declare that they have no competing interests.

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REFERENCES

1. Liu, M., K. Liang, J. Zhen, M. Zhou, X. Wang, Z. Wang, X. Wei, Y. Zhang, Y. Sun, Z. Zhou, H. Su, C. Zhang, N. Li, C. Gao, J. Peng, and F. Yi. 2017. Sirt6 deficiency exacerbates podocyte injury and proteinuria through targeting Notch signaling. *Nature Communications* 8 (1): 413. https://doi.org/10.1038/s41467-017-00498-4.
2. Noone, D.G., K. Iijima, and R. Parekh. 2018. Idiopathic nephrotic syndrome in children. *Lancet* 392 (10141): 61–74. https://doi.org/10.1016/S0140-6736(18)30536-1.
3. Asanuma, K., and P. Mundel. 2003. The role of podocytes in glomerular pathobiology. *Clinical and Experimental Nephrology* 7 (4): 255–259. https://doi.org/10.1007/s10157-003-0259-6.

4. Wiggins, R.C. 2007. The spectrum of podocytopathies: a unifying view of glomerular diseases. *Kidney International* 71 (12): 1205–1214. https://doi.org/10.1038/sj.ki.5002222.
5. Guijarro, C., and J. Egido. 2001. Transcription factor-kappa B (NF-kappa B) and renal disease. *Kidney International* 59 (2): 415–424. https://doi.org/10.1046/j.1523-1755.2001.059002415.x.
6. Zhang, Q., M.J. Lenardo, and D. Baltimore. 2017. 30 years of NF-kappaB: a blossoming of relevance to human pathobiology. *Cell* 168 (1–2): 37–57. https://doi.org/10.1016/j.cell.2016.12.012.
7. Mudge, S.J., K. Paizis, R.B. Auwardt, R.J. Thomas, and D.A. Power. 2001. Activation of nuclear factor-kappa B by podocytes in the autologous phase of passive Heymann nephritis. *Kidney International* 59 (3): 923–931. https://doi.org/10.1046/j.1523-1755.2001.059003923.x.
8. Zheng, L., R. Sinniah, and S.I. Hsu. 2006. In situ glomerular expression of activated NF-kappaB in human lupus nephritis and other non-proliferative proteinuric glomerulopathy. *Virchows Archiv* 448 (2): 172–183. https://doi.org/10.1007/s00428-005-0061-9.
9. Shimo, T., Y. Adachi, S. Yamanouchi, S. Tsuji, T. Kimata, K. Umezawa, M. Okigaki, J. Takaya, S. Ikehara, and K. Kaneko. 2013. A novel nuclear factor kappaB inhibitor, dehydroxymethylepoxyquinomicin, ameliorates puromycin aminonucleoside-induced nephrosis in mice. *American Journal of Nephrology* 37 (4): 302–309. https://doi.org/10.1159/000348803.
10. Yamashita, M., T. Yoshida, S. Suzuki, K. Homma, and M. Hayashi. 2017. Podocyte-specific NF-kappaB inhibition ameliorates proteinuria in adriamycin-induced nephropathy in mice. *Clinical and Experimental Nephrology* 21 (1): 16–26. https://doi.org/10.1007/s10157-016-1268-6.
11. Lopez-Rodriguez, C., J. Aramburu, L. Jin, A.S. Rakeman, M. Michino, and A. Rao. 2001. Bridging the NFAT and NF-kappaB families: NFAT5 dimerization regulates cytokine gene transcription in response to osmotic stress. *Immunity* 15 (1): 47–58.
12. Roth, I., V. Leroy, H.M. Kwon, P.Y. Martin, E. Feraille, and U. Hasler. 2010. Osmoprotective transcription factor NFAT5/TonEBP modulates nuclear factor-kappaB activity. *Molecular Biology of the Cell* 21 (19): 3459–3474. https://doi.org/10.1091/mbc.E10-02-0133.
13. Chung, I., Y.S. Hah, S. Ju, J.H. Kim, W.S. Yoo, H.Y. Cho, J.M. Yoo, S.W. Seo, W.S. Choi, and S.J. Kim. 2017. Ultraviolet B radiation stimulates the interaction between nuclear factor of activated T cells 5 (NFAT5) and nuclear factor-kappa B (NF-kappaB) in human lens epithelial cells. *Current Eye Research* 42 (7): 987–994. https://doi.org/10.1080/02713683.2016.1270327.
14. Li, C., M. Liu, L. Bo, W. Liu, Q. Liu, X. Chen, D. Xu, Z. Li, and F. Jin. 2016. NFAT5 participates in seawater inhalation induced acute lung injury via modulation of NF-kappaB activity. *Molecular Medicine Reports* 14 (6): 5033–5040. https://doi.org/10.3892/mmr.2016.5860.
15. Zhang, S.Y., M. Kamal, K. Dahan, A. Pawlak, V. Ory, D. Desvaux, V. Audard, M. Candelier, F. BenMohamed, M. Matignon, C. Christov, X. Decrouy, V. Bernard, G. Mangiapan, P. Lang, G. Guellaen, P. Ronco, and D. Sahali. 2010. C-mip impairs podocyte proximal signaling and induces heavy proteinuria. *Science Signaling* 3 (122): ra39. https://doi.org/10.1126/scisignal.2000678.
16. Srivastava, T., M. Sharma, K.H. Yew, R. Sharma, R.S. Duncan, M.A. Saleem, E.T. McCarthy, A. Kats, P.A. Cudmore, U.S. Alon, and C.J. Harrison. 2013. LPS and PAN-induced podocyte injury in an in vitro model of minimal change disease: changes in TLR profile. *Journal of Cell Communication and Signaling* 7 (1): 49–60. https://doi.org/10.1007/s12079-012-0184-0.
17. Wei, C., C.C. Moller, M.M. Altintas, J. Li, K. Schwarz, S. Zacchigna, L. Xie, A. Henger, H. Schmid, M.P. Rastaldi, P. Cowan, M. Kretzler, R. Parrilla, M. Bendayan, V. Gupta, B. Nikolic, R. Kalluri, P. Carmeliet, P. Mundel, and J. Reiser. 2008. Modification of kidney barrier function by the urokinase receptor. *Nature Medicine* 14 (1): 55–63. https://doi.org/10.1038/nm1696.
18. Ilatovskaya, D.V., O. Palygin, V. Chubinskiy-Nadezhdin, Y.A. Negulyaev, R. Ma, L. Birnbaumer, and A. Staruschenko. 2014. Angiotensin II has acute effects on TRPC6 channels in podocytes of freshly isolated glomeruli. *Kidney International* 86 (3): 506–514. https://doi.org/10.1038/ki.2014.71.
19. Ransom, R.F., N.G. Lam, M.A. Hallett, S.J. Atkinson, and W.E. Smoyer. 2005. Glucocorticoids protect and enhance recovery of cultured murine podocytes via actin filament stabilization. *Kidney International* 68 (6): 2473–2483. https://doi.org/10.1111/j.1523-1755.2005.00723.x.
20. Borges, C.M., A. Papadimitriou, D.A. Duarte, J.M. Lopes de Faria, and J.B. Lopes de Faria. 2016. The use of green tea polyphenols for treating residual albuminuria in diabetic nephropathy: A double-blind randomised clinical trial. *Scientific Reports* 6: 28282. https://doi.org/10.1038/srep28282.
21. Comper, W.D. 2009. Is the LPS-mediated proteinuria mouse model relevant to human kidney disease? *Nature Medicine* 15 (2): 133; author reply 133-134. https://doi.org/10.1038/nm0209-133a.
22. Zhou, Y., P. Castonguay, E.H. Sidhom, A.R. Clark, M. Dvela-Levitt, S. Kim, J. Sieber, N. Wieder, J.Y. Jung, S. Andreeva, J. Reichardt, F. Dubois, S.C. Hoffmann, J.M. Basgen, M.S. Montesinos, A. Weins, A.C. Johnson, E.S. Lander, M.R. Garrett, C.R. Hopkins, and A. Greka. 2017. A small-molecule inhibitor of TRPC5 ion channels suppresses progressive kidney disease in animal models. *Science* 358 (6368): 1332–1336. https://doi.org/10.1126/science.aal4178.
23. Fang, L., Y. Zhou, H. Cao, P. Wen, L. Jiang, W. He, C. Dai, and J. Yang. 2013. Autophagy attenuates diabetic glomerular damage through protection of hyperglycemia-induced podocyte injury. *PLoS One* 8 (4): e60546. https://doi.org/10.1371/journal.pone.0060546.
24. Mashmoushi, A.K., and J.C. Oates. 2015. Lipopolysaccharide induces inducible nitric oxide synthase-dependent podocyte dysfunction via a hypoxia-inducible factor 1alpha and cell division control protein 42 and Ras-related C3 botulinum toxin substrate 1 pathway. *Free Radical Biology & Medicine* 84: 185–195. https://doi.org/10.1016/j.freeradbiomed.2015.02.031.
25. Qiao, Y., A.L. Berg, P. Wang, Y. Ge, S. Quan, S. Zhou, H. Wang, Z. Liu, and R. Gong. 2016. MC1R is dispensable for the proteinuria reducing and glomerular protective effect of melanocortin therapy. *Scientific Reports* 6: 27589. https://doi.org/10.1038/srep27589.
26. Mezzano, S.A., M. Barria, M.A. Droguett, M.E. Burgos, L.G. Ardiles, C. Flores, and J. Egido. 2001. Tubular NF-kappaB and AP-1 activation in human proteinuric renal disease. *Kidney International* 60 (4): 1366–1377. https://doi.org/10.1046/j.1523-1755.2001.00941.x.
27. Sanz, A.B., M.D. Sanchez-Nino, A.M. Ramos, J.A. Moreno, B. Santamaria, M. Ruiz-Ortega, J. Egido, and A. Ortiz. 2010. NF-kappaB in renal inflammation. *Journal of the American Society of Nephrology* 21 (8): 1254–1262. https://doi.org/10.1681/ASN.2010020218.
28. Kim, Y.S., J.S. Kim, J.S. Kwon, M.H. Jeong, J.G. Cho, J.C. Park, J.C. Kang, and Y. Ahn. 2010. BAY 11-7082, a nuclear factor-kappaB inhibitor, reduces inflammation and apoptosis in a rat cardiac ischemia-reperfusion injury model. *International Heart Journal* 51 (5): 348–353.
29. Nagata, M. 2016. Podocyte injury and its consequences. *Kidney International* 89 (6): 1221–1230. https://doi.org/10.1016/j.kint.2016.01.012.
30. Ohga, S., K. Shikata, K. Yozai, S. Okada, D. Ogawa, H. Usui, J. Wada, Y. Shikata, and H. Makino. 2007. Thiazolidinedione

ameliorates renal injury in experimental diabetic rats through anti-inflammatory effects mediated by inhibition of NF-kappaB activation. *American Journal of Physiology Renal Physiology* 292 (4): F1141–F1150. https://doi.org/10.1152/ajprenal.00288.2005.

31. Buxade, M., G. Lunazzi, J. Minguillon, S. Iborra, R. Berga-Bolanos, M. Del Val, J. Aramburu, and C. Lopez-Rodriguez. 2012. Gene expression induced by Toll-like receptors in macrophages requires the transcription factor NFAT5. *The Journal of Experimental Medicine* 209 (2): 379–393. https://doi.org/10.1084/jem.20111569.
32. Berga-Bolanos, R., M. Alberdi, M. Buxade, J. Aramburu, and C. Lopez-Rodriguez. 2013. NFAT5 induction by the pre-T-cell receptor serves as a selective survival signal in T-lymphocyte development. *Proceedings of the National Academy of Sciences of the United States of America* 110 (40): 16091–16096. https://doi.org/10.1073/pnas.1215934110.
33. Lopez-Rodriguez, C., J. Aramburu, A.S. Rakeman, and A. Rao. 1999. NFAT5, a constitutively nuclear NFAT protein that does not cooperate with Fos and Jun. *Proceedings of the National Academy of Sciences of the United States of America* 96 (13): 7214–7219.
34. Brahler, S., C. Ising, H. Hagmann, M. Rasmus, M. Hoehne, C. Kurschat, T. Kisner, H. Goebel, S. Shankland, K. Addicks, F. Thaiss, B. Schermer, M. Pasparakis, T. Benzing, and P.T. Brinkkoetter. 2012. Intrinsic proinflammatory signaling in podocytes contributes to podocyte damage and prolonged proteinuria. *American Journal of Physiology Renal Physiology* 303 (10): F1473–F1485. https://doi.org/10.1152/ajprenal.00031.2012.
35. Mak, M.C., K.M. Lam, P.K. Chan, Y.B. Lau, W.H. Tang, P.K. Yeung, B.C. Ko, S.M. Chung, and S.K. Chung. 2011. Embryonic lethality in mice lacking the nuclear factor of activated T cells 5 protein due to impaired cardiac development and function. *PLoS One* 6 (7): e19186. https://doi.org/10.1371/journal.pone.0019186.