

# Upregulation of miR-126 inhibits podocyte injury in sepsis via EGFL6/DKC1 signaling pathway

JIANMING SU and LIMIN DING

Department of Emergency, The First Affiliated Hospital of Zhejiang Chinese Medical University,  
Hangzhou, Zhejiang 310006, P.R. China

Received September 3, 2020; Accepted February 8, 2021

DOI: 10.3892/mmr.2021.12012

**Abstract.** Sepsis-induced cardiorenal syndrome is one of the multiple organ dysfunctions observed in sepsis. It is determined by a primary dysfunction in one organ that leads to secondary injury to another organ. Studies have shown the involvement of microRNAs (miRs) in the diagnosis and prognosis of several pathologies. However, the implication of miR-126 in the podocyte damage associated with sepsis has not been evaluated until now. In the current study, the miR-126 expression was downregulated in a podocyte injury model together with downregulation of nephrin expression. The transfection of podocytes from podocyte injury group with miR-126 mimics demonstrated an increase in cell proliferation and a decrease in cell apoptosis. Bioinformatics analysis predicted that the target of miR-126 was epidermal growth factor-like domain multiple 6 (EGFL6) and dyskeratosis congenita 1 (DKC1) and these were confirmed by dual-luciferase reporter assay. miR-126 upregulation determined EGFL6 and DKC1 upregulation and prevented podocyte injury. The current study demonstrated that overexpression of miR-126 could protect podocytes from sepsis-induced injury through an EGFL6/DKC1 signaling pathway.

## Introduction

Sepsis is a pathological condition caused by a number of factors, including severe infections, burn, trauma and surgery (1-5). An imbalance between the secretion of pro-inflammatory and anti-inflammatory factors that lead to multiple organ failure appears in the pathophysiological process of sepsis (6). Sepsis is one of the leading causes of mortality in critical patients (7). Higher mortality in sepsis appears when cardiac injury or acute kidney injury develops, especially in intensive care unit

patients (8,9). Sepsis-induced cardiorenal syndrome is one of the multiple organ dysfunctions observed in sepsis. It is determined by a primary dysfunction in one organ that leads to secondary injury to another organ (10). Its main clinical manifestation is myocardial inhibition leading to decreased left ventricular systolic function and cardiac output (10). Podocytes are differentiated cells anchored on the basement membrane of the glomerulus. They maintain the structure of glomerulus, which is an important part of the glomerular filtration membrane (11). Toxic effects on podocytes that lead to podocyte losing integrity or functional damage are the main causes of proteinuria and glomerulosclerosis involved in the occurrence and development of a number of kidney diseases (12).

MicroRNAs (miRs) are endogenous small molecules of non-coding RNAs. Their biological effects are associated with gene expression regulation by modifying mRNA protein synthesis. miRNAs influence the protein synthesis at one or more mRNAs genes by targeting the 3' untranslated region (UTR), 5'UTR region and the coding sequence of the gene (13). Bioinformatics and research studies demonstrate that there are thousands of target genes for human miRs and more >5,000 target genes are regulated by miRs, indicating their involvement in various physiological and pathological processes (14-20). Environmental factors including diet, lifestyle, pollutants and carcinogens can influence the expression of miRs (21-25) and modulate important molecular pathways implicated in the pathogenesis of chronic diseases. Studies have shown that the profile of miRs has a great implication in the diagnosis and prognosis of several pathologies, including types of cancer (26-31). Disease-associated miRs can also serve as targets for personalized therapy (32-34). In the model of Dicer knockout mice, where miRs were knocked out, proteinuria, serious renal damage and changes in podocyte cytoskeleton proteins of podocytes are observed, showing the association between miR and podocyte integrity (35).

Studies have associated miR-126 with multiple functions in the organism, including the development of cardiovascular pathologies, Parkinson's disease, diabetes, diabetic nephropathy and types of cancer (36-40).

The present study hypothesized that miR-126 might be involved in the podocyte damage associated with sepsis. Lipopolysaccharide (LPS) is a part of the gram-negative bacterial cell wall that mediates systemic inflammation leading

---

*Correspondence to:* Dr Limin Ding, Department of Emergency, The First Affiliated Hospital of Zhejiang Chinese Medical University, 54 Youdian Road, Hangzhou, Zhejiang 310006, P.R. China  
E-mail: dinglimin8781@126.com

**Key words:** microRNA 126, epidermal growth factor-like domain multiple 6, dyskeratosis congenita 1, cardiorenal syndrome, podocytes toxicity, sepsis

to sepsis. LPS induces podocyte injury by mediating the release of pro-inflammatory factors (41). An *in vitro* model of LPS-induced injury in conditioned immortalized mouse podocytes was used to provide a theoretical basis for further studies clarifying the pathogenesis of sepsis-induced nephrotoxicity.

## Materials and methods

**Cell culture and transfection.** Mouse podocyte cells (SV40 MES 13 cell line; cat. no. CRL-1927; American Type Culture Collection) were cultured in Roswell Park Memorial Institute-1640 medium (RPMI-1640; HyClone; Cytiva) containing 10% fetal bovine serum (FBS; HyClone; Cytiva) and  $8 \times 10^4$  U/l recombinant mouse  $\gamma$ -interferon (Beijing Solarbio Science & Technology Co., Ltd.) in 5% CO<sub>2</sub> at 37°C in an incubator (Binder GmbH). The cells were cultured for 3-5 days and were then maintained in RPMI-1640 medium containing 5% FBS.

Mouse podocyte cells with a density of  $1.0 \times 10^5$  cells/well were inoculated in a 6-well plate overnight. The podocyte injury model was established by stimulation with 10 mg/l LPS for 12, 24 and 36 h. The normal control group was treated with the same volume of phosphate-buffered saline (PBS). The plates were incubated at 37°C for 12, 24 and 36 h. The expression of nephrin protein was detected by western blotting. According to the results of western blotting, the optimal time of LPS stimulation was selected.

The cells in the miR-126 mimic group and miR-mimic negative control (NC) group were treated separated with miR-126-mimic (5'-ACCTCCAGCTG GGTCGTAC CGTGAGTAATAATG-3') and miR-mimic NC (5'-CTCAAC TGGTGTCTCTGGA-3'), then mixed Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) until the final concentration in the RPMI-1640 medium was 20 nmol/l. Cells were incubated for 1 h then cultured in complete medium for 24-48 h. All reagents were purchased from Shanghai GenePharma Co., Ltd. and all experiments were performed in triplicate.

**Reverse transcription-quantitative (RT-q) PCR.** RT-PCR Eastrop™ Super total RNA Extraction kit (Promega Corporation) was used to extract total RNA from samples. RNA was reverse transcribed into cDNA using an RT kit (Thermo Fisher Scientific, Inc.). RNA extraction, cDNA synthesis and qPCR were performed according to the manufacturer's protocol. When cell density reached 80%, culture medium was discarded, and 1 ml lysate was added to each well of a 6-well plate for 10 min at room temperature. The reaction system (20  $\mu$ l) included: 1.0 cDNA, 10.0 SYBR-Green Master Mix (Thermo Fisher Scientific, Inc.), 0.5 upstream and downstream primers (SANGON Biotech Co., Ltd.; Table I) and 8  $\mu$ l ddH<sub>2</sub>O (Milli-q Academic A10; EMD Millipore). Each experiment was performed three times and U6 was used as the internal reference. The reaction conditions were as follows: 95°C pre denaturation for 10 min, 95°C for 10 sec, 60°C for 20 sec and 72°C for 30 sec (40 cycles). Quantification was performed via the 2<sup>- $\Delta\Delta C_q$</sup>  method (42).

**Western blotting.** Mouse podocyte cells ( $5 \times 10^5$ ) were collected, rinsed with PBS, mixed with 250  $\mu$ l RIPA Lysis Buffer (Thermo Fisher Scientific, Inc.) and left on ice for 5 min. Then the

mixture was transferred into a new EP tube and centrifuged at 3,000 x g, 4°C for 10 min. The supernatant obtained was used to determine the total protein concentration of the cells by the BCA Protein Assay kit (Thermo Fisher Scientific, Inc.). The protein samples were diluted with 5X loading buffer (Thermo Fisher Scientific, Inc.) and PBS, boiled in water bath for 5 min and denatured. The protein liquid after denaturation was added to 12% SDS-PAGE gel (Beijing Solarbio Science & Technology Co., Ltd.) and underwent 80 V constant pressure electrophoresis for 60-120 min, each well was loaded with 20  $\mu$ g protein. The SDS-PAGE gel was placed onto a nitrocellulose membrane to perform the constant current transfer. Bovine serum albumin (BSA; 3%; HyClone; Cytiva) was used for blocking at 4°C for 1 h. Following blocking, the nitrocellulose membrane was washed with PBS and incubated with monoclonal Anti-nephrin (1:1,000; rabbit antibody (Y17-R); cat no. ab136894, Abcam), anti-epidermal growth factor-like domain multiple 6 (EGFL6; 1:1,000; rabbit polyclonal mouse antibody 51182-T16, Abcam), anti-dyskeratosis congenita 1 (DKC1; 1:1,000; rabbit monoclonal antibody, EPR10398; Abcam) and anti-GAPDH (1:1,000; mouse monoclonal antibody; cat. no. K106390M, Beijing Solarbio Science & Technology Co., Ltd.), respectively. The membrane was incubated overnight at 4°C. Then the nitrocellulose membrane was washed with PBS buffer and incubated with Anti-mouse IgG for IP (HRP; 1:5,000; cat no. ab131368, Abcam) labeled with horseradish peroxidase at room temperature for 30 min. Then, the nitrocellulose membrane was washed with PBS buffer and Chemistar ECL Western Blotting Substrate (Ultra-sensitive ECL luminescent solution; Hanbio Biotechnology Co., Ltd.) was used for chemiluminescence. Kodak X (Kodak) was used to expose the nitrocellulose membrane for ~3 min. The X-ray film was scanned (Oxford Instruments plc) and the grey analysis of the strip was processed by Gel-Pro Analyzer software 4.0 (Media Cybernetics, Inc.). The grey value of each band was measured by software in three independent repeated experiments and the ratio of the grey value of the target gene product to that of the  $\beta$ -actin product was used as the relative expression of the protein.

**Cell viability assay using cell counting kit-8 (CCK8).** The treated cells of each group were inoculated into 96-well plates at a density of  $1 \times 10^4$  cells/well. The final volume of each well was 100  $\mu$ l. Each experiment was performed in triplicate. After 24 h, when the cells adhere to the wall, the original culture medium was replaced and the cells were incubated with the treatment according to their time points. Following incubation, the cell viability was determined using a CCK-8 kit (Thermo Fisher Scientific, Inc.). A volume of 10  $\mu$ l CCK8 solution was added to each well and cultured in 5% CO<sub>2</sub> at 37°C for 2 h. The absorbance (OD) was determined at 450 nm using a microplate reader. Cell survival rate was calculated using the following formula: Cell survival rate: OD value of experimental group-OD value of blank group/OD value of the control group-OD value of blank group x100%.

**Terminal deoxynucleotidyl transferase dUTP nick labeling (TUNEL) assay.** Cell apoptosis was determined using TransDetect® *In Situ* Fluorescein TUNEL Cell Apoptosis Detection kit (TransGen Biotech Co., Ltd.), according to the manufacturer's instructions. Briefly, the cells were washed

Table I. Primer sequence information (Sangon Biotech Co., Ltd.).

Name	Primer sequence (5'-3')	
Nephrin	Upstream primers	CCCTCCGGGACCCTACTG
	Downstream primers	TCTGGGAGGATGGGATTGG
miR-126	Upstream primers	CGGCAGGAACCTCCTTACTC
	Downstream primers	TGTGCCCTAGGGACGAAGGA
EGFL6	Upstream primers	TCTGTTTGCTCTTTGATTACCG
	Downstream primers	TTCCCTGTCTTCCACTTTTCAT
DKC1	Upstream primers	GCTAAGTTGGACACGTCTCAG
	Downstream primers	TGCAAGAGGTGTATAGTGTGTTG
GAPDH	Upstream primers	TGTGTCCGTCGTGGATCTGA
	Downstream primers	TTGCTGTTGAAGTCGCAGGAG

miR, microRNA; EGFL6, epidermal growth factor-like domain multiple 6; DKC1, dyskeratosis congenita 1.

with PBS and fixed with 4% paraformaldehyde or biyuntian (p0098) for 30-60 min. Then the cells were washed with PBS or Hank's balanced salt solution, followed by the addition of PBS containing 0.1% Triton X-100 and incubation on an ice bath for 2 min. To each sample, 50  $\mu$ l TUNEL detection solution was added and incubated for 60 min at 37°C, washed again with PBS and incubated with DAPI in PBS for 30 min at 30°C. After incubation, the samples were washed with PBS for three successive times. The samples were observed under the IX73 fluorescence microscope (Olympus Corporation; x400 magnification). The excitation wavelength range was 450-500 nm and the emission wavelength range was 515-565 nm (green fluorescence).

**Dual-luciferase reporter assay.** For the dual-luciferase reporter assay, the TransDetect® Double-Luciferase Reporter Assay kit (Firefly Luciferase and *Renilla* Luciferase and plasmids; TransGen Biotech Co., Ltd.) was used. The cells were cultured and transfected for 48 h with EGFL6 3'UTR wild-type (Wt)/mutant (Mut) reporter plasmids and DKC1 3'UTR Wt/Mut reporter plasmids with or without miR-126 mimic/control precursor plasmids using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.). Each experiment was performed in triplicate. Following transfection, the medium was discarded and the cells were lysed with cell lysis buffer. The luciferase activity was measured using a GloMax 96 microplate luminescent detector (Promega Corporation). Luciferase activity was normalized to that of *Renilla*.

**Statistical analysis.** SPSS 22.0 statistical software (IBM Corp.) was used for data analysis. The data were analyzed by t-test for the differences between 2 groups and one-way analysis of variance (ANOVA) for the comparison of >2 groups. ANOVA with Dunnett's post hoc test was used to compare the data.  $P < 0.05$  was considered to indicate a statistically significant difference.

## Results

**Podocyte injury model.** LPS (10  $\mu$ g/ml) was used to treat mouse immortalized podocytes for sepsis model establishment. RT-qPCR demonstrated that the mRNA expression of

nephrin was significantly downregulated with the prolongation of LPS treatment, reaching the lowest level after 36 h of culture (Fig. 1A), showing a time-dependent effect. The results of western blotting demonstrated that the protein expression of nephrin also significantly decreased 36 h after LPS stimulation (Fig. 1B). In addition, the expression level of miR-126 was downregulated with the prolongation of LPS treatment (Fig. 1C). These results confirmed the successful establishment of podocyte injury model and suggested that miR-126 served a role in podocyte function.

**Increased miR-126 inhibited the podocyte injury.** To investigate the specific role of differentially expressed miR-126 in septic podocyte injury, miR-126 mimics and miR-mimic NC were transfected into podocytes. Following transfection with miR-126 mimics and miR-mimic NC, RT-qPCR demonstrated that the expressions of miR-126 in the miR-mimic NC group and the control group were in the same level (Fig. 2A). Following transfection with miR-126 mimics, RT-qPCR demonstrated that the expression of miR-126 (Fig. 2A) and nephrin (Fig. 2B) in podocytes was promoted, indicating that upregulated miR-126 might mediate inhibition of podocyte injury. CCK-8 assay was used to detect the effect of miR-126 on the proliferation of podocytes. Results demonstrated that the growth of podocyte cells transfected with miR-126 was significantly promoted compared with podocyte injury group (Fig. 2C). Following transfection with miR-126 mimics, the growth of podocytes was promoted and the proliferation ability was increased.

**Increased miR-126 inhibited the podocyte apoptosis.** TUNEL assay results demonstrated that in the podocyte injury group the number of apoptotic cells was significantly increased compared with the control group, while the overexpression of miR-126 can alleviate injury and reduce the number of apoptotic cells (Fig. 3).

**miR-126 target 3'UTR binding sites of EGFL6/DKC1 mRNA to regulate their expression and inhibit podocyte injury.** Following co-transfection of miR-126 and Wt 3'UTR of EGFL6, luciferase

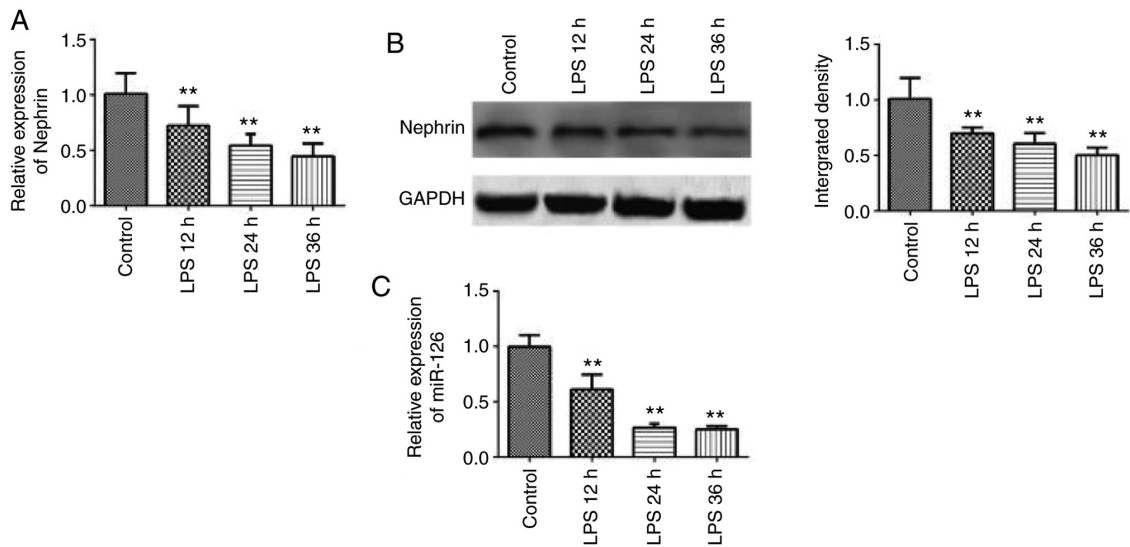


Figure 1. Podocyte injury model. (A) RT-qPCR results of the expression of nephrin in LPS-induced sepsis model of podocytes. (B) The expression of nephrin in LPS-induced sepsis model of podocytes by western blotting. (C) RT-qPCR results of the expression of miR-126 in LPS-induced sepsis model of podocytes. \*\* $P < 0.01$  vs. control. RT-qPCR, reverse transcription-quantitative PCR; LPS, lipopolysaccharide.

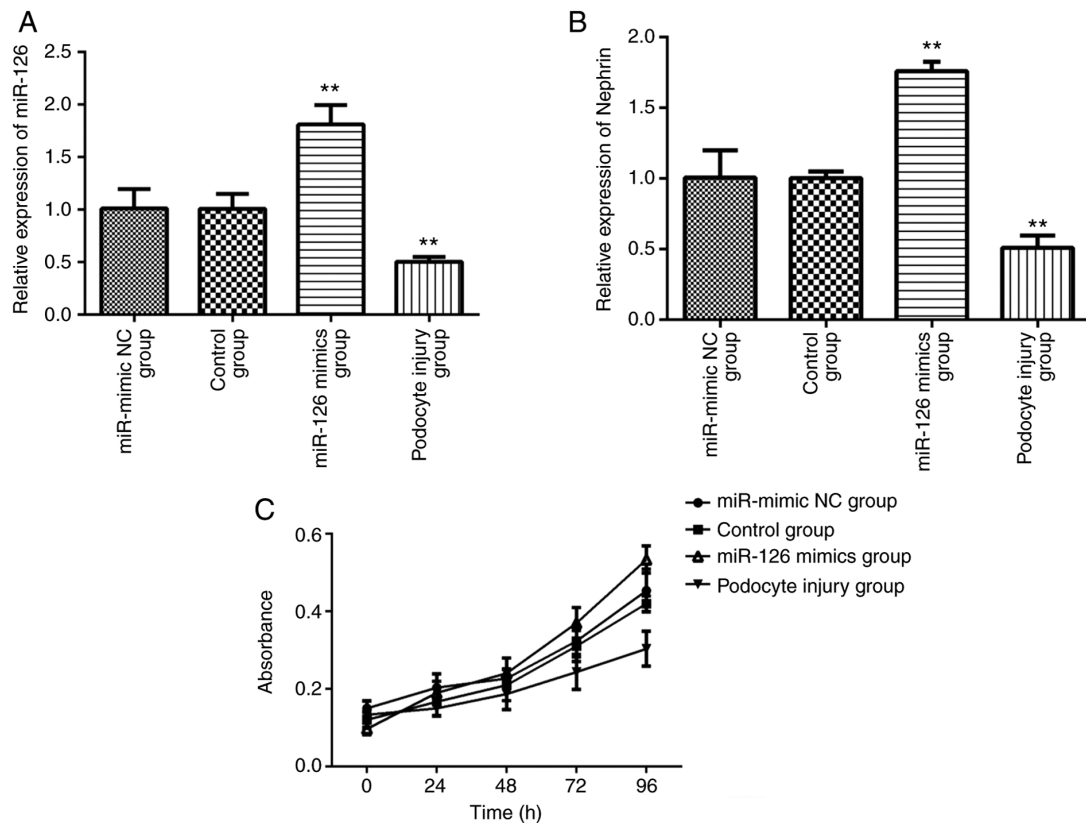


Figure 2. Increased miR-126 inhibits podocyte injury. (A) The expression of miR-126 following transfection with miR-126 mimics by RT-qPCR. (B) The expression of nephrin following transfection with miR-126 mimics by RT-qPCR. (C) Cell proliferation following transfection with miR-126 mimics by CCK8 assays. \*\* $P < 0.01$  vs. control. miR, microRNA; RT-qPCR, reverse transcription-quantitative PCR.

activity was significantly reduced ( $P < 0.001$ ; Fig. 4A). Similarly, as shown in Fig. 4B, luciferase activity was significantly reduced compared with the control group ( $P < 0.001$ ) after co-transfection of miR-126 and Wt 3'UTR of DKC1. When miR-126 was upregulated, the protein levels of both EGFL6 and DKC1 as determined by western blotting were increased (Fig. 5).

## Discussion

Glomerular podocytes are differentiated terminal cells with a weak capability of division and regeneration. Podocytes, endothelial cells and basement membrane act together as glomerular filtration barrier. Negative charge of podocytes can



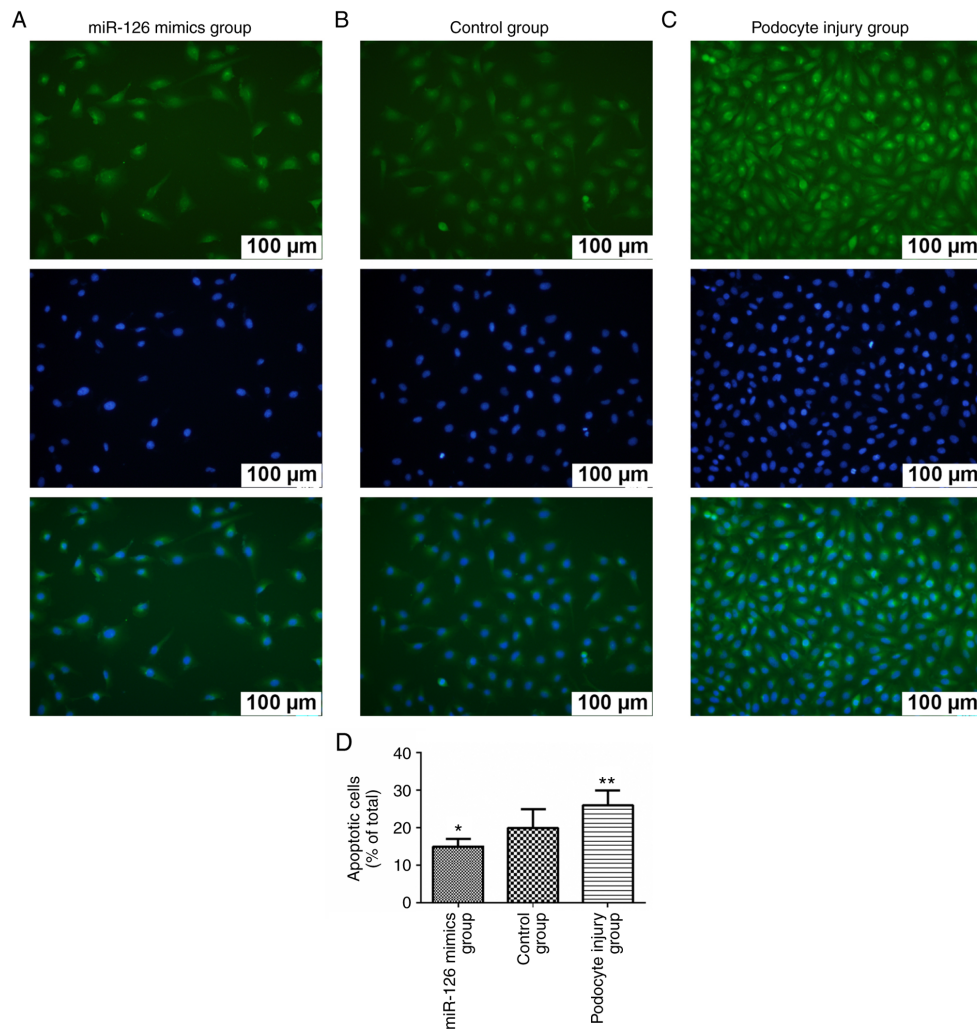


Figure 3. Apoptosis evaluation with TUNEL assay. (A) The TUNEL positive cells in miR-126 mimics group. (B) The TUNEL positive cells in control group. (C) The TUNEL positive cells in podocyte injury group. (D) The percentage of apoptotic cells. \*P<0.05, \*\*P<0.01.

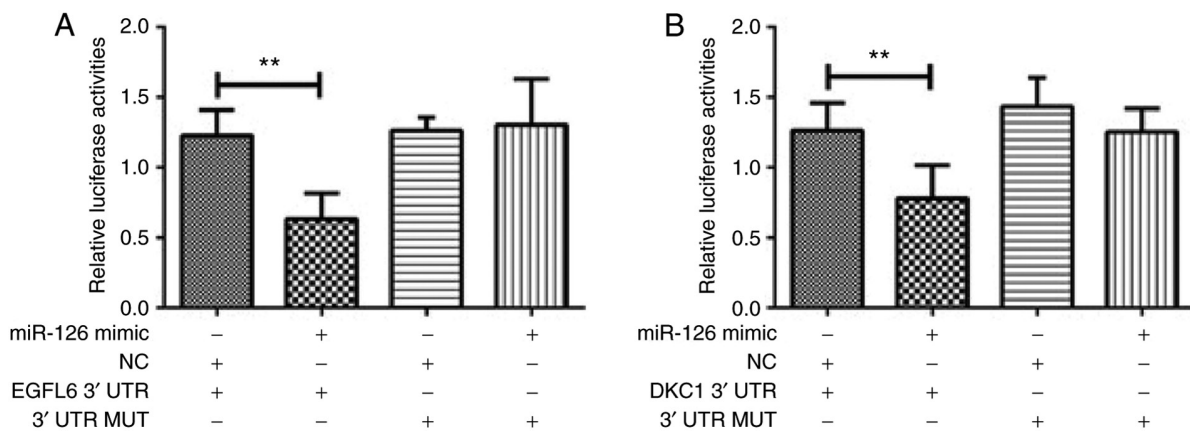


Figure 4. Target gene of miR-126 in the podocytes. (A) Dual-luciferase reporter assay: The luciferase activities were detected after co-transfected with EGFL6 3'UTR Wt/Mut reporter plasmids and miR-126/NC mimic for 48 h in the podocyte; (B) Dual-luciferase reporter assay: The luciferase activities were detected after co-transfected with DKC1 3'UTR Wt/Mut reporter plasmids and miR-126/NC mimic for 48 h in the podocyte. miR, microRNA; EGFL6, epidermal growth factor-like domain multiple 6; UTR, untranslated region; Wt, wild-type; Mut, mutant; DKC1, dyskeratosis congenita 1; NC, negative control group. \*\*P<0.01.

prevent loss (or alternatively deprivation) of albumin and other macromolecules (11,12). Inflammation is an important factor in promoting damage of podocytes and proteinuria formation (43). The loss of podocyte structure and function is closely

associated with inflammatory factors (11) and its mechanism has remains to be elucidated. The implication of inflammatory factors in podocyte damage has attracted the attention of a number of researchers aiming to find new therapeutic targets

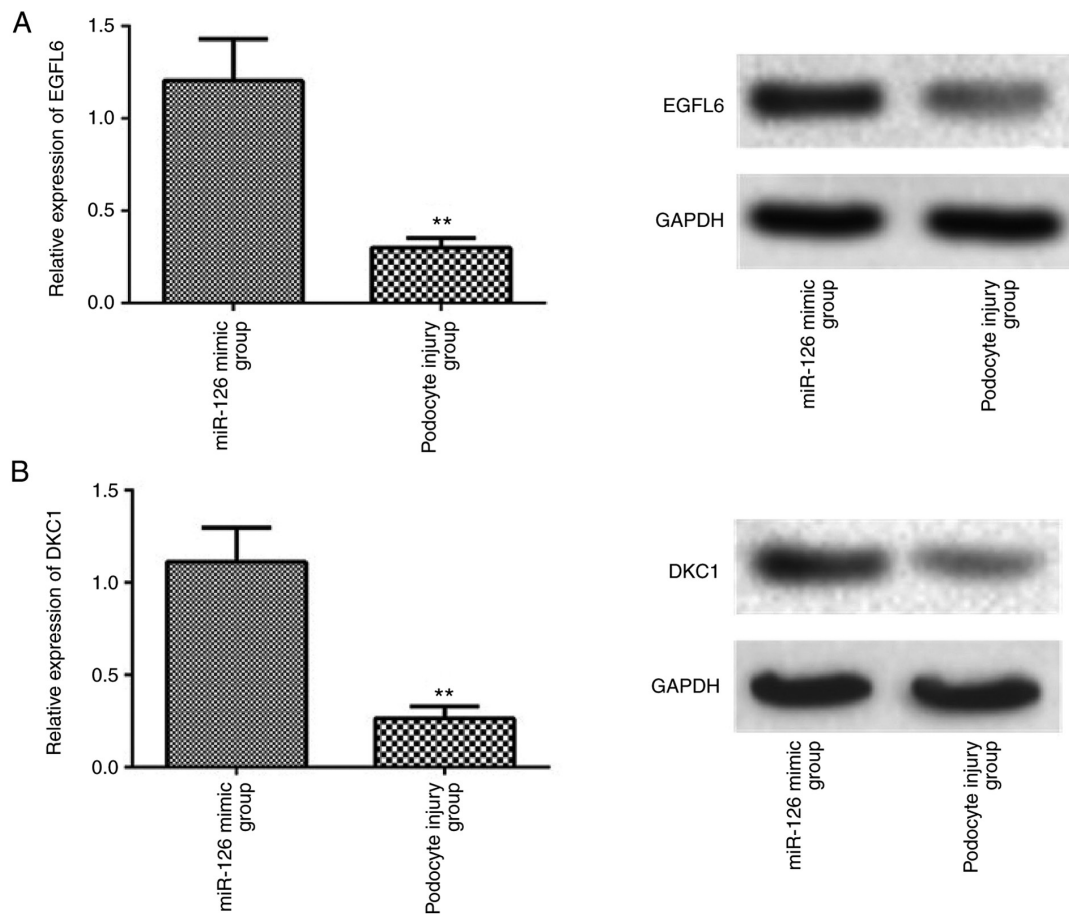


Figure 5. Protein levels of EGFL6 and DKC1 are increased when miR-126 is upregulated. Expression of (A) EGFL6 and (B) DKC1 proteins in miR-126 mimic group and podocyte injury group. EGFL6, epidermal growth factor-like domain multiple 6; DKC1, dyskeratosis congenita 1; miR, microRNA. \*\* $P < 0.01$ .

which can provide a fundamental theoretical basis for the diagnosis and treatment of kidney diseases.

The present study found that miR-126 was downregulated in sepsis-induced podocyte injury. Overexpression of miR-126 could protect LPS-induced podocytes damage through an EGFL6/DKC1 signaling pathway. Bioinformatics analysis and dual-luciferase reporter assay demonstrated that EGFL6 and DKC1 are the key targets of miR-126.

miRs serve a key role in the post-transcriptional regulation of gene expression and are involved in normal and pathological renal function (44). Upregulation of miR-27a, miR-21 and miR-370 and downregulation of miR-15b-5p and miR-34c is associated with podocytes damage in diabetic nephropathy (45-49). Downregulation of miR-120a-5p is associated with increased expression of M-type phospholipase A2 receptor, which determines podocyte apoptosis and the progression of membranous nephropathy (50). Henique *et al* (51) demonstrate that upregulation of miRNA-92a is associated with the development and progression of glomerulonephritis. Sepsis-induced acute kidney disease is associated with the miR-15a-5p-XIST-CUL3 regulatory axis (52). Upregulation of miR-27b is associated with puromycin aminonucleoside-induced podocytes damage by targeting adenosine receptor 2B (53). The present study focused attention on miR-126 as one of the main regulators of sepsis-induced podocytes injury, potentially by targeting EGFL6 and DKC1 proteins.

The EGFL6 gene is a member of the EGF superfamily. The members of EGF superfamily are implicated in a wide spectrum of functions in the organism including cell proliferation, cell cycle and developmental processes (54). EGFL6 has been associated with tumor angiogenic functions in several types of cancers, including hepatocellular carcinoma, ovarian and breast cancer (54-56). Its role in preventing LPS-induced podocytes damage has yet to be investigated. The present study is the first, to the best of the authors' knowledge, that associates increased EGFL6 protein expression with a protective kidney effect. Further studies are required to elucidate these findings and potential utilization as a therapeutic strategy.

DKC1 is a gene that provides instruction for dyskerin protein involved in telomere integrity (57). A previous study demonstrated that downregulation of miR-126 is associated with high glucose-induced ageing to human glomerular mesangial cells and transfection with miR-126 mimics can act as an inhibitor of telomere-p-53-p21-RB signaling pathway and delay the effects (58). The current study supported these findings, showing that the modulation of DKC1 could be involved in the protective mechanism of miR-126 against podocytes damage.

The signal pathway regulated by various miRs could be interpreted as the key pathophysiological mechanism of nephropathy. These miR-related signal pathway inhibitors or inducers are expected to become clinical therapeutic drugs in the future (59). Understanding the key mechanism of miRNA in the development and progression of renal injury will help

to identify new potential therapeutic targets and design new therapeutic strategies. At present, it has been found that these miRNAs serve a regulatory role in some specific molecular pathways (59). It is necessary to expand the interaction between different pathways of miRNA and construct the miRNA interaction network in nephrotic patients. This will contribute to the understanding of the specific mechanism of miRNA in functional renal injury and structural renal injury.

From the perspective of treatment, miR as a new diagnostic and therapeutic marker of nephropathy is a new (or recent) trend (51). However, it is still necessary to fully understand the exact regulatory mechanism and specific functions of each miR at the transcription and translation level.

In conclusion, the present study found that miR-126 was downregulated in sepsis-induced podocyte injury and that overexpression of miR-126 could protect podocytes through an EGFL6/DKC1 signaling pathway. However, more research is needed to clarify the exact function and mechanism of miRNAs as a specific treatment for nephropathy.

## Acknowledgements

Not applicable.

## Funding

This work was supported by Zhejiang Province Traditional Chinese Medicine Science and Technology Project (grant no. 2015ZA089).

## 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

JS and LD performed the experiments, analyzed and interpreted data and wrote the manuscript. Both authors were responsible for confirming the authenticity of the data. Both authors read and approved the final version of the 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

- Zlatian O, Balasoïu AT, Balasoïu M, Cristea O, Docea AO, Mitrut R, Spandidos DA, Tsatsakis AM, Bancescu G and Calina D: Antimicrobial resistance in bacterial pathogens among hospitalised patients with severe invasive infections. *Exp Ther Med* 16: 4499-4510, 2018.
- Călina D, Docea AO, Rosu L, Zlatian O, Rosu AF, Anghelina F, Rogoveanu O, Arsene AL, Nicolae AC, Drăgoi CM, *et al*: Antimicrobial resistance development following surgical site infections. *Mol Med Rep* 15: 681-688, 2017.
- Ungureanu A, Zlatian O, Mitroi G, Drocaș A, Țîrcă T, Călina D, Dehelean C, Docea AO, Izotov BN, Rakitskii VN, *et al*: *Staphylococcus aureus* colonisation in patients from a primary regional hospital. *Mol Med Rep* 16: 8771-8780, 2017.
- Calina D, Rosu L, Rosu AF, Ianosi G, Ianosi S, Zlatian O, Mitrut R, Oana DA, Rogoveanu O, Mitruț P, *et al*: Etiological diagnosis and pharmacotherapeutic management of parapneumonic pleurisy. *Farmacia* 64: 946-952, 2016.
- Tanase A, Colita A, Ianosi G, Neagoe D, Branisteanu DE, Calina D, Docea AO, Tsatsakis A and Ianosi SL: Rare case of disseminated fusariosis in a young patient with graft vs. host disease following an allogeneic transplant. *Exp Ther Med* 12: 2078-2082, 2016.
- Seymour CW, Liu VX, Iwashyna TJ, Brunkhorst FM, Rea TD, Scherag A, Rubenfeld G, Kahn JM, Shankar-Hari M, Singer M, *et al*: Assessment of clinical criteria for sepsis: For the third international consensus definitions for sepsis and septic shock (Sepsis-3). *JAMA* 315: 762-774, 2016.
- Marik PE: Patterns of death in patients with sepsis and the use of hydrocortisone, ascorbic acid, and thiamine to prevent these deaths. *Surg Infect (Larchmt)* 19: 812-820, 2018.
- Genga KR and Russell JA: Update of sepsis in the intensive care unit. *J Innate Immun* 9: 441-455, 2017.
- Shi B, Shi F, Xu K, Shi L, Tang H, Wang N, Wu Y, Gu J, Ding J and Huang Y: The prognostic performance of Sepsis-3 and SIRS criteria for patients with urolithiasis-associated sepsis transferred to ICU following surgical interventions. *Exp Ther Med* 18: 4165-4172, 2019.
- Ronco C, Bellasi A and Di Lullo L: Cardiorenal syndrome: An overview. *Adv Chronic Kidney Dis* 25: 382-390, 2018.
- Greka A and Mundel P: Cell biology and pathology of podocytes. *Annu Rev Physiol* 74: 299-323, 2012.
- Wharram BL, Goyal M, Wiggins JE, Sanden SK, Hussain S, Filipiak WE, Saunders TL, Dysko RC, Kohno K, Holzman LB, *et al*: Podocyte depletion causes glomerulosclerosis: Diphtheria toxin-induced podocyte depletion in rats expressing human diphtheria toxin receptor transgene. *J Am Soc Nephrol* 16: 2941-2952, 2005.
- Gambari R, Brognara E, Spandidos DA and Fabbri E: Targeting oncomiRNAs and mimicking tumor suppressor miRNAs: New trends in the development of miRNA therapeutic strategies in oncology (Review). *Int J Oncol* 49: 5-32, 2016.
- Roberts JT and Borchert GM: Computational prediction of MicroRNA target genes, target prediction databases, and web resources. *Methods Mol Biol* 1617: 109-122, 2017.
- Candido S, Lupo G, Pennisi M, Basile MS, Anfuso CD, Petralia MC, Gattuso G, Vivarelli S, Spandidos DA, Libra M, *et al*: The analysis of miRNA expression profiling datasets reveals inverse microRNA patterns in glioblastoma and Alzheimer's disease. *Oncol Rep* 42: 911-922, 2019.
- Bibaki E, Tsitoura E, Vasarmidi E, Margaritopoulos G, Trachalaki A, Koutoulaki C, Georgopoulou T, Spandidos DA, Tzanakis N and Antoniou KM: miR-185 and miR-29a are similarly expressed in the bronchoalveolar lavage cells in IPF and lung cancer but common targets DNMT1 and COL1A1 show disease specific patterns. *Mol Med Rep* 17: 7105-7112, 2018.
- Falzone L, Romano GL, Salemi R, Bucolo C, Tomasello B, Lupo G, Anfuso CD, Spandidos DA, Libra M and Candido S: Prognostic significance of deregulated microRNAs in uveal melanomas. *Mol Med Rep* 19: 2599-2610, 2019.
- Doukas SG, Vageli DP, Lazopoulos G, Spandidos DA, Sasaki CT and Tsatsakis A: The effect of NNK, a tobacco smoke carcinogen, on the miRNA and mismatch DNA repair expression profiles in lung and head and neck squamous cancer cells. *Cells* 9: 1031, 2020.
- Koutsaki M, Libra M, Spandidos DA and Zaravinos A: The miR-200 family in ovarian cancer. *Oncotarget* 8: 66629-66640, 2017.
- Sahu SC and Tsatsakis A: microRNAs: Potential biomarkers of toxicity: A special issue of the Journal Toxicology Reports. *Toxicol Rep* 7: 198-199, 2020.
- Falzone L, Grimaldi M, Celentano E, Augustin LSA and Libra M: Identification of modulated MicroRNAs associated with breast cancer, diet, and physical activity. *Cancers (Basel)* 12: 2555, 2020.
- Polo A, Crispo A, Cerino P, Falzone L, Candido S, Giudice A, De Petro G, Ciliberto G, Montella M, Budillon A, *et al*: Environment and bladder cancer: Molecular analysis by interaction networks. *Oncotarget* 8: 65240-65252, 2017.



23. Filetti V, Falzone L, Rapisarda V, Caltabiano R, Eleonora Graziano AC, Ledda C and Loreto C: Modulation of microRNA expression levels after naturally occurring asbestiform fibers exposure as a diagnostic biomarker of mesothelial neoplastic transformation. *Ecotoxicol Environ Saf* 198: 110640, 2020.
24. Sharifi-Rad J, Rodrigues CF, Sharopov F, Docea AO, Can Karaca A, Sharifi-Rad M, Kahveci Karıncaoglu D, Gülseren G, Şenol E, Demircan E, *et al*: Diet, lifestyle and cardiovascular diseases: Linking pathophysiology to cardioprotective effects of natural bioactive compounds. *Int J Environ Res Public Health* 17: 2326, 2020.
25. Sharifi-Rad M, Anil Kumar NV, Zucca P, Varoni EM, Dini L, Panzarini E, Rajkovic J, Tsouh Fokou PV, Azzini E, Peluso I, *et al*: Lifestyle, oxidative stress, and antioxidants: Back and forth in the pathophysiology of chronic diseases. *Front Physiol* 11: 694, 2020.
26. Finotti A, Allegretti M, Gasparello J, Giacomini P, Spandidos DA, Spoto G and Gambari R: Liquid biopsy and PCR-free ultrasensitive detection systems in oncology (Review). *Int J Oncol* 53: 1395-1434, 2018.
27. Silantiev AS, Falzone L, Libra M, Gurina OI, Kardashova KS, Nikolouzakakis TK, Nosyrev AE, Sutton CW, Mitsias PD and Tsatsakis A: Current and future trends on diagnosis and prognosis of glioblastoma: From molecular biology to proteomics. *Cells* 8: 863, 2019.
28. Nikolouzakakis TK, Vassilopoulou L, Fragkiadaki P, Mariolis Papasakos T, Papadakis GZ, Spandidos DA, Tsatsakis AM and Tsiaoussis J: Improving diagnosis, prognosis and prediction by using biomarkers in CRC patients (Review). *Oncol Rep* 39: 2455-2472, 2018.
29. Zaravinos A, Radojicic J, Lambrou GI, Volanis D, Delakas D, Stathopoulos EN and Spandidos DA: Expression of miRNAs involved in angiogenesis, tumor cell proliferation, tumor suppressor inhibition, epithelial-mesenchymal transition and activation of metastasis in bladder cancer. *J Urol* 188: 615-623, 2012.
30. Rizos E, Siafakas N, Skourti E, Papageorgiou C, Tsoporis J, Parker TH, Christodoulou DI, Spandidos DA, Katsantoni E and Zoumpourlis V: miRNAs and their role in the correlation between schizophrenia and cancer (Review). *Mol Med Rep* 14: 4942-4946, 2016.
31. Doukas SG, Vageli DP, Nikolouzakakis TK, Falzone L, Docea AO, Lazopoulos G, Kalbakis K and Tsatsakis A: Role of DNA mismatch repair genes in lung and head and neck cancer (Review). *World Acad Sci J* 1: 184-191, 2019.
32. Cheng Y, Yang M and Peng J: Correlation the between the regulation of miRNA-1 in c-Met-induced EMT and cervical cancer progression. *Oncol Lett* 17: 3341-3349, 2019.
33. Xiong DD, Xu WQ, He RQ, Dang YW, Chen G and Luo DZ: In silico analysis identified miRNA based therapeutic agents against glioblastoma multiforme. *Oncol Rep* 41: 2194-2208, 2019.
34. Liu C, Tong Z, Tan J, Xin Z, Wang Z and Tian L: MicroRNA-21-5p targeting PDCD4 suppresses apoptosis via regulating the PI3K/AKT/FOXO1 signaling pathway in tongue squamous cell carcinoma. *Exp Ther Med* 18: 3543-3551, 2019.
35. Krill KT, Gurdziel K, Heaton JH, Simon DP and Hammer GD: Dicer deficiency reveals microRNAs predicted to control gene expression in the developing adrenal cortex. *Mol Endocrinol* 27: 754-768, 2013.
36. Asgeirsdóttir SA, van Solingen C, Kurniati NF, Zwiers PJ, Heeringa P, van Meurs M, Satchell SC, Saleem MA, Mathieson PW, Banas B, *et al*: MicroRNA-126 contributes to renal microvascular heterogeneity of VCAM-1 protein expression in acute inflammation. *Am J Physiol Renal Physiol* 302: F1630-F1639, 2012.
37. Metzinger-Le Meuth V, Andrianome S, Chillon JM, Bengrine A, Massy ZA and Metzinger L: microRNAs are dysregulated in the cerebral microvasculature of CKD mice. *Front Biosci (Elite Ed)* 6: 80-88, 2014.
38. Zampetaki A, Kiechl S, Drozdov I, Willeit P, Mayr U, Prokopi M, Mayr A, Weger S, Oberhollenzer F, Bonora E, *et al*: Plasma microRNA profiling reveals loss of endothelial miR-126 and other microRNAs in type 2 diabetes. *Circ Res* 107: 810-817, 2010.
39. Wang S, Aurora AB, Johnson BA, Qi X, McAnally J, Hill JA, Richardson JA, Bassel-Duby R and Olson EN: The endothelial-specific microRNA miR-126 governs vascular integrity and angiogenesis. *Dev Cell* 15: 261-271, 2008.
40. Liu Y, Gao G, Yang C, Zhou K, Shen B, Liang H and Jiang X: Stability of miR-126 in Urine and Its Potential as a Biomarker for Renal Endothelial Injury with Diabetic Nephropathy. *Int J Endocrinol* 2014: 393109, 2014.
41. Sheng X, Zuo X, Liu X, Zhou Y and Sun X: Crosstalk between TLR4 and Notch1 signaling in the IgA nephropathy during inflammatory response. *Int Urol Nephrol* 50: 779-785, 2018.
42. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2<sup>-</sup>(Delta Delta C(T)) Method. *Methods* 25: 402-408, 2001.
43. Langham RG, Kelly DJ, Cox AJ, Thomson NM, Holthöfer H, Zaoui P, Pinel N, Cordonnier DJ and Gilbert RE: Proteinuria and the expression of the podocyte slit diaphragm protein, nephrin, in diabetic nephropathy: Effects of angiotensin converting enzyme inhibition. *Diabetologia* 45: 1572-1576, 2002.
44. Wolenski FS, Shah P, Sano T, Shinozawa T, Bernard H, Gallacher MJ, Wyllie SD, Varrone G, Cicia LA, Carsillo ME, *et al*: Identification of microRNA biomarker candidates in urine and plasma from rats with kidney or liver damage. *J Appl Toxicol* 37: 278-286, 2017.
45. Zhou Z, Wan J, Hou X, Geng J, Li X and Bai X: MicroRNA-27a promotes podocyte injury via PPAR $\gamma$ -mediated  $\beta$ -catenin activation in diabetic nephropathy. *Cell Death Dis* 8: e2658, 2017.
46. Chen X, Zhao L, Xing Y and Lin B: Down-regulation of microRNA-21 reduces inflammation and podocyte apoptosis in diabetic nephropathy by relieving the repression of TIMP3 expression. *Biomed Pharmacother* 108: 7-14, 2018.
47. Fu Y, Wang C, Zhang D, Chu X, Zhang Y and Li J: miR-15b-5p ameliorated high glucose-induced podocyte injury through repressing apoptosis, oxidative stress, and inflammatory responses by targeting Sema3A. *J Cell Physiol* 234: 20869-20878, 2019.
48. Xian Y, Dong L, Jia Y, Lin Y, Jiao W and Wang Y: miR-370 promotes high glucose-induced podocyte injuries by inhibiting angiotensin II type 1 receptor-associated protein. *Cell Biol Int* 42: 1545-1555, 2018.
49. Liu XD, Zhang LY, Zhu TC, Zhang RF, Wang SL and Bao Y: Overexpression of miR-34c inhibits high glucose-induced apoptosis in podocytes by targeting Notch signaling pathways. *Int J Clin Exp Pathol* 8: 4525-4534, 2015.
50. Liu D, Liu F, Wang X, Qiao Y, Pan S, Yang Y, Hu Y, Zhang Y, Tian F and Liu Z: MiR-130a-5p prevents angiotensin II-induced podocyte apoptosis by modulating M-type phospholipase A2 receptor. *Cell Cycle* 17: 2484-2495, 2018.
51. Henique C, Bollée G, Loyer X, Grahame F, Dhaun N, Camus M, Vernerey J, Guyonnet L, Gaillard F, Lazareth H, *et al*: Genetic and pharmacological inhibition of microRNA-92a maintains podocyte cell cycle quiescence and limits crescentic glomerulonephritis. *Nat Commun* 8: 1829, 2017.
52. Xu G, Mo L, Wu C, Shen X, Dong H, Yu L, Pan P and Pan K: The miR-15a-5p-XIST-CUL3 regulatory axis is important for sepsis-induced acute kidney injury. *Ren Fail* 41: 955-966, 2019.
53. Zheng Z, Hu H, Tong Y, Hu Z, Cao S, Shan C, Lin W, Yin Y and Li Z: MiR-27b regulates podocyte survival through targeting adenosine receptor 2B in podocytes from non-human primate. *Cell Death Dis* 9: 1133, 2018.
54. Noh K, Mangala LS, Han H-D, Zhang N, Pradeep S, Wu SY, Ma S, Mora E, Rupaimoole R, Jiang D, *et al*: Differential effects of EGFL6 on tumor versus wound angiogenesis. *Cell Rep* 21: 2785-2795, 2017.
55. Larimer BM and Deutscher SL: Identification of a peptide from in vivo bacteriophage display with homology to EGFL6: A candidate tumor vasculature ligand in breast cancer. *J Mol Biomark Diagn* 5: 178, 2014.
56. Mas VR, Maluf DG, Archer KJ, Yanek KC and Fisher RA: Angiogenesis soluble factors as hepatocellular carcinoma noninvasive markers for monitoring hepatitis C virus cirrhotic patients awaiting liver transplantation. *Transplantation* 84: 1262-1271, 2007.
57. Ballew BJ and Savage SA: Updates on the biology and management of dyskeratosis congenita and related telomere biology disorders. *Expert Rev Hematol* 6: 327-337, 2013.
58. Cao DW, Jiang CM, Wan C, Zhang M, Zhang QY, Zhao M, Yang B, Zhu DL and Han X: Upregulation of miR-126 delays the senescence of human glomerular mesangial cells Induced by high glucose via telomere-p53-p21-Rb signaling pathway. *Curr Med Sci* 38: 758-764, 2018.
59. Yu X, Odenthal M and Fries JW: Exosomes as miRNA carriers: Formation-function-future. *Int J Mol Sci* 17: 2028, 2016.