Department of Rheumatology and Immunology[1]; The Central Laboratory[2], The Affiliated Hospital of Qingdao University, Qingdao, China

MiR-130a protects against lipopolysaccharide-induced glomerular cell injury by upregulation of Klotho

Hongda Liang[1, #], Kun Yang[2, #], Miaomiao Xin[1], Xiangping Liu[2], Lei Zhao[1], Bin Liu[1], Jibo Wang[1, *]

Received March 23, 2017, accepted April 27, 2017

**Corresponding author: Jibo Wang, Department of Rheumatology and Immunology, The Affiliated Hospital of Qingdao University, No. 1677, Wutaishan Road, Qingdao 266555, China*
wangjibo7788@126.com
[#] These authors contributed equally to this study.

Pharmazie 72: 468–474 (2017) *doi: 10.1691/ph.2017.7525*

Aims: Lupus nephritis is a frequent and serious complication of systemic lupus erythematosus (SLE). Therefore, better understanding regarding the underlying mechanism of renal tubular injury induced by SLE, is beneficial to develop different therapeutic strategies for lupus nephritis. The study aimed to investigate the role of miR-130a against lipopolysaccharide-induced glomerular cell injury. *Methods:* HK-2 cells (human renal proximal tubule cells) were used for detecting miR-130a levels. Cells were divided into scramble, miR-130 mimic, siNC, si-miR-130a and si-Klotho groups apoptosis and CCK-8 assays were performed to investigate the cell apoptosis and proliferation rates. qRT-PCR, ELISA, and western blotting were performed to detect the proteins and their expressions. *Results:* LPS induced inflammatory injury in HK-2 cells by inducing cell apoptosis ($P < 0.01$) and by expressing the inflammatory factors such as IL-1β, IL-6, IL-8 and TNF-α in HK-2 cells. LPS increased the expression of miR-130a compared to control group of cells ($P < 0.01$). miR-130a was highly expressed in HK-2 cells ($P < 0.001$). Overexpression of miR-130a reversed LPS-induced apoptosis ($P < 0.05$), increased expression of inflammatory mediators and decreased cell viability ($P < 0.05$), and miR-130a knockdown in HK-2 cells revealed to just the opposite effects upon treatment with LPS. Western blotting results showed that overexpression of miR-130a promoted the expression of Klotho and activated the PI3K/AKT pathway but inhibited Wnt and NF-κB pathways. Conclusions: These findings demonstrated that miR-130a promoted PI3K/AKT pathway but inhibited Wnt and NF-κB pathways through upregulation of Klotho. Furthermore, miR-130a protects against lipopolysaccharide-induced glomerular cell injury by upregulating Klotho expression.

1. Introduction

Systemic lupus erythematosus (SLE) is a common chronic autoimmune disease (Qingjuan et al. 2016; Gu et al. 2016). SLE might affect many organs, including heart, joints, skin, lung, liver, kidney, blood vessels, and the nervous system (Gu et al. 2016). SLE usually occurs in young women (lupus fact sheet 2016). It has been reported that 40-50% SLE patients also suffer from lupus nephritis (Yee et al. 1990), which is a frequent and serious complication of SLE (Farid et al. 2013; Qingjuan et al. 2016). The main pathological change involved in lupus nephritis is mesangial cell proliferation (Seret et al. 2012). Inhibition of mesangial cell proliferation can alleviate the pathological changes associated with glomerulosclerosis (Hirai et al. 2006; Pfeilschifter 1994). Hence, mesangial cells play a major role in the progression of glomerular injury. Therefore, it is crucial to understand the mechanism of renal tubular injury induced by SLE as it might help in development of novel drug targets of lupus nephritis.

Many factors released from both immune and non-immune cells play major roles in the pathophysiology of glomerular disease (Pfeilschifter 1994). Mesangial cells function as smooth muscle-like cells, help in the production of extracellular matrix, and also take part in release of different cytokines related to renal disease severity (Ortiz et al. 1994). Mesangial cells are one of the major intraglomerular sources of tumor necrosis factor-α (TNF-α) which is both expressed in and released from the cell membrane following lipopolysaccharide (LPS) challenge (Baud et al. 1989; Fouqueray 1992). LPS also induces the PI3K-AKT pathway in the mesangial cells (Peairs et al. 2009). Other inflammatory mediators released by mesangial cells include cytokines such as IL-1β, IL-6, IL-8, and TNF-α which contribute in the pathogenesis of lupus (Suryaprabha et al. 1991).

Klotho is a single transmembrane 130 KDa protein encoded by the Klotho gene. A 70 kD protein, the secreted form of Klotho, is produced by alternative splicing (Chen et al. 2007; Kuroo et al. 1997; Matsumura et al. 1998; Shirakiiida et al. 1998; Tohyama et al. 2004) and this may in turn can be released into blood (Bloch et al. 2009; Huang 2010). Furthermore, klotho might exert multiple systemic biological actions on distant organs (Carpenter et al. 2010; Goetz and Potts, 2010; Liu et al. 2007). The cleaved extracellular domain of membrane Klotho, which is referred to as soluble Klotho (sKl) functions as a β-glucuronidase (Hu et al. 2010; Matsumura et al. 1998) and sialidase (Cha et al. 2009, 2008). Klotho is primarily synthesized in kidney and brain although it is expressed in multiple organs (Peairs et al. 2009). A recent study investigated the association between genetic variations in the KLOTHO gene and high mortality rates in hemodialysed patients, and the study have also concluded that this can be modified by activated vitamin D supplementation (Friedman et al. 2009). This modification of KLOTHO gene may subsequently affect the ultimate outcome in chronic kidney disease patients.

miRNAs are a class of small, non-coding RNAs that enter the RNA interference (RNAi) pathway to regulate the expression of

Abbreviations

LPS: lipopolysaccharides; PI3K/AKT: phosphatidylinositol 3'-kinase; NF-κB: Nuclear factor-κB

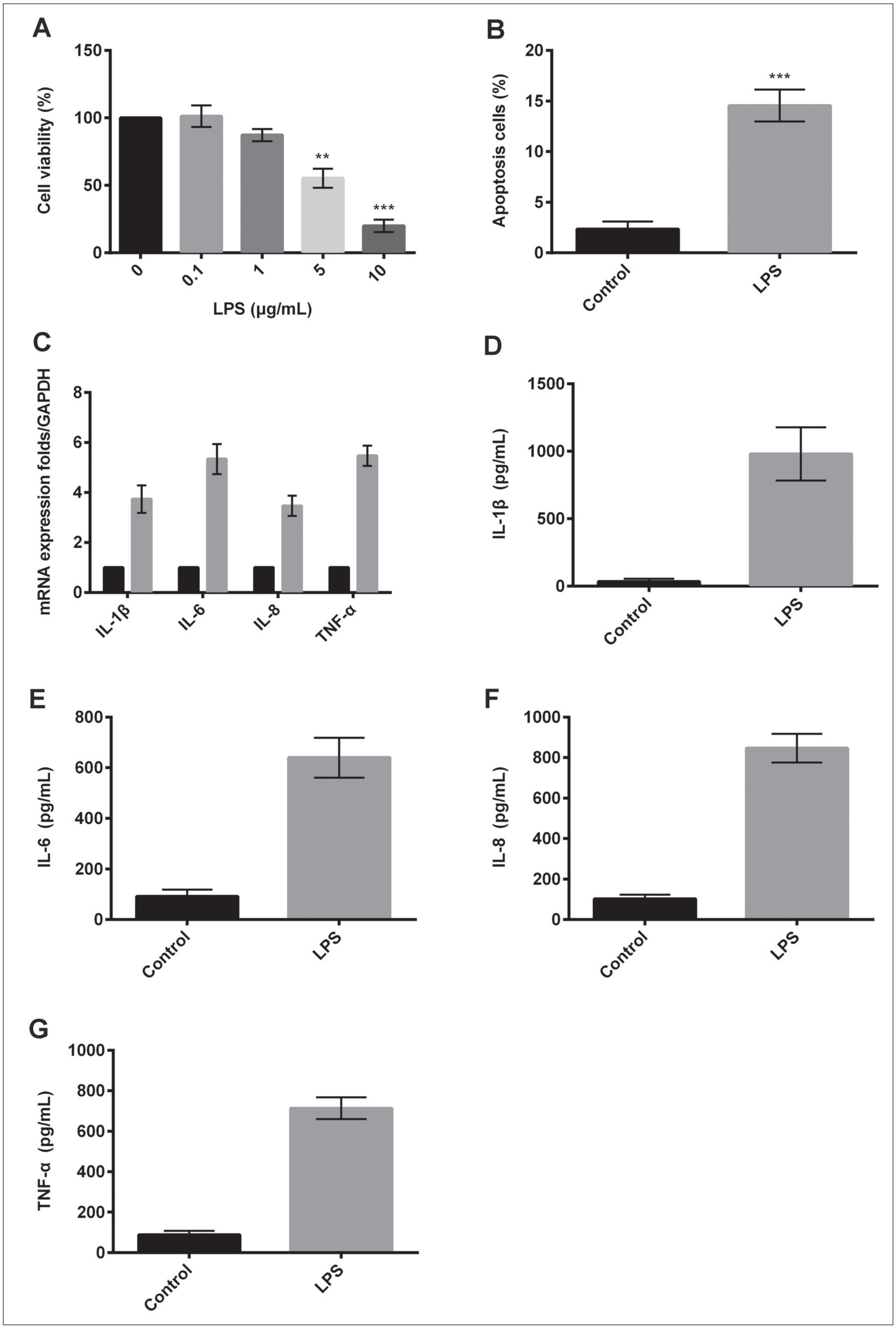

Fig. 1: LPS induced inflammatory injury in HK-2 cells. a) HK-2 cells were treated with different concentrations of LPS. The cell survival rate was about 50% when the cells were treated with 5 g/mL LPS, and the cell survival rate is about 20% when the cells were treated with 10 g/mL LPS ($P<0.01$). Therefore, 5 g/mL was chosen in further experiments; b) LPS induced HK-2 cell apoptosis ($P<0.001$); C-G) LPS induced high expression of inflammatory factors inHK-2 cells. **$P<0.01$, ***$P<0.001$.

protein-encoding genes at the post-transcriptional level. They bind to the 3'UTR of their target genes or induce mRNA degradation and cause posttranscriptional repression of the target gene or block protein translation. miRNAs play a crucial roles in a number of biological functions, such as cell proliferation, differentiation, and apoptosis and may also be involved in the pathogenesis of

cancers or other diseases by regulating the expression of key genes (Qingjuan et al. 2016). Along with these, a number of miRNAs are highly expressed in the kidneys (Tian et al. 2008) and abnormal expression of these miRNAs can contribute in the pathogenesis of many kidney diseases including diabetic nephropathy and renal cancer (Kato et al. 2009; Neal et al. 2010). Few miRNAs with abnormal expressions were seen in the blood serum, urine, and renal tissue of patients with lupus nephritis (Qingjuan et al. 2016). However, these results were not explored extensively. miR-130a is a carcinogenic gene. For example, miR-130a can promote the proliferation and angiogenesis in gastric cancer cells by combining with Runx3 (Lee et al. 2015). miR-130a can promote the proliferation, migration and invasion by inhibiting the expression of Ras-relative protein (RAB)-5A (Liu et al. 2012).
Hence, in the present study we investigated the role of miR-130a against lipopolysaccharide-induced glomerular cell injury.

2. Investigations and results

2.1. LPS induced inflammatory injury of HK-2 cells

HK-2 cells were treated with different concentrations (0, 0.1, 1, 5 and 10 mg/mL) of LPS to determine the cell viability. The results demonstrated that the cell survival rate was about 50% when the cells were treated with 5 mg/mL LPS (P<0.01), and the cell survival rate was about 20% when the cells were treated with 10 mg/mL LPS (P<0.001). Therefore, 5 mg/mL was chosen for further study experiments (Fig. 1A). Furthermore, cell apoptosis assay was performed to determine the HK-2 cell apoptosis which showed statistically significant differences (P<0.001) between the LPS treated group (approximately 15%) and the control group (<5%) (Fig.1B). Also, LPS induced high expression of inflammatory factors such as IL-1β, IL-6, IL-8, and TNF-α were seen in HK-2 cells. ELISA results revealed that inflammatory cytokines release by the LPS treated cells (IL-1β: approximately 1000 pg/mL; IL-6: >600 pg/mL; IL-8: >800 pg/mL; TNF-α: nearly 800 pg/mL) was significantly higher than in the control group (Fig. 1C-G, P<0.05). These results suggested that 5 mg/mL of LPS induced glomerular cell injury by facilitating HK-2 cell apoptosis and high expression of inflammatory cytokines.

2.2. LPS increased the expression of miR-130a

qRT-PCR was performed to detect miR-130a expression in both LPS treated and control group cells. The results demonstrated a statistically significant (P<0.01) expression of miR-130a in the LPS treated group (mean=2.23) compared to the control group (Mean=1.00). This suggested that miR-130a expression was significantly enhanced following exposure to LPS (Fig. 2).

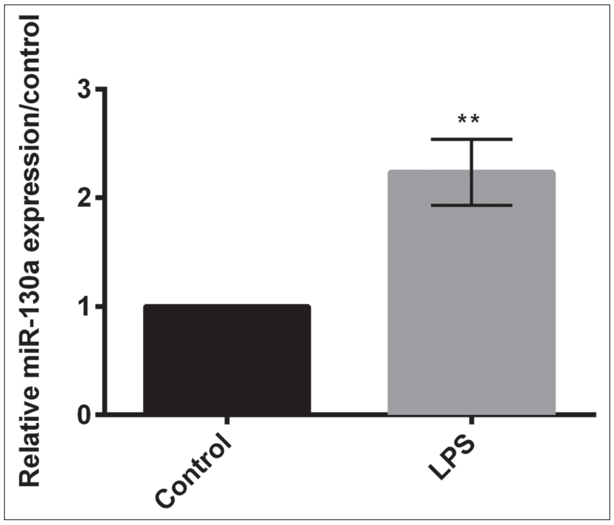

Fig. 2: LPS increased the expression of miR-130a, **P<0.01.

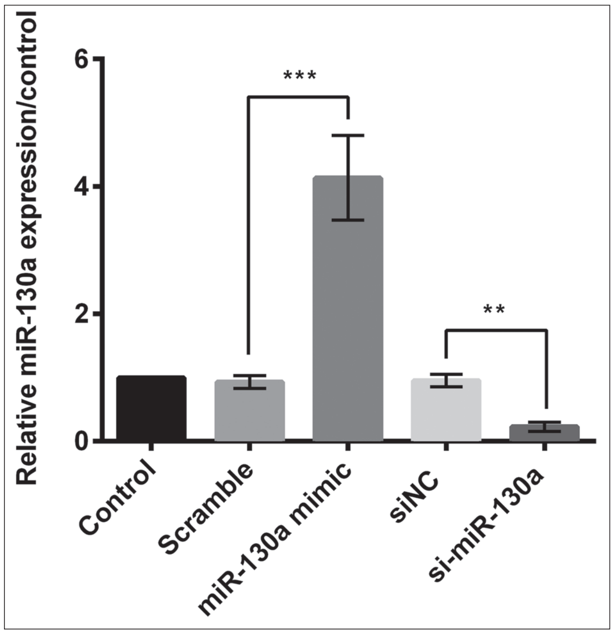

Fig. 3: Abnormal expression of miR-130a in HK-2 cells, **P<0.01, ***P<0.001.

2.3. Abnormal expression of miR-130a in HK-2 cells

qRT-PCR was performed to investigate the expression of miR-130a. miR-130a expression was significantly (P<0.001) higher compared to scramble. Also, miR-130a mimic was significantly expressed compared to control, siNC, and si-miR-130a. These results suggested that miR-130a was highly expressed in HK-2 cells transfected with miR-130a (si-miR-130a mimics) and similarly, expression of miR-130a was suppressed significantly in HK-2 cells transfected with si-miR-130a (si-miR-130a) (Fig. 3).

2.4. Overexpression of miR-130a reduced LPS-induced damage in HK-2 cells

Cell apoptosis assay, proliferation assay, and ELISA were performed to investigate the expression of miR-130a and its association with LPS-induced damage in HK-2 cells. Cell viability results revealed that miR-130a mimic, treated by LPS, showed a significant increase in the cell viability compared to scramble treated with LPS (P<0.05, Fig. 4A). This suggested that overexpression of miR-130a increased cell viability, and knockdown of miR-130a revealed opposite results. Apoptosis assay demonstrated that miR-130a mimic group of cells, treated by LPS, showed a significant reduction in the cell apoptosis rate compared to the scramble group of cells treated with LPS (P<0.05) which suggested that overexpression of miR-130a reduced cell apoptosis, and *vice versa* (Fig. 4B). ELISA was performed to detect the expression levels of inflammatory cytokines (IL-1β, IL-6, IL-8, and TNF-α). These results showed that overexpression of miR-130a following exposure to LPS led to decreased expression of inflammatory factors (IL-1β, IL-6, IL-8, and TNF-α) compared to scramble, siNC, and si-miR-130a group of cells treated with LPS (Fig. 4C), although the difference was significant only between miR-130a group of cells and scramble group of cells exposed to LPS. Knockdown of miR-130a (si-miR-130a) showed a decrease in expression of different inflammatory mediators compared to other group of cells, although the difference was significant only between si-NC and si-miR-130a (Fig. 4C-G).

2.5. miR-130a promoted the expression of Klotho

Western blotting was performed to detect the expression of Klotho, a protein found in chronic renal failure patients, in different groups of HK-2 cells, namely control, scramble, miR-130a mimic, siNC,

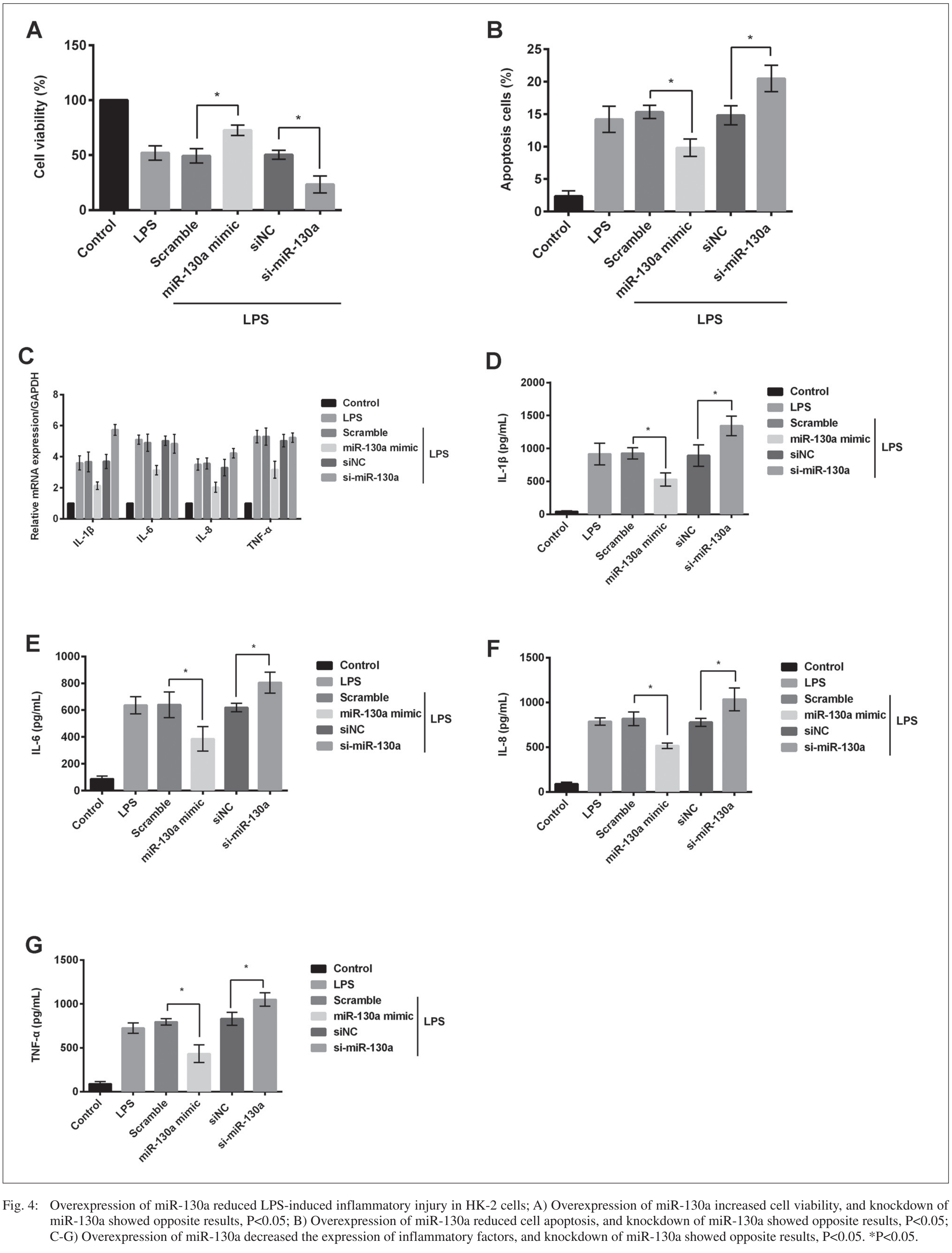

Fig. 4: Overexpression of miR-130a reduced LPS-induced inflammatory injury in HK-2 cells; A) Overexpression of miR-130a increased cell viability, and knockdown of miR-130a showed opposite results, P<0.05; B) Overexpression of miR-130a reduced cell apoptosis, and knockdown of miR-130a showed opposite results, P<0.05; C-G) Overexpression of miR-130a decreased the expression of inflammatory factors, and knockdown of miR-130a showed opposite results, P<0.05. *P<0.05.

and si-miR-130a. Results revealed that Klotho was overexpressed in miR-130a mimic group compared to scramble, siNC, si-miR-130a groups. This suggested that overexpression of miR-130a increased the expression of Klotho, while knockdown of miR-130a suppressed expression of Klotho (Fig. 5).

2.6. miR-130a activated PI3K/AKT pathway but inhibited Wnt and NF-κB by upregulation of Klotho

Western blotting was performed to detect the expression of Klotho in HK-2 cells. Assessment of transfection efficiency revealed reduced expression of Klotho in si-Klotho group of HK-2 cells

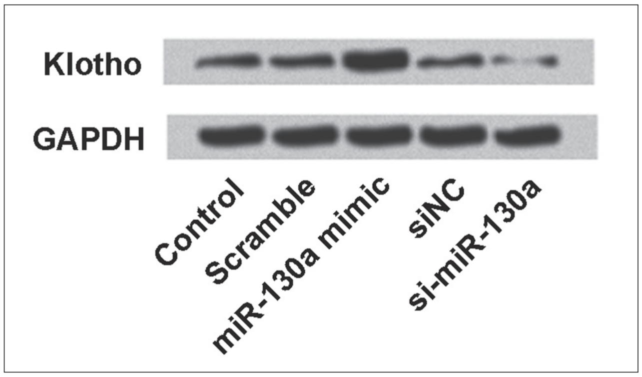

Fig. 5: miR-130a promoted the expression of Klotho. Overexpression of miR-130a increased the expression of Klotho, and knockdown of miR-130a showed opposite results.

compared to negative control (siNC), (Fig. 6). Western blotting results revealed that although the expression of PI3K and AKT were just the same in both miR-130a mimic and miR-130a mimic+-si-Klotho groups (cells overexpressing miR-130a but with knockdown of Klotho expression), however other proteins associated with the PI3K/AKT pathway like p-PI3K and p-AKT expressions were lower in mR-103a+si-Klotho group of cells. These results revealed that miR-130a activated PI3K/AKT by upregulating Klotho. Furthermore, analysis of western blotting results revealed that all the proteins associated with Wnt pathway are increased in miR-130a+si-Klotho group compared to miR-130a. This suggested that miR-130a inhibited Wnt by upregulating Klotho (Fig. 6).

3. Discussion

In the present study, we explored the role of miR-130a on LPS-induced inflammatory injury in HK-2 cells. The results showed that the expression of miR-130a was increased by administration of LPS in HK-2 cells. Moreover, we found that the overexpression of miR-130a could alleviate the LPS-induced injury in HK-2 cells, while knockdown of miR-130a aggravated the injury. Furthermore, the results showed that miR-130a promoted the PI3K/AKT pathway but inhibited Wnt and NF-κB pathways through upregulation of Klotho. This paper lays a foundation for further study regarding role of miR-130a in the regulation of inflammatory injury, and also provides a novel strategy for the treatment of lupus nephritis.

Our study has demonstrated that LPS increased the expression of miR-130a (Fig. 2). Similarly, a report by Zhou et al. (2010) reported that LPS could increase miR-130a expression in human biliary epithelial cells. This might be due to the formation of NF-κB/miR-130a/TNF-α feedback loop which may contribute to the low TNF-α concentration that suppresses apoptosis in carcinogenesis.

Results of our study demonstrated that miR-130a was abnormally expressed in the human renal proximal tubule cells (Fig. 3). Similarly, abnormal expression of miR-130a was reported in patients with the autoimmune disease immune thrombocytopenia (Zhao et al. 2014). This might be due to aberrant miRNA expression involved in the pathogenesis of autoimmune diseases including ITP. Identifying specific miRNA involved in the pathological type helps in the development of new targets for diagnosis and treatment.

Other results demonstrated that overexpression of miR-130a reduced LPS-induced inflammatory injury in HK-2 cells (Fig. 4). Overexpression of miR-130a increased cell viability, and knockdown of miR-130a showed opposite results (Fig. 4A). Overexpression of miR-130a reduced cell apoptosis, and *vice versa* with miR-130a knockdown (Fig. 4B). Overexpression of miR-130a decreased the expression of inflammatory mediators, while knockdown of miR-130a showed opposite results (Figure 4C-G). In contrast to our results, Meng et al. demonstrated that overexpression of miR-130a in endothelial progenitor cells (EPC) promoted migration, differentiation, colony formation, and tubule formation, while reduced levels of miR-130a contributes to EPC dysfunction in type II diabetes mellitus. The same study demonstrated that inhibition of miR-130a increased while overexpression of miR-130a decreased Runx3 mRNA and protein levels in EPCs.

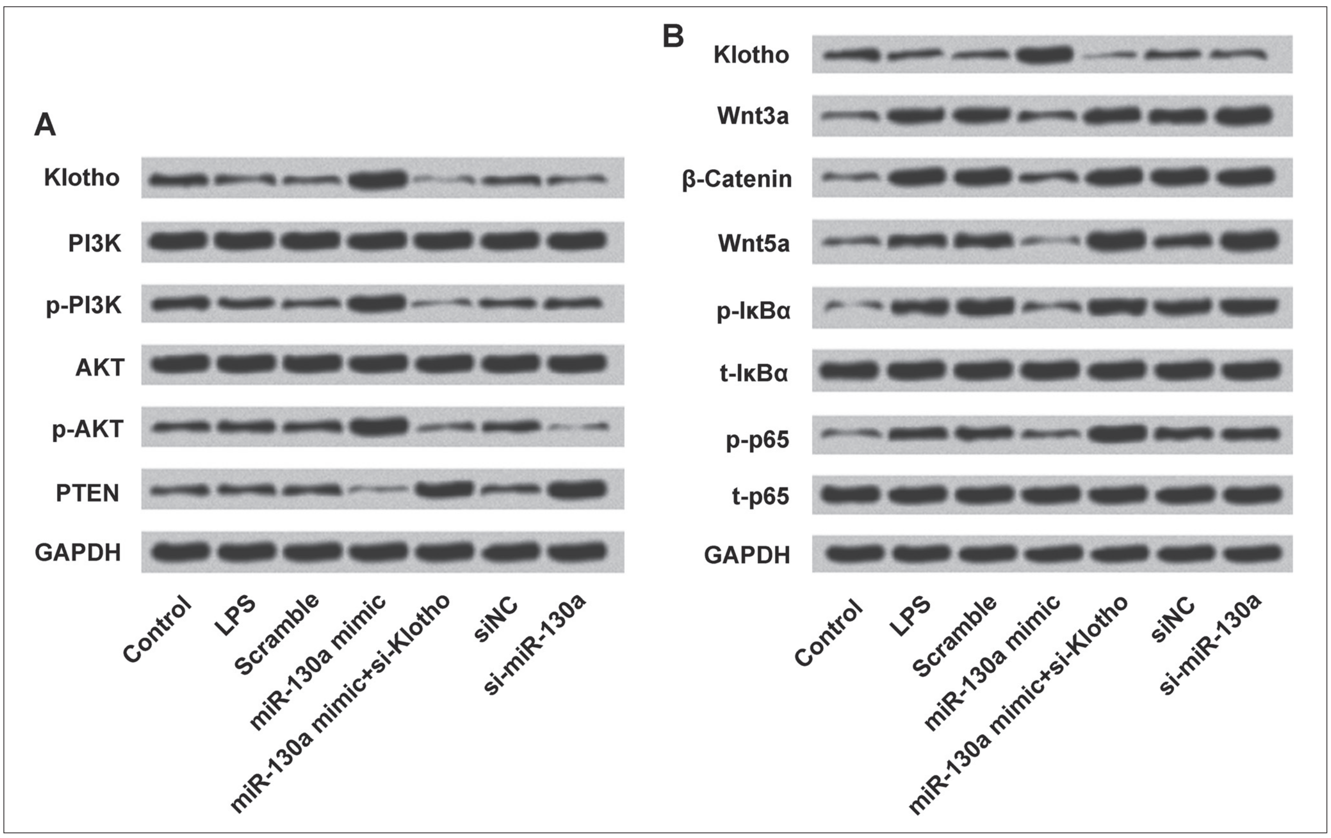

Fig. 6: miR-130a activated PI3K/AKT pathway but inhibited Wnt and NF-κB by upregulation of Klotho.

In contrast, knockdown of Runx3 expression in EPCs derived from patients with diabetes mellitus (DM) promotes colony formation, proliferation, migration and differentiation of EPCs, thus rescuing EPC dysfunction due to miR-130a deficiency in DM (Meng et al. 2013).

In our study, miR-130a promoted the expression of Klotho (Fig. 5). Few studies have demonstrated that Klotho deficiency in acute kidney injury (AKI) is a pathogenic factor that increases the degree of acute kidney damage and contributes to its long-term consequences. Restoration by exogenous supplementation or stimulation of endogenous Klotho might prevent and ameliorate injury, promote recovery, and suppress fibrosis to mitigate development of CKD (Hu and Moe 2012; Hu et al. 2010).

In our study, miR-130a activated the PI3K/AKT pathway but inhibited Wnt and NF-κB by upregulation of Klotho (Fig. 6). Our results were supported by studies which demonstrated that PI3K/AKT helps in the regulation of DM (Meng et al. 2013; Peairs et al. 2009). In contrast to our results, Zhang et al. (2014) demonstrated that NF-κB-modulated miR-130a that targets TNF-α in cervical cancer cells, which showed NF-κB and miR-130a promotes growth of human cervical cancer cells.

In conclusion, these findings demonstrated that miR-130a was highly expressed in HK-2 cells exposed to LPS, suggesting that miR-130a might be involved in the regulation of inflammatory damage in renal tubular cells. Our study thus helps in understanding of the underlying molecular mechanism regarding miR-130a mediated alleviation of lupus nephritis and might provide a new potential biomarker of diagnostic and therapeutic value for LPS-induced glomerular injury.

4. Experimental

4.1. Cell culture and treatment

HK-2 cells (human renal proximal tubule cells) were obtained from ATCC (American Type Culture Collection, Manassas, VA, USA). They were cultured in Dulbecco's Modified Eagle's Medium/Nutrient Mixture F-12 (DMEM-F12, 3:1) supplemented with 10% fetal bovine serum in a humidified 5 % CO_2 atmosphere at 37 °C. The cells were maintained in growth medium in a 75 cm^2 flask. Fresh medium was added to cells every 3 days until confluence was achieved. All experiments were done using cells at passage 10 or below. The cells were treated by LPS for 12 h.

4.2. miRNAs transfection

The scramble, miR-130 mimic, siNC and si-miR-130a were synthesized by GenePharma Co (Shanghai, China). Cell transfections were conducted using Lipofectamine 3000 reagent (Invitrogen) following the manufacturer's protocol.

4.3. Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNAs from cultured cells were extracted using RNA pure Rapid Extraction Kit (Bioteke Corporation, Beijing, China) according to the manufacturer's instructions. For reverse transcription of miRNA, single-step cDNA synthesis was used by adding poly (A) tail to the 3′end of miRNAs with oligo (dT) adaptor primer and Super M-MLV reverse transcriptase (Bioteke Corporation, Beijing, China). For mRNA, total RNAs were reverse transcribed in a reaction system containing random primers and M-MLV reverse transcriptase. Subsequently, the reverse transcription products (cDNA) were amplified by using RT-PCR with SYBR green Master Mix, which was performed in Exicycler 96 Real -Time Quantitative Thermal Block (BIONEER, Daejeon, South Korea). U6 was used as an internal control for miRNA expression analysis, while GAPDH was used as the internal control for determination of mRNA expression levels. The RT-PCR conditions were as follows: initial 10 min incubation at 95 °C, then 40 cycles consisting of 95 °C for 10 s, 60 °C for 20 s and 72°C for 30 s, followed by 5 min incubation at 4 °C. Relative quantification analysis was conducted using the $2^{-\Delta\Delta CT}$ method. Each sample was analyzed in triplicate, and all experiments were carried out three times independently.

4.4. Cell counting kit-8 (CCK-8) assay

HK-2 cells were seeded in 96-well plates with 5000 cells/well, cell proliferation was assessed by a CCK-8 assay (Dojindo Molecular Technologies, Gaithersburg, MD). Briefly, after stimulation, the CCK-8 solution was added to the culture medium, and the cultures were incubated for 1 h at 37 °C in humidified 95 % air and 5 % CO_2. The absorbance was measured at 450 nm using a Microplate Reader (Bio-Rad, Hercules, CA).

4.5. Apoptosis assay

Apoptosis analysis was performed to identify and quantify the apoptotic cells by using Annexin V-FITC/PI apoptosis detection kit (Beijing Biosea Biotechnology, Beijing, China). The cells (100,000 cells/well) were seeded in 6 well-plates. Treated cells were washed twice with cold PBS and were resuspended in buffer. The adherent and floating cells were combined and treated according to the manufacturer's instruction and measured with flow cytometer (Beckman Coulter, USA) to differentiate apoptotic cells (Annexin-V positive and PI-negative) from necrotic cells (Annexin-V and PI-positive).

4.6. Enzyme-linked immunosorbent assay (ELISA)

Culture supernatant was collected from 24-well plates and concentrations of inflammatory cytokines were measured by ELISA using protocols supplied by the manufacturer (R&D Systems, Abingdon, UK) and normalized to cell protein concentrations.

4.7. Western blot analysis

The protein used for western blotting was extracted using RIPA lysis buffer (Beyotime Biotechnology, Shanghai, China) supplemented with protease inhibitors (Roche, Guangzhou, China). The proteins were quantified using the BCA™ Protein Assay Kit (Pierce, Appleton, WI, USA). The western blot system was established using a Bio-Rad Bis-Tris Gel system according to the manufacturer's instructions. GAPDH antibody was purchased from Sigma. Primary antibodies were prepared in 5 % blocking buffer at a dilution of 1:1,000. Primary antibody was incubated with the membrane at 4 °C overnight, followed by wash and incubation with secondary antibody marked by horseradish peroxidase for 1 h at room temperature. After rinsing, the polyvinylidene difluoride (PVDF) membrane carried blots and antibodies were transferred into the Bio-Rad ChemiDoc™ XRS system, and then 200 μl Immobilon Western Chemiluminescent HRP Substrate (Millipore, MA, USA) was added to cover the membrane surface. The signals were captured and the intensity of the bands was quantified using Image Lab™ Software (Bio-Rad, Shanghai, China).

4.8. Statistical analysis

All experiments were repeated three times. The results of multiple experiments are presented as mean±SD. Statistical analyses were performed using Graphpad 6.0 statistical software. P-values were calculated using one-way analysis of variance (ANOVA). A P-value of <0.05 was considered to be statistically significant.

Conflict of interests and financial disclosure: None declared.

References

Baud L, Oudinet JP, Bens M, Noe L, Peraldi MN, Rondeau E, Etienne J, Ardaillou R (1989) Production of tumor necrosis factor by rat mesangial cells in response to bacterial lipopolysaccharide. Kidney Int 35: 1111-1118.

Bloch L, Sineshchekova O, Reichenbach D, Reiss K, Saftig P, Kuro-O M, Kaether C (2009) Klotho is a substrate for alpha-, beta- and gamma-secretase. FEBS Lett 583: 3221-3224.

Carpenter TO, Insogna KL, Zhang JH, Ellis B, Nieman S, Simpson C, Olear E, Gundberg CM (2010) Circulating levels of soluble klotho and FGF23 in X-linked hypophosphatemia: circadian variance, effects of treatment, and relationship to parathyroid status. J Clin Endocrinol Metabol 95: E352-357.

Cha SK, Hu MC, Kurosu H, Kuro-O M, Moe O, Huang CL (2009) Regulation of renal outer medullary potassium channel and renal K(+) excretion by Klotho. Mol Pharmacol 76: 38-46.

Cha SK, Ortega B, Kurosu H, Rosenblatt KP, Kuro-O M, Huang CL (2008) Removal of sialic acid involving Klotho causes cell-surface retention of TRPV5 channel via binding to galectin-1. Proc Nat Acad Sci USA 105: 9805-9810.

Chen CD, Podvin S, Gillespie E, Leeman SE, Abraham CR (2007) Insulin stimulates the cleavage and release of the extracellular domain of Klotho by ADAM10 and ADAM17. Proc Nati Acad Sci USA 104: 19796-19801.

Farid EM, Hassan AB, Abalkhail AA, El-Agroudy AE, Arrayed SA, Al-Ghareeb SM (2013) Immunological aspects of biopsy-proven lupus nephritis in Bahraini patients with systemic lupus erythematosus. Saudi J Kidney Dis Transplant 24: 1271-1279.

Fouqueray B (1992) Tumor necrosis factor alpha and mesangial cells. Kidney Int 41: 600-603.

Friedman DJ, Afkarian M, Tamez H, Bhan I, Isakova T, Wolf M, Ankers E, Ye J, Tonelli M, Zoccali C (2009) Klotho variants and chronic hemodialysis mortality. J Bone Min Res 24: 1847-1855.

Goetz R, Potts JT (2010) Isolated C-terminal tail of FGF23 alleviates hypophosphatemia by inhibiting FGF23-FGFR-Klotho complex formation. Proc Nat Acad Sci 107: 407-412.

Gu Z, Tan W, Ji J, Feng G, Meng Y, Da Z, Guo G, Xia Y, Zhu X, Shi G, Cheng C (2016) Rapamycin reverses the senescent phenotype and improves immunoregulation of mesenchymal stem cells from MRL/lpr mice and systemic lupus erythematosus patients through inhibition of the mTOR signaling pathway. Aging 8: 1102-1114.

Hirai T, Masaki T, Kuratsune M, Yorioka N, Kohno N (2006) PDGF receptor tyrosine kinase inhibitor suppresses mesangial cell proliferation involving STAT3 activation. Clin Exp Immunol 144: 353-361.

Hu MC, Moe OW (2012) Klotho as a potential biomarker and therapy for acute kidney injury. Nature Rev Nephrol 8: 423-429.

Hu MC, Shi MJ, Pastor J, Nakatani T, Lanske B, Razzaque MS, Rosenblatt KP, Baum MG, Kuro oM, Moe OW (2010) Klotho: a novel phosphaturic substance acting as an autocrine enzyme in the renal proximal tubule. FASEB J 24: 3438-3450.

Huang CL (2010) Regulation of ion channels by secreted Klotho: mechanisms and implications. Kidney Int 77: 855.

Kato M, Putta S, Wang M, Yuan H, Lanting L, Nair I, Gunn A, Nakagawa Y, Shimano H, Todorov I (2009) TGF-beta activates Akt kinase through a microRNA-dependent amplifying circuit targeting PTEN. Nature Cell Biol 11: 881-889.

Kuroo M, Matsumura Y, Aizawa H, Kawaguchi H, Suga T, Utsugi T, Ohyama Y, Kurabayashi M, Kaname T, Kume E (1997) Mutation of the mouse klotho gene leads to a syndrome resembling ageing. Nature 390: 45-51.

Lee SH, Jung YD, Choi YS, Lee YM (2015) Targeting of RUNX3 by miR-130a and miR-495 cooperatively increases cell proliferation and tumor angiogenesis in gastric cancer cells. Oncotarget 6: 33269-33278.

Liu B, Wen X, Cheng Y (2012) Survival or death: disequilibrating the oncogenic and tumor suppressive autophagy in cancer. Cell Death Dis 4: 377-377.

Liu H, Fergusson MM, Castilho RM, Liu J, Cao L, Chen J, Malide D, Rovira II, Schimel D, Kuo CJ (2007) Augmented Wnt signaling in a mammalian model of accelerated aging. Science 317: 803.

Lupus fact sheet (2016). http://www.lupusresearchinstitute.org/lupus-facts/lupus-fact-sheet.

Matsumura Y, Aizawa H, Shirakiiida T, Nagai R, Kuroo M, Nabeshima Y (1998) Identification of the human klotho gene and its two transcripts encoding membrane and secreted klotho protein. Biochem Biophys Res Comm 242: 626-630.

Meng S, Cao J, Zhang X, Fan Y, Fang L, Wang C, Lv Z, Fu D, Li Y (2013) Downregulation of microRNA-130a contributes to endothelial progenitor cell dysfunction in diabetic patients via its target Runx3. Plos One 8: e68611.

Neal CS, Michael MZ, Rawlings LH, Mb VDH, Gleadle JM (2010) The VHL-dependent regulation of microRNAs in renal cancer. BMC Med 8: 64.

Ortiz A, Gómez-Chiarri M, Alonso J, Bustos C, Gómez-Guerrero C, López-Armada MJ, Gómez-Garre D, Palacios I, Ruíz-Ortega M, Gutierrez S (1994) The potential role of inflammatory and fibrogenic cytokines in the glomerular diseases. J Lipid Med Cell Signal 9: 55-74.

Peairs A, Radjavi A, Davis S, Li L, Ahmed A, Giri S, Reilly CM (2009) Activation of AMPK inhibits inflammation in MRL/lpr mouse mesangial cells. Clin Exp Immunol 156: 542–551.

Pfeilschifter J (1994) Mesangial cells orchestrate inflammation in the renal glomerulus. Physiology 9: 271-276.

Qingjuan L, Xiaojuan F, Wei Z, Chao W, Pengpeng K, Hongbo L, Sanbing Z, Jun H, Min Y, Shuxia L (2016) MiR-148a-3p overexpression contributes to glomerular cell proliferation by targeting PTEN in lupus nephritis. Am J Physiol Cell Physiol 310: ajpcell.00129.02015.

Seret G, Le Meur Y, Renaudineau Y, Youinou P (2012) Mesangial cell-specific antibodies are central to the pathogenesis of lupus nephritis. Clin Devel Immunol 2012: 579670.

Shirakiiida T, Aizawa H, Matsumura Y, Sekine S, Iida A, Anazawa H, Nagai R, Kuroo M, Nabeshima Y (1998) Structure of the mouse klotho gene and its two transcripts encoding membrane and secreted protein. FEBS Letters 424: 6-10.

Suryaprabha P, Das UN, Ramesh G, Kumar KV, Kumar GS (1991) Reactive oxygen species, lipid peroxides and essential fatty acids in patients with rheumatoid arthritis and systemic lupus erythematosus. Prostaglandins Leukot Essent Fatty Acids 43: 251-255.

Tian Z, Greene AS, Pietrusz JL, Matus IR, Liang M (2008) MicroRNA-target pairs in the rat kidney identified by microRNA microarray, proteomic, and bioinformatic analysis. Genome Res 18: 404-411.

Tohyama O, Imura A, Iwano A, Freund JN, Henrissat B, Fujimori T, Nabeshima Y (2004) Klotho is a novel beta-glucuronidase capable of hydrolyzing steroid beta-glucuronides. J Biol Chem 279: 9777-9784.

Yee AM, Yip YK, Fischer HD, Buyon JP (1990) Serum activity that confers acid lability to alpha-interferon in systemic lupus erythematosus: its association with disease activity and its independence from circulating alpha-interferon. Arthritis Rheumatol 33: 563-568.

Zhang J (2014) NF-κB-modulated miR-130a targets TNF-α in cervical cancer cells. J Translat Med 12: 1-14.

Zhao H, Li H, Du W, Zhang D, Ge J, Xue F, Zhou Z, Yang R (2014) Reduced MIR130A is involved in primary immune thrombocytopenia via targeting TGFB1 and IL18. Br J Haematol 166: 767–773.

Zhou R, Hu G, Gong AY, Chen XM (2010) Binding of NF-kappaB p65 subunit to the promoter elements is involved in LPS-induced transactivation of miRNA genes in human biliary epithelial cells. Nucl Acids Res 38: 3222-3232.