

The protective effect of the TUG1/miR-197/MAPK1 axis on lipopolysaccharide-induced podocyte injury

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Abstract. The podocyte, a type of glomerular epithelial cell, is the key constituent of the filtration barrier layer in the kidney. Previous studies have shown that long non-coding RNA (lncRNA)-taurine-upregulated gene 1 (TUG1) served a protective role in diabetes-induced podocyte damage. The aim of the present study was to investigate the potential role of TUG1 in the progress of podocyte injury induced by lipopolysaccharide (LPS), and explore the underlying mechanisms. The results showed that TUG1 expression was suppressed in LPS-induced podocytes. Enhanced TUG1 expression by exogenous recombinant vector regulated the expression of podocyte associated proteins [Nephrin, Podocin and CCAAT/enhancer-binding protein (CHOP)]. A marked decrease was observed in the level the albumin influx in cells transfected with TUG1. Further study indicated that microRNA (miR)-197 is a potential target of TUG1. The enhanced level of miR-197 induced by LPS was inhibited in cells transfected with TUG1. The decreased Nephrin and Podocin expression, upregulated CHOP expression and the increased albumin influx were slightly enhanced by miR-197 mimic transfection, while markedly suppressed by miR-197 inhibitor transfection in LPS-induced podocytes. Mitogen-activated protein kinase (MAPK) protein was predicted as a potential target of miR-197. The downregulated expression of phosphorylated-MAPK/MAPK induced by LPS was significantly suppressed by TUG1 transfection in podocytes. In addition to this, autophagy was promoted by TUG1 transfection via the

elevation of the Beclin1 and light chain (LC)3 II/LC3 I levels, and suppressing p62 expression. However, the p38 MAPK inhibitor SB203580 reversed the changes that TUG1 induced in the levels of Beclin1, LC3 II/LC3 I and p62. Taken together, these results demonstrated that LPS-induced podocyte injury could be alleviated by the TUG1/miR-197/MAPK1 axis.

Introduction

Podocytes are terminally differentiated glomerular cells with elaborate extensions of the cell body and play an important role in maintaining the structural and functional integrity of the filtration barrier (1). Studies have indicated that podocytes damage and the reduction in podocyte density are the main contributors to the development of proteinuria and glomerulosclerosis (2). Thus, podocytes serve as vital role in preventing proteinuria and glomerulosclerosis from getting worse.

Long non-coding RNAs (lncRNAs), a heterogeneous class of long (>200 nucleotides) 5 transcripts, is characterized by an apparent lack of protein-coding potential (3). lncRNAs have attracted much attention of scientists for its diagnostic, prognostic, and predictive potential (4). lncRNA taurine-upregulated gene 1 (TUG1) was firstly identified in a genomic screen as a part of photoreceptors and overexpressed in response to retinal development in mouse retinal cells (5). Researchers have indicated that TUG1 could be a predictive biomarkers of kidney neoplasms. Besides, accumulated studies have demonstrated that TUG1 contributed to the development of chronic kidney diseases (6). However, the underlying molecular mechanisms are not fully understood.

Researches have shown that TUG1 has moderation effects on various diseases via interacting with microRNAs (miRNAs or miRs), including miR-204-5p (7), miR-144-3p (8), miR-382 (9) and so on. Studies over the last decades have suggested that miRNAs served as pathogenic or therapeutic factor to be involved in the development of a variety of renal diseases, such as chronic kidney disease (10), kidney fibrosis (11), type 2 diabetic kidney disease (12) and glucose-induced podocyte apoptosis (13). According to miRDB, at least two binding sites were identified between miR-197 and TUG1. Besides, TargetScan and miRwalk have predicted the high binding possibility between MAPK1 and miR-197. As we all know, MAPK pathway could induce cell

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Abbreviations: MAPK, mitogen-activated protein kinase; LPS, lipopolysaccharide; lncRNAs, long non-coding RNAs; TUG1, taurine-upregulated gene 1; miRNA/miR, microRNA; TNF- α , tumor necrosis factor- α

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autophagy in various types of cells (14), and autophagy played a positive role in defending podocyte against lipopolysaccharide (LPS)-induced damage (15). Thus, miR-197 may be a potential biomarker and a regulation factor in the treatment of podocyte injury.

In this study, we investigated the underlying mechanisms of TUG1 in treating LPS-induced podocytes injury. Our results strongly demonstrated that TUG1 could protect podocyte against LPS-induced injury by targeting miR-197 through activating MAPK pathway.

Materials and methods

Cell culture. The mouse immortalized podocyte cell line MPC5 was purchased from National Infrastructure of Cell Line Resource. Cells were cultivated at 33°C with RPMI-1640 containing 10% FBS and recombinant mouse interferon- γ (50 U/ml, Toyobo Co., Osaka, Japan) to propagate cells. After reaching confluence, cells were incubated without interferon- γ to induce cell differentiation into the podocyte lineage at 37°C for more than 6 days. The culture medium was changed every three days. Then, cells were divided into different groups.

Cell transfection. SB203580 was purchased from Calbiochem (San Diego, CA, USA) and dissolved in dimethyl sulfoxide (DMSO). pcDNA-TUG1, pcDNA vector, miR-197 mimic and miR-197 inhibitor were purchased from Addgene (Cambridge, MA, USA). MPC5 cells were transfected with pcDNA-TUG1 (40 μ g/ml), pcDNA vector (40 μ g/ml), miR-197 mimic (80 nM) or miR-197 inhibitor (160 nM) using Lipofectamine[®] 2000 (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) according to manufacturers instruction. 48 h after transfection, transfected MPC5 podocytes and normal MPC5 podocytes were treated with lipopolysaccharide (LPS; 1 μ g/ml) or the combination of LPS (1 μ g/ml) and SB203580 (10 μ M) for 24 h for further experiments.

Western blot assay. The total protein was isolated from MPC5 cells in different groups, and separated by SDS-PAGE and transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). After blocking with 5% BSA and washing with 0.05% Tween-20, membranes were incubated with primary antibodies [Podocin, Nephlin, CCAAT/enhancer-binding protein (CHOP), p-MAPK, MAPK, Beclin1, p62, light chain (LC)3 and β -actin] (Abcam, Cambridge, UK) at 4°C overnight. Then, all membranes were incubation with horseradish peroxidase (HRP)-conjugated secondary antibodies for 1.5 h at room temperature. The bands were analyzed using ImageJ [National Institutes of Health (NIH), Sacaton, AZ, USA].

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) assay. Total RNAs were extracted from MPC5 podocytes in different groups using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. RT-qPCR was conducted using Applied Biosystems StepOnePlus[™] system. The RT-PCR primers for Podocin, Nephlin, CHOP, TUG1 and miR-197 purchased from GeneCopoeia Inc. (Rockville, MD, USA). The

specific primers were as follows: Podocin (forward: 5'-GCA TCAAGCCCTCTGGATTAG-3' and reverse: 3'-AGACGG AGATCAACCTTGTGATA-5'); Nephlin (forward: 5'-ATG GGAGCTAAGGAAGCCACA-3' and reverse: 3'-CCACAC CACAGCTTAAGTGTGTC-5'), CHOP (forward: 5'-TTGCCC TCTTATTGGTCCAGC-3' and reverse: 3'-TAGCGACTGTTC TGTTCCAC-5'). GAPDH was used as the internal control. The relative levels were measured using the $2^{-\Delta\Delta C_q}$ analysis method (16).

Albumin influx assay. Filtration barrier function of podocyte monolayers was assessed using albumin influx assay. Briefly, 5×10^3 podocytes were seeded into a 3 μ m pores trans-well filter coated with collagen (Corning Incorporated, Corning, NY, USA), then incubated for 10 days. After that, MPC5 podocytes were serum-starved overnight and washed with PBS containing 1 mmol/l $MgCl_2$ and 1 mmol/l $CaCl_2$. Then, top chamber was filled with 0.15 ml RPMI-1640 and the bottom chamber with 1 ml RPMI-1640 containing 40 mg/ml of bovine serum albumin at 37°C with 5% CO_2 . Cells were cultured for 24 h. Albumin concentration was measured using an ELISA kit (Thermo Fisher) according to the manufacturer's instructions.

Statistical analysis. SPSS 19.0 software (IBM Corp., Armonk, NY, USA) was used to analyze all the data. The data are presented as the mean \pm standard deviation. Analysis was performed using one-way analysis of variance followed by a Bonferroni post hoc test. Statistical significance was assigned at $P < 0.05$.

Results

TUG1 was downregulated in LPS impaired podocyte. To investigate whether TUG1 expression was related to LPS-induced damage, MPC5 podocytes were treated with LPS (1 μ g/ml) for 24 h. Results indicated that the protein expressions of Podocin and Nephlin were remarkably suppressed while CHOP significantly elevated in LPS group compared with control group (Fig. 1A, C and E). The mRNA expression was consistent with protein expression (Fig. 1B, D and F). LPS treatment result in a greater albumin influx across the podocyte monolayer compared with control (Fig. 1G). These results suggested that the function of MPC5 podocytes was impaired by LPS. Besides that, the relative mRNA expression of TUG1 was dramatically inhibited in LPS group in comparison with control group (Fig. 1H), indicating that TUG1 was downregulated in LPS damaged podocyte.

Overexpression of TUG1 alleviates LPS-induced podocyte injury. To explore whether TUG1 could attenuate the cell damage induced by LPS, MPC5 podocytes were transfected with pcDNA-TUG1. As shown in Fig. 2A, the relative expression of TUG1 was largely enhanced in TUG1 group, indicating the high transfection efficiency. The reduced Podocin and Nephlin expression along with the increased CHOP expression induced by LPS compared with control were significantly inhibited by TUG1 transfection (Fig. 2B-E). Moreover, the elevated albumin influx caused by LPS compared with control was remarkably repressed by

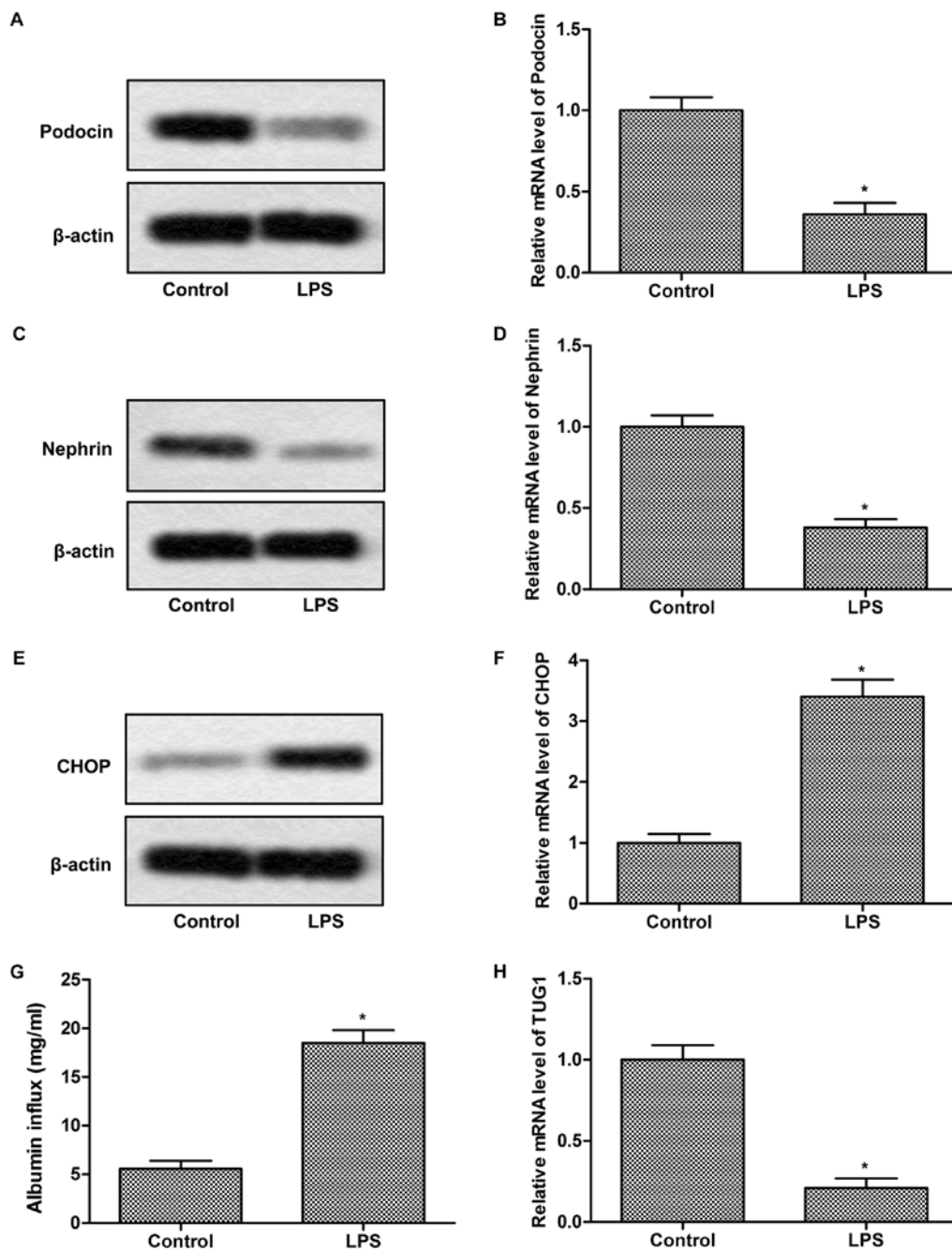


Figure 1. TUG1 is downregulated in LPS damaged podocyte. Podocytes were randomly divided into 2 groups: The control group consisted of cells without treatment; and the LPS group was comprised of cells that were treated with LPS. The protein and relative mRNA expressions of (A and B) Podocin, (C and D) Nephrin and (E and F) CHOP were detected using western blotting and RT-qPCR, respectively. (G) Albumin influx assay was applied to assess the glomerular filtration function. (H) The relative mRNA expression of TUG1 was also measured using RT-qPCR. The experiments were repeated at least 3 times, and data is presented as the mean \pm standard deviation. *P<0.05 vs. control group. TUG1, taurine-upregulated gene 1; LPS, lipopolysaccharide; CHOP, CCAAT/enhancer-binding protein; RT-qPCR, reverse transcription-quantitative polymerase chain reaction.

TUG1 overexpression (Fig. 2F). These results suggested that LPS-induced podocyte injury could be alleviated by TUG1 overexpression.

miR-197 is a direct target of TUG1. The targeting relationship between miR-197 and MAPK1 was predicted by bioinformatics (Fig. 3A). To further evaluate the potential relationship between miR-197 and TUG1, RT-qPCR and western blotting were conducted in podocytes transfected with pcDNA-TUG1/pcDNA-vector/ miR-197 mimic/miR-197

inhibitor. As illustrated in Fig. 3B, the expression of miR-197 was significantly increased in LPS group compared with control group. The elevated level of miR-197 in cells treated with LPS was inhibited by transfection with TUG1 (Fig. 3C). Besides that, the reduced Nephrin and Podocin expression along with the enhanced CHOP expression were slightly enhanced by TUG1 mimic transfection while remarkably suppressed by TUG1 inhibitor transfection (Fig. 3D-G). Moreover, the increased albumin influx induced by LPS was slightly enhanced by TUG1 mimic transfection while

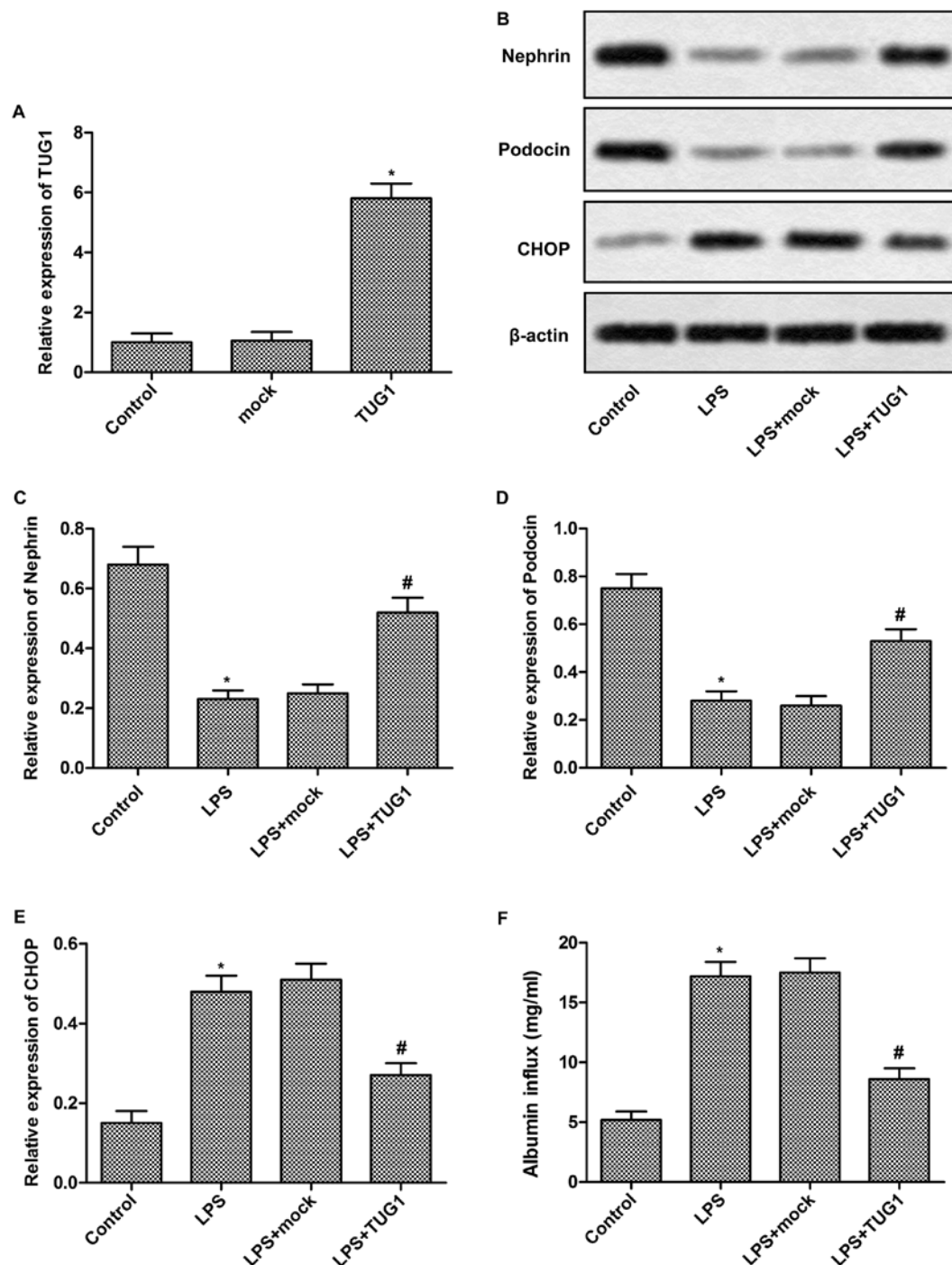
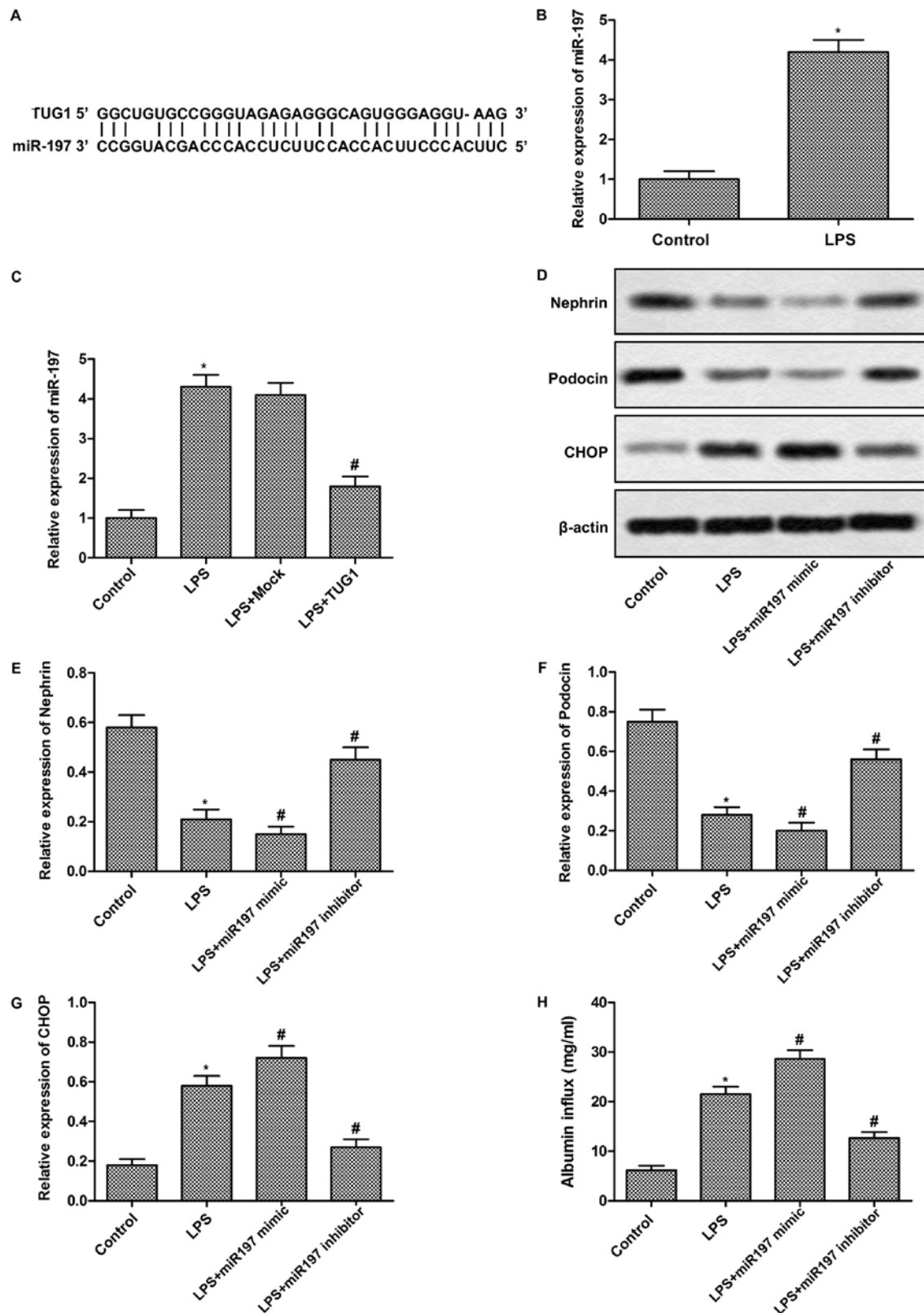


Figure 2. Overexpression of TUG1 alleviates LPS-induced podocyte injury. (A) Podocytes were randomly divided into 3 groups: Control group, cells without treatment; the mock group, cells transfected with pcDNA vector; and cells transfected with pcDNA-TUG1. The relative mRNA expression of TUG1 was assessed using reverse transcription-quantitative polymerase chain reaction. Podocytes were then randomly divided into 4 groups: Control group, cells without treatment; LPS group, cells induced by LPS; LPS+mock group, cells transfected with pcDNA vector that were induced by LPS; and LPS+TUG1 group, cells transfected with pcDNA-TUG1 that were induced by LPS. (B) The protein expressions of Nephrin, Podocin and CHOP were measured using western blot analysis. Data summary and analysis of the expression of (C) Nephrin, (D) Podocin and (E) CHOP in podocyte cells according to the results of western blotting. (F) Albumin influx assay was used to evaluate the glomerular filtration function. The experiments were repeated at least 3 times, and data is presented as the mean \pm standard deviation. * $P < 0.05$ vs. control group; # $P < 0.05$ vs. TUG1, taurine-upregulated gene 1; LPS group. LPS, lipopolysaccharide; CHOP, CCAAT/enhancer-binding protein.

remarkably inhibited by TUG1 inhibitor transfection (Fig. 3H). These results indicated that miR-197 is a potential target of TUG1.

lncRNA TUG1 aggravates podocytes damage via regulating MAPK pathway. The target sequences of miR-197 in the

3'-UTR region of MAPK1 was predicted through bioinformatics analysis (Fig. 4A). To detect the regulatory relationship between TUG1 and MAPK1, MPC5 podocytes were transfected with pcDNA-TUG1/pcDNA-vector. As illustrated in Fig. 4B, the decreased expression of p-MAPK/MAPK induced by LPS compared with control was strongly



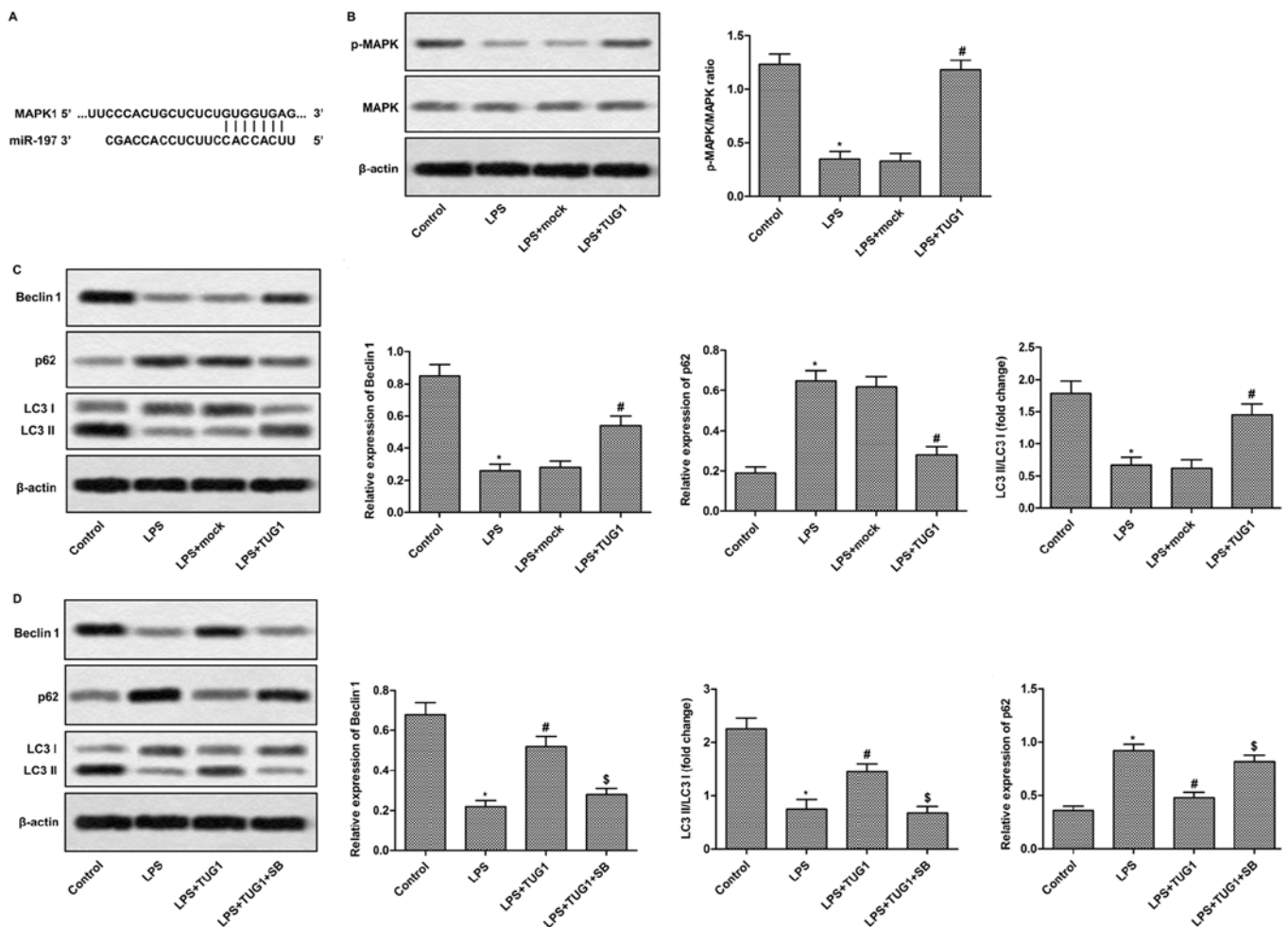


Figure 4. miR-197 aggravates podocyte damage by regulating the MAPK signaling pathway. (A) The targeting association between miR-197 and MAPK1 was predicted by bioinformatics. (B and C) Podocytes were randomly divided into 4 groups: Control group, cells without treatment; LPS group, cells induced by LPS; LPS+mock group, cells transfected with pcDNA vector that were induced by LPS; and LPS+TUG1 group, cells transfected with pcDNA-TUG1 that were induced by LPS. (B) The expression of p-MAPK/MAPK was measured using western blotting. (C) The levels of Beclin1, p62 and LC3 I/ LC3 II were evaluated using western blotting. (D) Podocytes were randomly divided into 4 groups: Control group, cells without treatment; LPS group, cells induced by LPS; LPS+TUG1 group, cells transfected with pcDNA-TUG1 that were induced by LPS; and LPS+TUG1+SB group, cells transfected with pcDNA-TUG1 were treated with LPS and SB. The expression level of Beclin1, p62 and LC3 I/ LC3 II were detected using western blot analysis. The experiments were repeated at least 3 times, and data is presented as the mean \pm standard deviation. * $P < 0.05$ vs. control group; # $P < 0.05$ vs. LPS group; \$ $P < 0.05$ vs. LPS+TUG1 group. MAPK, mitogen-activated protein kinase; LPS, lipopolysaccharide; TUG1, taurine-upregulated gene 1; LC3, light chain 3; p-, phosphorylated; SB, SB203580 (inhibitor).

inhibited by TUG1 transfection. Besides, the decreased Beclin1, LC3 II/LC3 I along with upregulated p62 levels were vastly suppressed by TUG1 transfection, indicating TUG1 could regulate cell autophagy via regulating MAPK pathway (Fig. 4C). To further validate this result, SB203580 was added into cells to inactivate MAPK pathway. Results indicated that SB203580 could reverse all the changes in the expressions of Beclin1, LC3 II/LC3 I and p62 caused by TUG1 (Fig. 4D). These results suggested that lncRNA TUG1 could aggravate podocytes damage via regulating MAPK pathway indirectly.

Discussion

The podocyte is a kind of special glomerular epithelial cell and key constituent of the layer of the filtration barrier in the kidney (17). Intact kidney filtration barrier maintains the balance of proteins in the blood (18). If the podocytes are impaired, plasma albumin can leak into the urinary space,

leading to the deterioration of kidney diseases and hypoalbuminemia. If the albuminuria persist and increase in patients, it will develop into systemic diseases, such as anemia, postural hypotension, bradyarrhythmia. Thus, treatment for podocyte damage is extremely important to the prevention of various renal diseases and related systemic diseases.

It has been suggested and supported by researchers that lncRNAs have extensive biological functions. Abnormal expression of lncRNAs is linked to a variety of diseases, such as cancer as well as neurological, cardiac and renal diseases (19,20). Among these lncRNAs, Long and colleagues observed that the expression of TUG1 was remarkably inhibited in the podocytes of diabetic mice. Podocyte-specific overexpression of TUG1 could elevate PGC-1 α expression, resulting in the improvement of mitochondrial bioenergetics (21). Similarly, in our research, overexpression of TUG1 could protect podocyte from LPS-induced damage, evidences were the reduced Nephlin, Podocin expression, elevated CHOP

expressions along with the enhanced albumin influx were vastly inhibited by TUG1 transfection. However, how did TUG1 worked need further exploration.

Previous studies have shown that TUG1 acted as a regulation role in a variety of diseases through interacting with miRNAs. miRNAs, existed in eukaryotic cells, could regulate various physiological biological metabolism through combining with 3'-UTR of target genes mRNA, thereby promoting mRNA cleavage or suppressing the translation of mRNA (22). More and more researchers have demonstrated that many miRNAs were involved in the development of multiple kidney diseases. For example, miR-21 acts as an oncogene in renal cancer via inducing cell proliferation and invasion (23). miR-214, -132, -21 and -15b have been testified to participate in the renal fibrosis process (10). It is predicted by TargetScan and miRwalk that miR-197 has a close relationship to TUG1, at least two binding sites were found between them. Our research further provided evidences that miR-197 may be a target of TUG1 through detecting the miR-197 content in podocytes transfected with TUG1. In addition to this, the decreased Nephhrin, Podocin expression, upregulated CHOP expressions along with the increased albumin influx induced by LPS were slightly enhanced by miR-197 mimic transfection while significantly suppressed by miR-197 inhibitor transfection in podocytes.

Apoptotic cell death is a genetically programmed mechanism that allows the cell to commit suicide (24), and plays a vital role in the development of multiple organ dysfunction in LPS-induced sepsis (25). miRNAs serve as the useful laboratory markers of organ failure during sepsis and have various effects on the processes of pro-apoptosis or anti-apoptosis (26,27). According to the previous report, miR-197 induced apoptosis and suppressed tumorigenicity in multiple myeloma xenograft models (28). Report also indicate that the level of miR-197 was elevated in severe acute viral hepatitis with coagulopathy (29). Thus, cell apoptosis regulated by miR-197 may be involved in the pathological process of LPS-induced podocytes injury. Autophagy serves a vital role in keeping the balance between anabolism and catabolism, maintaining protein quality control and removing impaired proteins, organelles and lipids (30). The results obtained from the current studies have demonstrated that the dysfunction of podocyte autophagy taken responsibility for the development of various kidney diseases, including diabetic nephropathy (31), immunoglobulin A Nephropathy (32) and proteinuric kidney disease (33). Besides, Tan *et al* (15) pointed out that the podocytes autophagy was remarkably suppressed by LPS, which result in LPS-induced injury of podocytes. The inducing autophagy ability of MAPK pathway was demonstrated by many researchers in multiple diseases (34,35). Thus, MAPK pathway may be a major target in treating LPS-induced podocytes injury. According to the prediction of TargetScan and miRwalk, miR-197 may inactivate MAPK pathway via binding to MAPK1, and then restrain the autophagy of podocyte. Considering that miR-197 is a target of TUG1, TUG1 might induce the autophagy of podocytes via motivating MAPK pathway indirectly. Our study demonstrated that TUG1 transfection could remarkably inhibit the increased p-MAPK/MAPK level in LPS-induced podocytes. Besides that, the reduced Beclin1, p62 and LC3 II/I and increased p62 expression caused by LPS were remarkably inhibited by TUG1 transfection. Moreover, the p38 MAPK

inhibitor SB203580 could reverse all the changes caused by TUG1 in LPS-induced podocytes.

All in all, our study indicated that TUG1 level was down-regulated in podocytes impaired by LPS. Elevated TUG1 by transfection suppressed the LPS-induced alteration of podocyte injury related proteins (Nephhrin, Podocin and CHOP) expression and elevated albumin influx. Further research demonstrated that miR-197 was a direct target of TUG1. TUG1 was confirmed to restore the function of podocytes via sponging miR-197 to enhance the level of p-MAPK/MAPK, thereby inducing autophagy. In summary, the TUG1-miR-197-MAPK pathway may act as a potential new target for LPS-induced podocytes injury therapy.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

DZ analyzed and interpreted the data regarding cell culture, transfection and western blot analysis, and conducted the statistical analysis. ZL was involved in RT-qPCR analysis. HZ was responsible for the Albumin influx assay, as well as the study design and drafting of the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Kriz W, Gretz N and Lemley KV: Progression of glomerular diseases: Is the podocyte the culprit? *Kidney Int* 54: 687-697, 1998.
2. Ijpeelaar DH, Schulz A, Koop K, Schlesener M, Bruijn JA, Kerjaschki D, Kreutz R and de Heer E: Glomerular hypertrophy precedes albuminuria and segmental loss of podoplanin in podocytes in Munich-Wistar-Fröster rats. *Am J Physiol Renal Physiol* 294: F758-F767, 2008.

3. Djebali S, Davis CA, Merkel A, Dobin A, Lassmann T, Mortazavi A, Tanzer A, Lagarde J, Lin W, Schlesinger F *et al*: Landscape of transcription in human cells. *Nature* 489: 101-108, 2012.
4. Ponting CP, Oliver PL and Reik W: Evolution and functions of long noncoding RNAs. *Cell* 136: 629-641, 2009.
5. Young TL, Matsuda T and Cepko CL: The noncoding RNA taurine upregulated gene 1 is required for differentiation of the murine retina. *Curr Biol* 15: 501-512, 2005.
6. Li SY and Susztak K: The long noncoding RNA Tug1 connects metabolic changes with kidney disease in podocytes. *J Clin Invest* 126: 4072-4075, 2016.
7. Yu C, Li L, Xie F, Guo S, Liu F, Dong N and Wang Y: lncRNA TUG1 sponges miR-204-5p to promote osteoblast differentiation through upregulating Runx2 in aortic valve calcification. *Cardiovasc Res* 114: 168-179, 2018.
8. Cao J, Han X, Qi X, Jin X and Li X: TUG1 promotes osteosarcoma tumorigenesis by upregulating EZH2 expression via miR-144-3p. *Int J Oncol* 51: 1115-1123, 2017.
9. Zhao L, Sun H, Kong H, Chen Z, Chen B and Zhou M: The lncRNA-TUG1/EZH2 axis promotes pancreatic cancer cell proliferation, migration and EMT phenotype formation through sponging Mir-382. *Cell Physiol Biochem* 42: 2145-2158, 2017.
10. Lv W, Fan F, Wang Y, Gonzalez-Fernandez E, Wang C, Yang L, Booz GW and Roman RJ: Therapeutic potential of microRNAs for the treatment of renal fibrosis and CKD. *Physiol Genomics* 50: 20-34, 2018.
11. Park EJ, Jung HJ, Choi HJ, Cho JI, Park HJ and Kwon TH: miR-34c-5p and CaMKII are involved in aldosterone-induced fibrosis in kidney collecting duct cells. *Am J Physiol Renal Physiol* 314: F329-F342, 2018.
12. Xie Y, Jia Y, Cuihua X, Hu F, Xue M and Xue Y: Urinary exosomal MicroRNA profiling in incipient type 2 diabetic kidney disease. *J Diabetes Res* 2017: 6978984, 2017.
13. Wang X, Lin B, Nie L and Li P: microRNA-20b contributes to high glucose-induced podocyte apoptosis by targeting SIRT7. *Mol Med Rep* 16: 5667-5674, 2017.
14. Xie H, Li J, Gao H, Wang J, Li C, Xu Y and Liu C: Total flavone of desmodium styracifolium relieved apoptosis and autophagy of COM-induced HK-2 cells by regulating KIM-1 via p38/MAPK pathway. *Mol Cell Biochem* 442: 169-175, 2018.
15. Tan X, Chen Y, Liang X, Yu C, Lai Y, Zhang L, Zhao X, Zhang H, Lin T, Li R and Shi W: Lipopolysaccharide-induced podocyte injury is mediated by suppression of autophagy. *Mol Med Rep* 14: 811-818, 2016.
16. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2⁻($\Delta\Delta C_T$) method. *Methods* 25: 402-408, 2001.
17. Guo B, Lyu Q, Slivano OJ, Dirks R, Christie CK, Czyzyk J, Hezel AF, Gharavi AG, Small EM and Miano JM: Serum response factor is essential for maintenance of podocyte structure and function. *J Am Soc Nephrol* 29: 416-422, 2018.
18. Guan Y, Nakano D, Zhang Y, Li L, Liu W, Nishida M, Kuwabara T, Morishita A, Hitomi H, Mori K, *et al*: A protease-activated receptor-1 antagonist protects against podocyte injury in a mouse model of nephropathy. *J Pharmacol Sci pii*: S1347-8613: 30128-30137, 2017.
19. Feng M, Tang PM, Huang XR, Sun SF, You YK, Xiao J, Lv LL, Xu AP and Lan HY: TGF- β mediates renal fibrosis via the Smad3-ErbB4-IR long noncoding RNA axis. *Mol Ther* 26: 148-161, 2018.
20. Huang HW, Xie H, Ma X, Zhao F and Gao Y: Upregulation of lncRNA PANDAR predicts poor prognosis and promotes cell proliferation in cervical cancer. *Eur Rev Med Pharmacol Sci* 21: 4529-4535, 2017.
21. Long J, Badal SS, Ye Z, Wang Y, Ayanga BA, Galvan DL, Green NH, Chang BH, Overbeek PA and Danesh FR: Long noncoding RNA Tug1 regulates mitochondrial bioenergetics in diabetic nephropathy. *J Clin Invest* 126: 4205-4218, 2016.
22. Tana C, Giamberardino MA and Cipollone F: microRNA profiling in atherosclerosis, diabetes, and migraine. *Ann Med* 49: 93-105, 2017.
23. Chen J, Gu Y and Shen W: MicroRNA-21 functions as an oncogene and promotes cell proliferation and invasion via TIMP3 in renal cancer. *Eur Rev Med Pharmacol Sci* 21: 4566-4576, 2017.
24. Fulda S, Gorman AM, Hori O and Samali A: Cellular stress responses: Cell survival and cell death. *Int J Cell Biol* 2010: 214074, 2010.
25. Wei WY, Ma ZG, Zhang N, Xu SC, Yuan YP, Zeng XF and Tang QZ: Overexpression of CTRP3 protects against sepsis-induced myocardial dysfunction in mice. *Mol Cell Endocrinol* 476: 27-36, 2018.
26. Liu S, Liu C, Wang Z, Huang J and Zeng Q: microRNA-23a-5p acts as a potential biomarker for sepsis-induced acute respiratory distress syndrome in early stage. *Cell Mol Biol (Noisy-le-grand)* 62: 31-37, 2016.
27. Portt L, Norman G, Clapp C, Greenwood M and Greenwood MT: Anti-apoptosis and cell survival: A review. *Biochim Biophys Acta* 1813: 238-259, 2011.
28. Yang Y, Li F, Saha MN, Abdi J, Qiu L and Chang H: miR-137 and miR-197 induce apoptosis and suppress tumorigenicity by targeting MCL-1 in multiple myeloma. *Clin Cancer Res* 21: 2399-2411, 2015.
29. Weseslindtner L, Macheleidt I, Eischeid H, Strassl R, Hofer H, Popow-Kraupp T, Dienes HP, Holzmann H and Odenthal M: Micro RNAs mir-106a, mir-122 and mir-197 are increased in severe acute viral hepatitis with coagulopathy. *Liver Int* 36: 353-360, 2016.
30. Glick D, Barth S and Macleod KF: Autophagy: Cellular and molecular mechanisms. *J Pathol* 221: 3-12, 2010.
31. Liu J, Li QX, Wang XJ, Zhang C, Duan YQ, Wang ZY, Zhang Y, Yu X, Li NJ, Sun JP and Yi F: β -Arrestins promote podocyte injury by inhibition of autophagy in diabetic nephropathy. *Cell Death Dis* 7: e2183, 2016.
32. Liang S, Jin J, Lin B, Gong J, Li Y and He Q: Rapamycin induces autophagy and reduces the apoptosis of podocytes under a stimulated condition of immunoglobulin A nephropathy. *Kidney Blood Press Res* 42: 177-187, 2017.
33. Zhou L and Liu Y: Wnt/ β -catenin signalling and podocyte dysfunction in proteinuric kidney disease. *Nat Rev Nephrol* 11: 535-545, 2015.
34. Xu S, Niu P, Chen K, Xia Y, Yu Q, Liu N, Li J, Li S, Wu L, Feng J, *et al*: The liver protection of propylene glycol alginate sodium sulfate preconditioning against ischemia reperfusion injury: Focusing MAPK pathway activity. *Sci Rep* 7: 15175, 2017.
35. Zhang L, Niu W, He Z, Zhang Q, Wu Y, Jiang C, Tang C, Hu Y and Jia J: Autophagy suppression by exercise pretreatment and p38 inhibition is neuroprotective in cerebral ischemia. *Brain Res* 1587: 127-132, 2014.