Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Fractalkine Deficiency Attenuates LPS-Induced Acute Kidney Injury and Podocytes Apoptosis by Targeting PI3K/Akt Signal Pathway

Qiming Gong
Youjiang Medical University for Nationalities
Jingxue Ma
Youjiang Medical University for Nationalities
Xiuhong Pan
Youjiang Medical University for Nationalities
Yan Jiang
Youjiang Medical University for Nationalities
Fafen Yang
Youjiang Medical University for Nationalities
Yanwu You (youyanwu@163.com)
Affiliated hospital of Youjiang Medical University for Nationalities https://orcid.org/0000-0002-2612-5317

Research Article

Keywords: Fractalkine, acute kidney injury, podocytes apoptosis, PI3K/Akt signal pathway

Posted Date: May 27th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-528182/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Version of Record: A version of this preprint was published at Clinical and Experimental Nephrology on April 8th, 2022. See the published version at https://doi.org/10.1007/s10157-022-02218-9.

Abstract

Podocytes injury is a major biomarker of primary glomerular disease that leads to massive proteinuria and kidney failure. The increased production of chemokine Fractalkine (FKN, CX3CL1) implicated as a hallmark in multiple inflammatory disease. However, the underlying mechanism of FKN on podocytes injury remains unknown. Here, we studied the effects of FKN in LPS-induced acute kidney injury (AKI) in wild type (WT) and FKN knockout (FKN-KO) mice. LPS stimulation resulted in kidney damage, increased expression of Bcl-2 family apoptosis protein while decreased the podocytes marker protein (nephrin and podocin) abundance compared with the control. LPS-induced FKN-KO mice exhibited reduced lethality and attenuated inflammatory cells infiltration, podocytes apoptosis and PI3K/Akt signal pathway inhibition compared with WT mice. Depletion of FKN served a protective effect in LPS-induced AKI by activating the PI3K/Akt signal pathway. In cultured podocytes, the interaction between FKN and PI3K/Akt signal pathway is well confirmed. FKN knockdown reduced podocytes apoptosis by regulating the Bcl-2 family, while this protective effect was reversed by co-administration of PI3K/Akt inhibitor (LY294002). These studies reveal a novel mechanistic property of FKN, PI3K/Akt signal and podocytes apoptosis.

Introduction

Sepsis is a frequently cause of acute kidney injury (AKI), contributing to 70% mortality of the sepsis patients in the intensive care unit (ICU)[1]. Studies have shown that patients with immunosuppression are at the highest mortality caused by the malfunction of apoptosis progression[2]. Podocytes are specifical glomerular epithelial cells that maintain glomerular blood filtration barrier[3]. Recent studies have shown that podocytes apoptosis are important factors that participate in the development of kidney disease[4]. However, its underlying mechanism largely elusive.

The specific ligand for CX3CR1 is chemokine Fractalkine (FKN, CX3CL1), which is an important molecular to modulates leukocyte migration and adhesion function and acts as a catalyst in the inflammatory response[5, 6]. Recently, a targeted therapy addresses the inhibition of FKN has been shown to have anti-fibrosis, anti-inflammatory, anti-tumor effects, holding important potential value in health and disease[7–9]. The protective effects of FKN antibody injected in MRL/Lpr mice were studied by our group previously[10]. However, exactly how FKN regulates podocytes injury remains unknown.

PI3K/Akt is widely distributed in mammalian immune organs as anti-apoptosis signal[11]. Studies demonstrated that Akt phosphorylation promoted cell proliferation while the expression of apoptosis proteins (such as Bax and Cyt-c) were promoted by preventing Akt phosphorylation[12, 13]. However, whether a causal link between FKN deficiency and PI3K/Akt signal activation in podocytes injury remains unknown. Here we investigate the protective effect of FKN deficiency on podocytes apoptosis and to further explore the role of PI3K/Akt signal in this process.

Materials And Methods

Experimental Animals

C57BL/6 mice were purchased from Shanghai Genechem Animal Co. Ltd (NO. SYXK 2015-0008). The guide RNA (CX3CL1-sgRNA1: ctggcaggttatcacgggttggg and CX3CL1-sgRNA2: TGGCAGTAACTCATACGTCCTGG) were constructed using the CRISPR/Cas9 technique. Transgenic founder mice were generated by microinjection embryos, then mated to wild-type mice to obtain independent lines of FKN knockout (FKN-KO, $FKN^{-/-}$) mice. All mice were kept in the specific pathogen-free (SPF) of the Youjiang Medical University for Nationalities (NO. SYXK 2017-0004). All procedures were performed according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the ethics committee of Youjiang Medical University for Nationalities.

8-10 weeks old WT and FKN-KO mice (n=5 each) were randomized into four groups: Control group: WT mice intraperitoneal (IP) injection of Saline, LPS group: WT mice IP injection of LPS (10mg/kg, 24h) (Sigma-Aldrich, St. Louis, MO, USA), FKN-KO group: FKN-KO mice IP injection of Saline, FKN-KO+LPS group: FKN-KO mice IP injection of LPS (10mg/kg, 24h). For survival test, mice were IP injected with LPS and monitored every 2 hours for 48 hours.

Cell Culture and Treatments

Immortalized mouse podocytes were obtained from the Cell Center of Fudan University (Shanghai, China). Podocytes were cultured in RPIM 1640 medium (Gibco, Australia) with 10% FBS (Gibco, Australia) and were maintained at 37°C, 5% CO_2 incubator. Cells were divided into four group: Control group, Si-NC group: podocytes were transfected with siRNA against scramble control (siRNA-control) (Shanghai Genechem Co., Ltd.), FKN-KD group: podocytes were transfected with siRNA against FKN (siRNA-FKN) (Shanghai Genechem Co., Ltd.), FKN-KD+LY294002 (a PI3K/A Akt signal inhibitor) (Selleck Chemicals, USA): FKN-KD podocytes infected with LY294002 (20 μM/mL, 48 h)[14].

Analysis of Blood Urea Nitrogen (BUN) and creatinine (Scr) activities.

All mice serum were collected and centrifugated at 4000 rpm for 15 min. BUN and Scr levels were examined by using the commercial kits (Jiancheng Bioengineering *Institute*, *Nanjing*, China) according to the manufacturer's instructions.

Histopathology

Kidney samples were fixed with formalin at 4℃ overnight. Kidney were embedded with paraffin and cut into 4-μM sections. Then hematoxylin and eosin (HE) staining were prepared and visualized under a light microscope (Olympus, Japan).

Immunofluorescence Staining

All samples were fixed with 4% paraformaldehyde for 30 min at room temperature. After being washed three times with PBS, samples were incubated with 0.1% Triton X-100 for 20 min and blocked with goat

serum for 30 min. Then kidney sections were incubated with anti-nephrin and anti-Bax (Affinity Biosciences, OH, USA), and podocytes were incubated with anti-Bax, anti-p-Akt (Affinity Biosciences, OH, USA) at 4℃, overnight. Next, samples were incubated with FITC and Fluor594-conjugated secondary antibodies (Affinity Biosciences, OH, USA) for 1 h at room temperature. The nuclei were stained with DAPI (5 μg/mL, Solarbio, Beijing, China) for 10 min and imaged by a fluorescence microscope (Olympus, Japan).

Western Blot Assay

Kidney tissues and podocytes were lysed with RIPA buffer (Solarbio, Beijing, China). Protein concentration were measured by a BCA assay kit (Solarbio, Beijing, China). Proteins (50 μg) were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), transferred to the polyvinylidene fluoride (PVDF) membrane, and incubated with anti-FKN, anti-Bax, anti-Bcl-2 and anti-Cyt-c, anti-nephrin, anti-podocin, anti-Akt, anti-p-Akt, (Affinity Biosciences, OH, USA) overnight at 4℃. Then incubated with horseradish peroxidase-linked secondary antibody (Affinity Biosciences, OH, USA) for 50 min at room temperature. The protein expression was quantified using ImageJ.

Co-immunoprecipitation Analysis

Co-IP assay was conducted according to the manufacturer's instruction of Co-IP kit (TK277274, Thermo Fisher Scientific). Briefly, Podocytes total protein was extracted by IP lysis buffer and quantified by BCA assay. The podocytes lysates were incubated with anti-FKN, anti-Akt, anti-p-Akt and protein A/G agarose beads at 4℃ overnight. Then the beads were washed with pre-cold IP lysis buffer and 50 μL of immunoprecipitated proteins were collected. the precipitated proteins were identified by Western blot assay.

Flow Cytometric Assay

After treatment, podocytes were adjusted to 1×10^6 cells/mL and stained with Annexin V-FITC and propidium iodide using Annexin V-FITC Apoptosis kit (BD Biosciences, USA). The apoptosis rate was measured by flow cytometry (BD Biosciences, USA).

Statistical Analysis

Statistical analysis was performed using SPSS 23.0 software. The data were expressed as mean ± standard deviation. The Student t test was used to determine the statistical difference between two groups. Differences between more than two groups were determined by ANOVA. $P < 0.05$ was considered statistically significant.

Results

Depletion of FKN Ameliorates LPS-induced Acute Kidney Injury

After LPS injection, WT mice started dying around 26 h and had 0 % survival rate on 48 h. In contrast, FKN-KO mice began to die at 36 h and had 28 % survival rate on 48 h (Fig.1**a**). We also assessed renal function by serum levels of BUN and Scr: Both indices were increased after LPS stimulation in WT mice, while these increases were ameliorated significantly in FKN-KO mice (Fig.1**b**, **c**). The kidney histopathology showed similar results than the serologic test (Fig.1**d**). The kidney tissues of Sham-treated WT mice were similar to the FKN-KO mice. After LPS stimulation, WT mice exhibited severe kidney injury, including enlarged glomerular with dilation of their capillaries and infiltrated inflammatory cells. However, FKN-KO mice showed less changes in morphology and a reduction in inflammatory cells infiltration. These results indicated that FKN Depletion exhibited a protective effect against LPS-induced AKI.

Depletion of FKN Attenuates LPS-induced Kidney Podocytes Apoptosis via PI3K/AKT Signal Pathway

Apoptosis progression is a critical mechanism of podocytes injury in kidney disease. LPS-induced podocytes apoptosis in mice kidney was assessed by Western Blot and Immunofluorescence Staining. The expression of pro-apoptosis Bax, Cyt-c protein were increased while the anti-apoptosis Bcl-2 protein, nephrin and podocin were decreased in kidney of WT mice stimulated with LPS, while the induction was reversed in FKN-KO mice (Fig.2**a**). After LPS stimulation, the localization of Bax were enhanced in the kidney tissues of WT mice and inhibited in FKN-KO mice, while the localization of nephrin were lost in the kidney tissues of WT mice and recovered in FKN-KO mice (Fig.2**b**). Therefore, FKN Depletion suppressed LPS-induced kidney podocytes apoptosis.

We further examined the biological effects of PI3K/Akt signal by LPS in mice kidney. Phosphorylated Akt (p-Akt) expression were decreased in WT mice with LPS injection, while the expression of p-Akt was not significantly different in FKN-KO mice (Fig.2**a**). We speculated that FKN Depletion reversed the LPS-induced PI3K/Akt signal inhibition in mice kidney.

FKN Interacted Directly with PI3K/AKT Signal Pathway in Podocytes

Co-IP assay was performed to examine the interactions between FKN and PI3K/Akt with extracts of podocytes. In Fig.3, immunoprecipitation of FKN pull down both Akt and p-Akt. Presence of interaction between FKN and PI3K/Akt in podocytes were indicated.

Inhibition of PI3K/AKT Signal Pathway Reverse the Anti-apoptosis Effect of FKN-knockdown in Podocytes

Apoptosis progression is hypothesized as the major mechanism of podocytes injury. As shown in Fig.4, FKN knockdown decreased the apoptosis rate of podocytes, while LY294002 (PI3K/Akt inhibitor) increased the podocytes apoptosis rate in FKN knockdown group. To provide explain the anti-apoptosis effect of PI3K/Akt signal in podocytes after FKN knockdown treatment, the protein expression of Akt, p-Akt, Bcl-2, Bax and Cyt-c were detected by Western Blot. FKN knockdown decreased the expression of Cyt-c and Bax protein while increased the expression of Bcl-2 and p-Akt protein compared with the control.

However, LY294002 treatment reversed the effect of FKN knockdown (Fig.5**a**). Similarly, Immunofluorescent staining found that FKN knockdown increased p-Akt nuclear translocation while decreased Bax protein abundance in podocytes cytoplasm compared with the control, whereas the results were reversed by co-treatment with LY294002 (Fig.5**b**, **c**).

Discussion

Although the increasing number of studies suggest the importance of chemokines on the regulation of kidney disease, the current findings of molecular biological function of chemokines in the kidney are limited and their roles of anti-inflammation in kidney disease remain to be exploration. In the present study, we explored the protective effects of chemokine FKN deficiency by regulating the progression of apoptosis using a mice model of AKI and cultured podocytes. This study demonstrated that the protective effect of FKN deficiency is mediated by the suppression of podocytes apoptosis progression through the PI3K/Akt signal pathway, which providing an evidence of therapeutic potential of FKN deficiency in AKI.

Sepsis is defined as a systemic inflammatory response syndrome caused by infection and acted as a major cause of AKI[15]. The pathophysiological characteristics of sepsis are the initial severe inflammatory phase, which lasts for several days, followed by a more lasting period of immunosuppression[16]. Novel therapeutic mechanism for sepsis-induced AKI is urgently needed[17, 18]. In this study, LPS was used to established a sepsis-induced AKI model in mice[19]. We found significantly elevated FKN expression in LPS-induced AKI mice. Functionally, FKN had pro-inflammatory effects such as adhesion and chemotaxis inducing the recruitment of lymphocytes[20]. In vivo model, intraperitoneal administered with LPS for 24 h significantly increased the levels of serum creatinine and BUN, which a sensitive marker of AKI. Depletion of FKN attenuated LPS-induced serum creatinine and BUN to suppress proinflammatory cell infiltration and kidney damage.

The imbalance of body’s immune function caused by apoptosis progression dysregulation is key factor inducing the immunosuppression of sepsis patients[21, 22]. Apoptosis is a carefully regulated cell death process and mediated by multiple genes. The mitochondrial pathway was recognized the classical apoptotic signal pathway[23]. The dysfunction of the heterodimer Bax/Bcl-2 in the mitochondria leads to the release of Cyt-c, which activates downstream apoptosis-related proteases and further amplifies the apoptosis progression[24]. In our study, the production of Bax and Cyt-c protein were induced by LPS in mice kidney, whereas the expression of Bcl-2, nephrin and podocin protein were decreased. The pretreatment of FKN Depletion reduced the apoptosis-related proteins while increased the podocytes-related proteins compared with LPS group. Moreover, FKN knockdown decreased the Bax, Cyt-c while increased Bcl-2 protein expression in cultured podocytes, which is consistent with the findings in vivo. We found that FKN is closely associated with apoptosis progression and confirmed under pathogenic conditions. Inhibition of podocytes apoptosis progression was an important determination of AKI amelioration, which attributed to the deficiency of chemokine FKN.

We selected the PI3K/Akt signal pathway as the follow-up anti-inflammatory mechanism for experiments. Akt is a major downstream factor of PI3K signal and have many cellular functions including proliferation, differentiation and anti-apoptosis[25–27]. It was reported that the Akt phosphorylation attenuated sepsis-induced cardiomyocyte apoptosis[28]. Inhibition of PI3K/Akt signal suppressed podocytes mesenchymal trans-differentiation[29]. The previous study of our research group have demonstrated that the activation of PI3K/Akt signal effectively suppressed AngII-induced podocytes apoptosis[30]. In our vivo model, the phosphorylation of Akt was inhibited by LPS while activated by FKN Depletion. FKN Depletion ameliorated LPS-induced AKI by promoted the activation of PI3K/Akt signal pathway. In cultured podocytes, FKN knockdown suppressed podocytes apoptosis progression by promoting Akt phosphorylation, while this effect was reversed by co-treatment with PI3K/Akt inhibitor.

In conclusion, chemokine FKN depletion can ameliorate sepsis-induced AKI in mice. A potential mechanism is that FKN deficiency suppressed podocytes apoptosis progression by promoting PI3K/Akt signal pathway. This study may provide a novel therapeutic approach for kidney inflammatory disease.

Declarations

Ethics Approval and Consent to Participate

All animal experiments in the present study were approved by the Animal Care and Use Committee of the Youjiang Medical University for Nationalities.

Consent for publication

Not applicable

Availability of data and materials

The dataset supporting the conclusions of this article is included within the article.

Funding

This research was supported by the National Natural Science Foundation of China (81860296), the Natural Science Foundation of Guangxi (2017GXNSFDA198005 and 2018GXNSFAA281038) and the Innovation Project of Guangxi Graduate Education (YCSW2020236).

Authors' contributions

Qiming Gong, Jingxue Ma, Xiuhong Pan and Yan Jiang carried out the experimental work and conceived of the study and participated in its design and coordination. Qiming Gong and Yanwu You drafted the manuscript. All authors read and approved the final manuscript.

Acknowledgements

Our thanks go to all researchers from science center of Youjiang Medical University for Nationalities who helped to carry out this project.

Compliance with Ethical Standards

Competing interests

The authors declare that they have no competing interests.

References

1. Sygitowicz G, Sitkiewicz D. Molecular mechanisms of organ damage in sepsis: an overview. Braz J Infect Dis 2020, 24:552-560.
2. Yoo H, Lee JY, Park J, Yang JH, Suh GY, Jeon K. Association of Plasma Level of TNF-Related Apoptosis-Inducing Ligand with Severity and Outcome of Sepsis. J Clin Med 2020, 9.
3. Garg P. A Review of Podocyte Biology. Am J Nephrol 2018, 47 Suppl 1:3-13.
4. Nagata M. Podocyte injury and its consequences. Kidney Int 2016, 89:1221-30.
5. Liu W, Jiang L, Bian C, Liang Y, Xing R, Yishakea M, et al. Role of CX3CL1 in Diseases. Arch Immunol Ther Exp (Warsz) 2016, 64:371-83.
6. Ostuni MA, Hermand P, Saindoy E, Guillou N, Guellec J, Coens A, et al. CX3CL1 homo-oligomerization drives cell-to-cell adherence. Sci Rep 2020, 10:9069.
7. Chen G, Zhou Z, Sha W, Wang L, Yan F, Yang X, et al. A novel CX3CR1 inhibitor AZD8797 facilitates early recovery of rat acute spinal cord injury by inhibiting inflammation and apoptosis. Int J Mol Med 2020, 45:1373-1384.
8. Luong VH, Utsunomiya A, Chino T, Doanh LH, Matsushita T, Obara T, et al. Inhibition of the Progression of Skin Inflammation, Fibrosis, and Vascular Injury by Blockade of the CX(3) CL1/CX(3) CR1 Pathway in Experimental Mouse Models of Systemic Sclerosis. Arthritis Rheumatol 2019, 71:1923-1934.
9. Korbecki J, Simińska D, Kojder K, Grochans S, Gutowska I, Chlubek D, et al. Fractalkine/CX3CL1 in Neoplastic Processes. Int J Mol Sci 2020, 21 (10):3723.
10. Fu D, Senouthai S, Wang J, You Y. FKN Facilitates HK-2 Cell EMT and Tubulointerstitial Lesions via the Wnt/β-Catenin Pathway in a Murine Model of Lupus Nephritis. Front Immunol 2019, 10:784.
11. Hoxhaj G, Manning BD. The PI3K-AKT network at the interface of oncogenic signalling and cancer metabolism. Nat Rev Cancer 2020, 20:74-88.
12. Jia X, Wen Z, Sun Q, Zhao X, Yang H, Shi X, et al. Apatinib suppresses the Proliferation and Apoptosis of Gastric Cancer Cells via the PI3K/Akt Signaling Pathway. J buon 2019, 24:1985-1991.
13. Chen S, Peng J, Sherchan P, Ma Y, Xiang S, Yan F, et al. TREM2 activation attenuates neuroinflammation and neuronal apoptosis via PI3K/Akt pathway after intracerebral hemorrhage in mice. J Neuroinflammation 2020, 17:168.

14. Wang Q, Li R, Xiao Z, Hou C. Lycopene attenuates high glucose-mediated apoptosis in MPC5 podocytes by promoting autophagy via the PI3K/AKT signaling pathway. Exp Ther Med 2020, 20:2870-2878.
15. Gómez H, Kellum JA. Sepsis-induced acute kidney injury. Curr Opin Crit Care 2016, 22:546-553.
16. Bergmann CB, Beckmann N, Salyer CE, Hanschen M, Crisologo PA, Caldwell CC. Potential Targets to Mitigate Trauma- or Sepsis-Induced Immune Suppression. Front Immunol 2021, 12:622601.
17. Liu J, Xie H, Ye Z, Li F, Wang L. Rates, predictors, and mortality of sepsis-associated acute kidney injury: a systematic review and meta-analysis. BMC Nephrol 2020, 21:318.
18. Agapito Fonseca J, Gameiro J, Marques F, Lopes JA. Timing of Initiation of Renal Replacement Therapy in Sepsis-Associated Acute Kidney Injury. J Clin Med 2020, 9(5):1413.
19. Ding X, Tong Y, Jin S, Chen Z, Li T, Billiar TR, et al. Mechanical ventilation enhances extrapulmonary sepsis-induced lung injury: role of WISP1-αvβ5 integrin pathway in TLR4-mediated inflammation and injury. Crit Care 2018, 22:302.
20. Chen X, Wei Q, Hu Y, Wang C. Role of Fractalkine in promoting inflammation in sepsis-induced multiple organ dysfunction. Infect Genet Evol 2020, 85:104569.
21. Brady J, Horie S, Laffey JG. Role of the adaptive immune response in sepsis. Intensive Care Med Exp 2020, 8:20.
22. Menassa J, Nedeva C, Pollock C, Puthalakath H. TLR4: the fall guy in sepsis? Cell Stress 2020, 4:270-272.
23. Zhang J, Wang C, Kang K, Liu H, Liu X, Jia X, et al. Loganin Attenuates Septic Acute Renal Injury with the Participation of AKT and Nrf2/HO-1 Signaling Pathways. Drug Des Devel Ther 2021, 15:501-513.
24. Carpenter R, Brady MF. BAX Gene. StatPearls. Treasure Island (FL): StatPearls Publishing Copyright © 2021, StatPearls Publishing LLC. 2021.
25. Jiang N, Dai Q, Su X, Fu J, Feng X, Peng J. Role of PI3K/AKT pathway in cancer: the framework of malignant behavior. Mol Biol Rep 2020, 47:4587-4629.
26. Li T, Zhang L, Jin C, Xiong Y, Cheng YY, Chen K. Pomegranate flower extract bidirectionally regulates the proliferation, differentiation and apoptosis of 3T3-L1 cells through regulation of PPARγ expression mediated by PI3K-AKT signaling pathway. Biomed Pharmacother 2020, 131:110769.
27. Liu C, Chen K, Wang H, Zhang Y, Duan X, Xue Y, et al. Gastrin Attenuates Renal Ischemia/Reperfusion Injury by a PI3K/Akt/Bad-Mediated Anti-apoptosis Signaling. Front Pharmacol 2020, 11:540479.
28. Yang S, Wang Y, Gao H, Wang B. MicroRNA-30a-3p overexpression improves sepsis-induced cell apoptosis in vitro and in vivo via the PTEN/PI3K/AKT signaling pathway. Exp Ther Med 2018, 15:2081-2087.
29. Tu Q, Li Y, Jin J, Jiang X, Ren Y, He Q. Curcumin alleviates diabetic nephropathy via inhibiting podocyte mesenchymal transdifferentiation and inducing autophagy in rats and MPC5 cells. Pharm Biol 2019, 57:778-786.

30. Wang J, Fu D, Senouthai S, You Y. Critical roles of PI3K/Akt/NF-κB survival axis in angiotensin II-induced podocyte injury. Mol Med Rep 2019, 20:5134-5144.

Figures

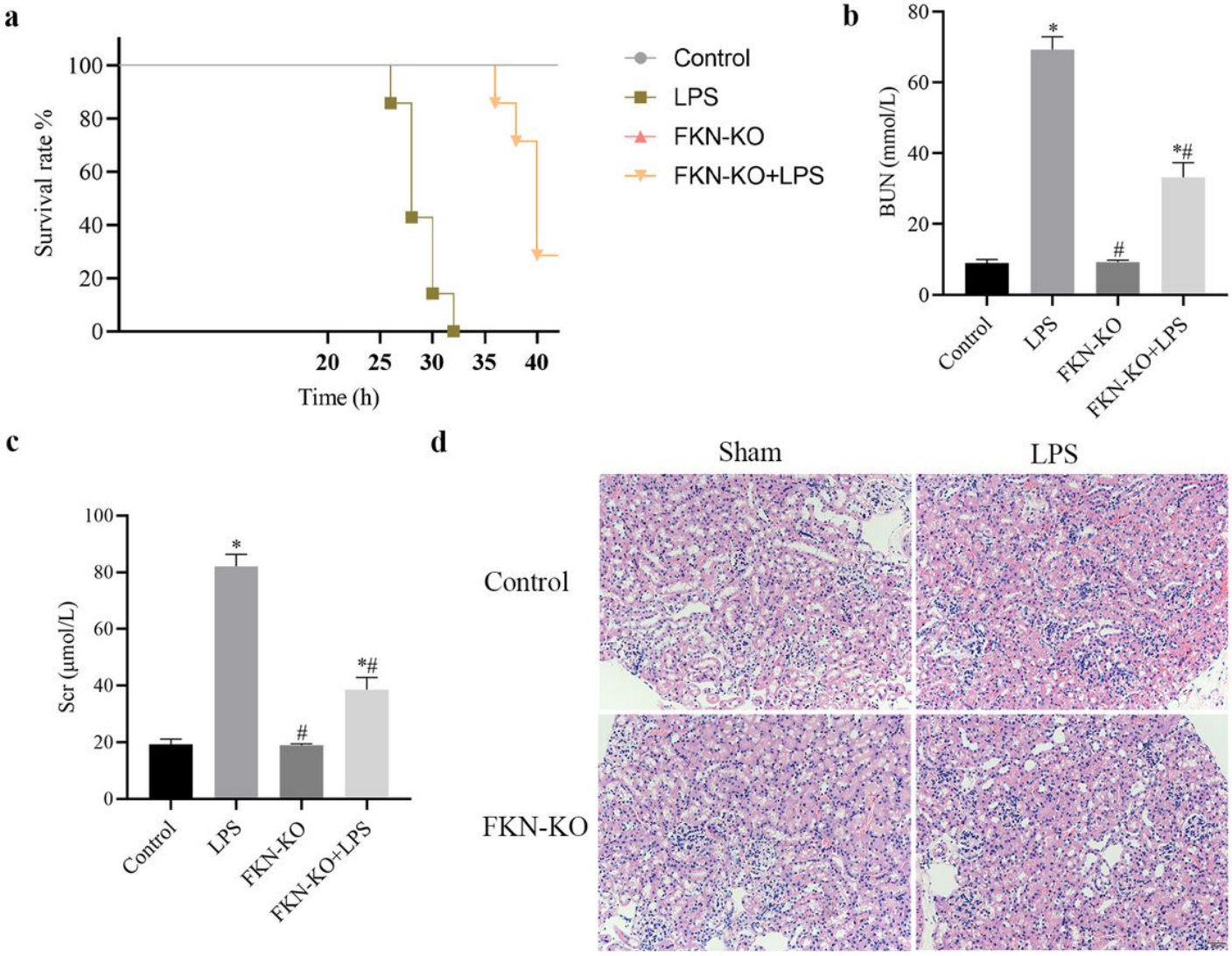

Figure 1

Depletion of FKN reduces kidney damage in LPS-induced mice. a, Survival rate (n=7) of control and FKN-knockout mice injected with LPS. B and C, Serum levels of Blood Urea Nitrogen (BUN) (b) and creatinine (Scr) (c). d, Haematoxylin-eosin staining of kidney sections (×200).

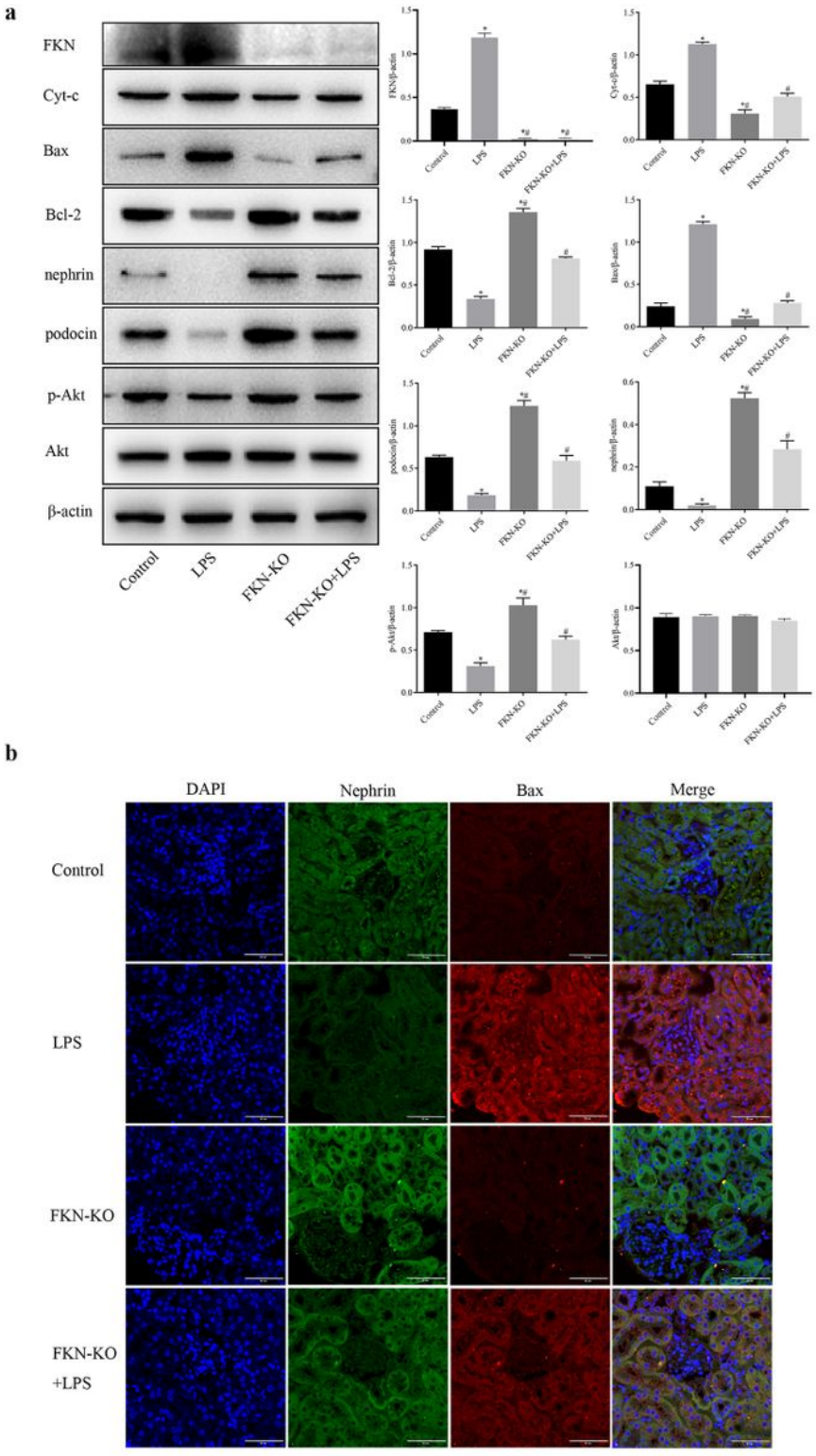

Figure 2

Reduced podocytes apoptosis and activation of PI3K/AKT signal pathway in kidney of LPS-induced FKN-knockout mice. a, Western Blot and quantitative assay showing the protein expression of FKN, Cyt-c, Bax, Bcl-2, nephrin, podocin, p-Akt, Akt in mice liver tissues. *p<0.05 compared to the Control group, #p<0.05 compared to the LPS group. b, Localization of nephrin and Bax protein were evaluated by immunofluorescence assay. (×600).

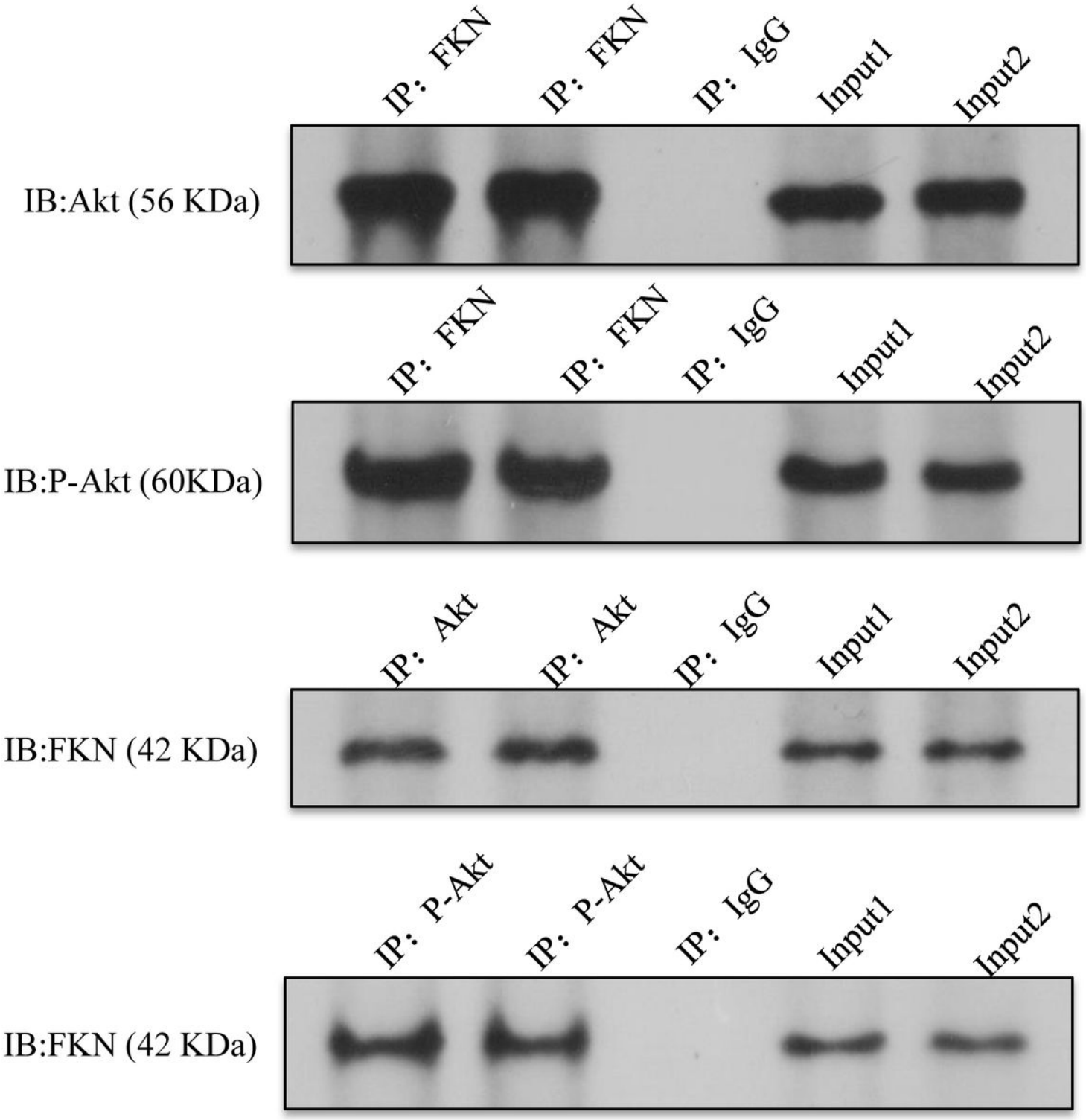

Figure 3

Co-IP assay verified the correlation of FKN with Akt and p-Akt in podocytes. Immune-precipitates were subjected to immunoblotting analysis with anti-FKN, anti-p-Akt, and anti-Akt. IP, immunoprecipitation, IB, immunoblot.

Figure 4

FKN-knockdown inhibits podocytes apoptosis rate. The apoptosis rates were detected by flow cytometry assay after treatment with FKN-knockdown and PI3K/AKT signal pathway inhibitor LY294002. *P < 0.05 compared with the Control group.

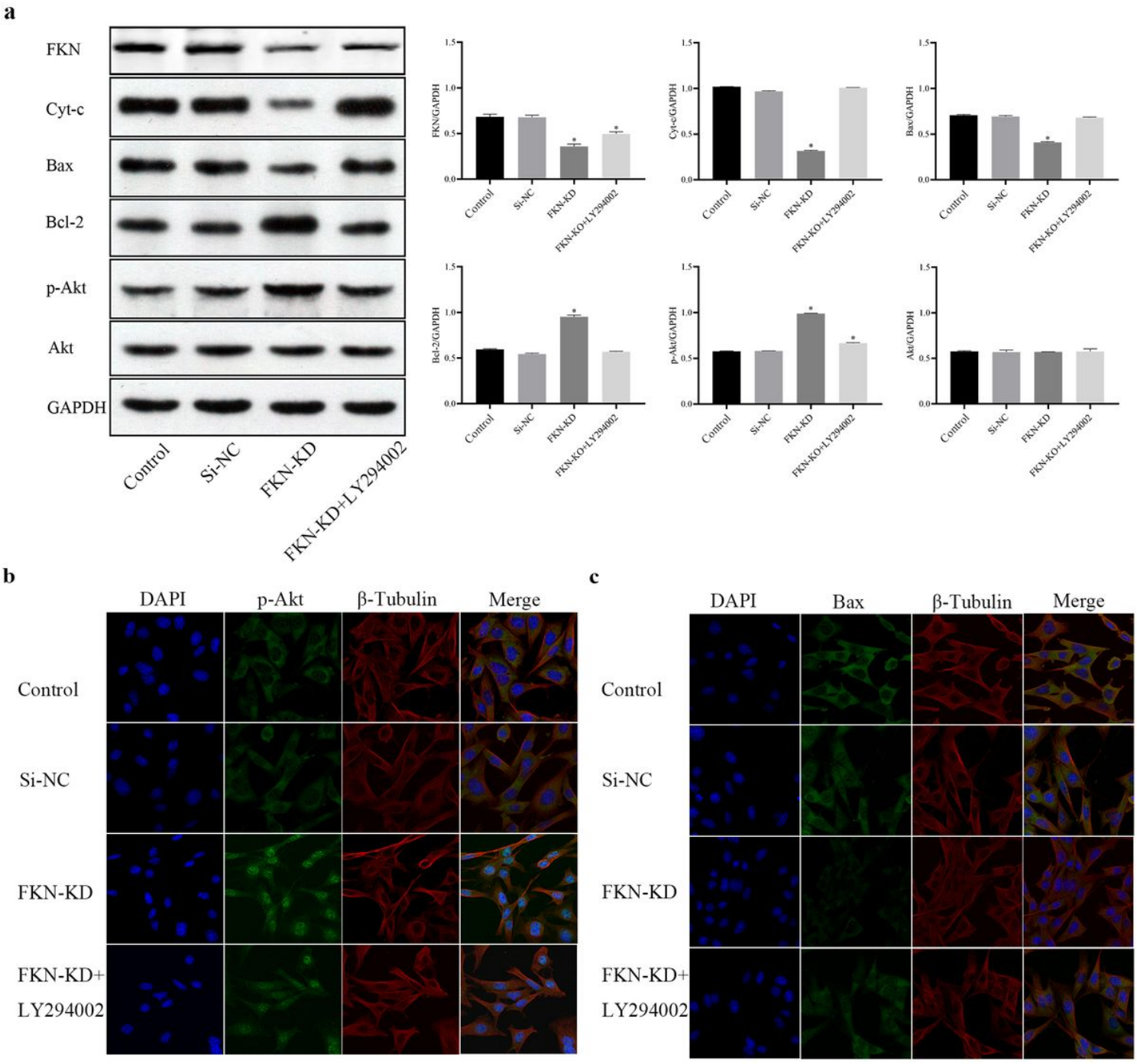

Figure 5

FKN-knockdown reduces apoptosis-related protein expression by activating PI3K/AKT signal pathway. a, Western Blot and quantitative assay showing the protein expression of FKN, Cyt-c, Bax, Bcl-2, p-Akt, Akt in podocytes. *p<0.05 compared to the Control group. b and c, Localization of p-Akt (B) and Bax (C) protein were evaluated by immunofluorescence assay. (×800).