RNA & DISEASE 2019; 6: e450. doi: 10.14800/rd.450; © 2019 by Jihye Yoo, *et al*.
http://www.smartscitech.com/index.php/rd

ARTICLE

Long non-coding RNA Rpph1 promotes inflammation in diabetic nephropathy

Jihye Yoo, Minsuh Kim, Dongwon Kim, Sungho Moon

Department of Nephology, Pundang Hospital, Dohang University of Science and Technology, South Korea.

Correspondence: Sungho Moon
E-mail: shmoon-2016@gmail.com
Received: February 09, 2019
Published: October 08, 2019

As the main complication of diabetes-related kidney damage, diabetic nephropathy (DN) is a complex clinical problem worldwide. As long non-coding RNAs (lncRNAs) play vital roles in DN, we would like to investigate the effect of lncRNA Rpph1 on glomerular mesangial inflammatory cytokines in diabetic nephropathy (DN). Compared with normal mice, Rpph1 was significantly up-regulated in kidney tissues of DN mice. Meanwhile, high-glucose cultured mesangial cells showed significantly up-regulation of Rpph1 expression in cultured mesangial cells. In addition, bioinformatics confirmed that Rpph1 does not have the ability to encode proteins. Moreover, the levels of TNF-α and MCP-1 were significantly up-regulated after overexpression of Rpph1 while down-regulation of Rpph1 results in the decreased expression of TNF-α and MCP-1. In conclusion, the lncRNA Rpph1 is highly expressed in mesangial cells of DN mice and the expression of inflammation-related factors is enhanced in high glucose environment which suggest that Rpph1 may be associated with the development of diabetic nephropathy.

Keywords: diabetic nephropathy; Rpph1; mesangial cells; TNF-α; MCP-1

To cite this article: Jihye Yoo, *et al*. Long non-coding RNA Rpph1 promotes inflammation in diabetic nephropathy. RNA Dis 2019; 6: e450. doi: 10.14800/rd.450.

Diabetes is a multi-pathogenic metabolic disease caused by absolute insulin deficiency or insulin utilization disorders, and persistent high blood sugar. Diabetes has multiple complications that cause long-term damage and dysfunction in a variety of organs, including the eyes, kidneys, and heart. Diabetic nephropathy (DN) is a serious complication of diabetes, in which renal fibrosis and activation of myofibroblasts are the main causes of end-stage severe renal damage [1]. Persistent hyperglycemia is considered to be the driving force for the development of DN, but in fact, the pathogenesis of DN is numerous, involving various mechanisms and factors such as inflammation and oxidative stress. Recent studies have shown that renal inflammation is essential for the promotion of DN development, and inflammation may be a key factor in stimulating known biochemical and metabolic disorders in DN. Although inflammation has been considered as an important link in the occurrence and development of DN, its specific mechanism is still inconclusive, and the current methods of diagnosis and treatment are still not ideal [2].

Long non-coding RNAs (lncRNAs) lack the ability to encode proteins and play a key role in many life processes, both in the cytoplasm and in the nucleus, with lengths greater than 200 nt. Studies have found that lncRNA affects the occurrence and development of inflammation in a variety of diseases and is associated with DN inflammation [3]. However, the exact role of lncRNA in DN inflammation remains unclear. In this study, DN mouse kidney tissue and normal mouse kidney tissue were sent for detection. The second-generation sequencing technology was used to find the abnormally expressed lncRNA in the tissue. It was found that

the expression of Rpph1 lncRNA was significantly higher in the kidney tissue of DN mice. Normal mouse kidney tissue. Therefore, further research on Rpph1 was conducted to investigate whether Rpph1 is associated with DN inflammation and its specific potential role. Then, the expression of Rpph1 was detected in high-low glucose cultured mesangial cells, and the overexpression plasmid and siRNA of Rpph1 were constructed. After transfected into mesangial cells, the efficiency was verified by qRT-PCR and the inflammation-related factor MCP-1 was detected. The expression of TNF-α explores the possible role of Rpph1 in DN inflammation and provides a new idea for the treatment of DN.

Materials and methods

Mouse kidney tissue extraction

DN model mouse B6.BKS(D)-Leprdb were purchased from Pundang Biomedical Research Institute and kept in an SPF lab. When the diabetic nephropathy mice and normal mice reached 15 weeks of age, the mouse kidney tissues were extracted.

Cell culture and transfection

The mesangial cells were cultured in low glucose (5 mmol/L) and DMEM high glucose (25 mmol/L) medium, and the fetal bovine serum content was 15%. The cells were named L-MC group and H-MC. group. Further, the mesangial cells cultured with high glucose in the liposome Lipofectamine 2000 were named as H-MC mock group, and the mesangial cells cultured in high glucose transfected with siRNA negative control were named H-MC siNC group. The high-glycemic mesangial cells stained with Rpph1 siRNA were named H-MC siRpph1 group; in the low glucose cultured mesangial cells, only the liposome Lipofectamine 3000 was added to the L-MC mock group, which was transfected. The no-load plasmid was named L-MC pcDNA3.1 and the Rpph1 overexpression plasmid was designated as L-MC Rpph1(+). The cells were inoculated with 6-well plates, and the cells were transfected when the cells proliferated to 75%-80%. After 5 hours, the cells were replaced with medium containing 15% serum. After 24 hours, the RNA was extracted, and the expression and interference efficiency were detected by qRT-PCR.

LncRNA-Rpph1 encoding protein ability analysis

Analysis of lncRNA-Rpph1 protein coding using the online software ORF Finder (www.ncbi.nlm.nih.gov/orffinder/) and CPC2 (Coding Potential Calculator 2) (http://cpc2.cbi.pku.edu.cn/) ability.

Construction of 1.5 pcDNA3.1(+)-Rpph1 eukaryotic expression plasmid

Rpph1 primers were designed using Primer Premier 5.0 software, and Pubmed's online alignment primers were consistent and specific. The plasmid expression vector was pcDNA3.1(+), and Rpph1 was subjected to restriction enzyme scanning analysis. The control plasmid cloning site was added to the BamHI and EcoRI sites in the upstream and downstream primers of Rpph1 to amplify the full-length sequence of Rpph1. Its primer sequence is upstream: 5'-GGATCCAGTG-GGCGGAGGAAGCTCATC-3', downstream: 5'-GATATC-AGGGCGGGGGAGAGTAGTCTGAA-3'. PCR amplification of Rpph1, agarose gel electrophoresis and purification, approximately 343 bp, configured with double digestion system (BamHI and EcoRI), then incubated at 37 °C for 1 h, added T4 ligase 16 °C overnight to connect Rpph1 PCR product to true On the nuclear expression vector, E. coli DH5α competent cells were transformed according to the procedure, 150 μL was inoculated onto a plate containing ampicillin (50 μg/mL), positive monoclonals were selected by plate streaking, and cultured overnight, and the results were observed. Add 15 mL of medium to a 50 mL plastic centrifuge tube, add antibiotics, shake at 250 r/min, shaken at 37 °C for 13 to 16 h, extract the plasmid according to the kit procedure, measure the plasmid concentration, and take the corresponding plasmid for double digestion. , agarose gel electrophoresis and sequencing identified whether the connection was successful. Pubmed online alignment results, if the amplified target fragment and the mouse Rpph1 gene sequence homology, indicating that the plasmid was successfully constructed. The empty plasmid was used as a control group.

Design and synthesis of Rpph1 small interfering RNA

Design 3 lines of Rpph1 online by DSIR (http://biodev.extra.cea.fr/DSIR/DSIR.html) and siDirect version 2.0 (http://sidirect2.rnai.jp/) with reference to the mouse Rpph1 gene sequence. siRNAs sequence. Sequence 1: 5'-CCAGCAGTG-CGAGTTCAAT-3'; sequence 2: 5'-CGAGTTCAATGGCTG-AGGT-3'; sequence 3: 5'-GGCCTCATAACCCAATTCA-3'. The siRNA sequence was synthesized by Pundong Biotechnology Company, and the interference efficiency was identified by qRT-PCR.

qRT-PCR detection of Rpph1 expression

TRIzol extracts tissue and cellular total RNA, reverse transcription according to the PrimerScriptTM instructions, and preserves cDNA at -20 °C. The primers for the design of Rpph1 and β-actin are then more specific than the ones and synthesized by the company. Upstream of Rpph1: 5'-CAGAC-TGGGCAGGAGAAGCC-3', downstream: 5'-TCACCTCA-GCCATTGAACTCG-3'; upstream of β-actin: 5'-GCTCAGT-AACAGTCCGCCTAG-3', downstream: 5'-AGTGTGACGT-TGACATCCG-3'. Using cDNA as a template, a PCR reaction system was used, and the system was 10 μL, and three replicate wells were set for each detection index. The PCR

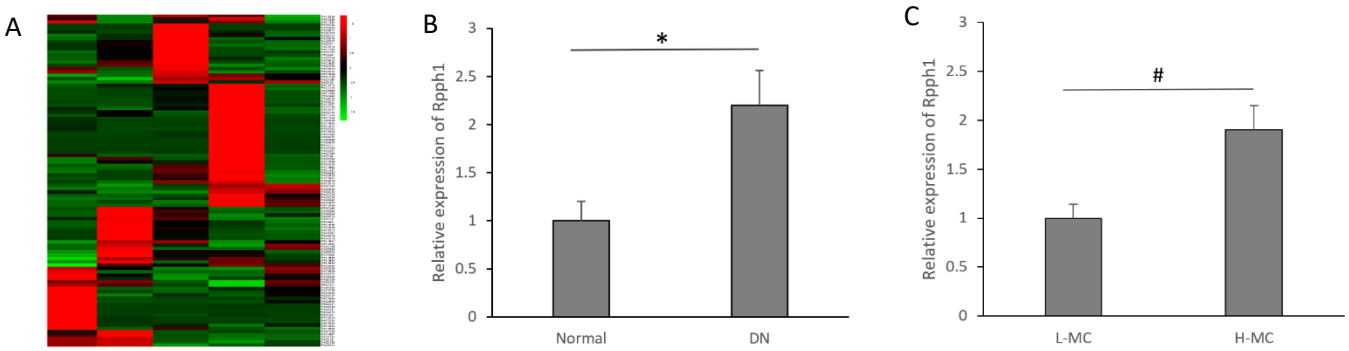

Figure 1. qRT-PCR detection of Rpph1 expression in kidney and mesangial cells of DN mice. A: Rpph1 levels were significantly up-regulated in kidney tissues of DN mice compared to kidney tissues of normal control mice by RNA-sequencing (RNA-seq) ($P < 0.05$). B: Rpph1 level in diabetic nephropathy mice and normal mouse kidneys (*$P < 0.01$, compared with the Normal group). C: Rpph1 levels in mesangial cells cultured in high glucose and low glucose (#$P < 0.05$, compared with L-MC Group comparison).

procedure was as follows: pre-denaturation at 95 °C for 3 min, 95 °C for 5 s, 58 °C for 34 s, and 72 °C for 60 s for 40 cycles. The relative quantitative data of Rpph1 was analyzed by 2-ΔΔCt method.

FISH detection of Rpph1 subcellular localization

The experimental procedure was carried out according to the kit instructions, and the localization of Rpph1 was detected in mesangial cells cultured in high glucose and mesangial cells cultured in low glucose. The cells were grouped into three groups: H-MC, L-MC, Control (only DAPI, cells without Rpph1 probe). The cells were seeded in 24-well plates with slides and cultured in a cell incubator for 24 h. The experimental procedure was as follows: PBS was washed in 24-well plate 4 times, PBS was aspirated, then 100 μL of 4% paraformaldehyde was added to each well, and allowed to stand at room temperature for 10 min, 0.5% Triton X-100 was added, and 100 μL of 0.5% was added to each well. Triton X-100, 5 min, increased cell permeability, washed 4 times in PBS for 5 min each time, pre-hybridization and hybridization solution were preheated in an oven at 37 °C for 30 min before use, and 100 μL per well before hybridization. The pre-hybrid solution was placed in a 37 ° C incubator for 20 min. The pre-hybrid solution was gently aspirated with a pipette tip, and the lncRNA-Rpph1 FISH probe and the hybridization solution were diluted 1:50, and 100 μL of the mixed solution was added to each well, protected from light, and hybridized overnight in a 37 ° C incubator. The next day, 4×SSC, 2×SSC, and 1×SSC were preheated at 50 °C, and the cells were washed three times in a high concentration to a low concentration. The tip was sucked off the SSC, 10 μL of DAPI working solution was added to each well, allowed to stand for 8 min, washed three times with PBS, and finally the photograph was taken to obtain an image.

Western blot analysis of TNF-α and MCP-1 expression

Rpph1 siRNA and overexpression plasmid were transfected into mesangial cells cultured in high glucose and low glucose respectively. After 2 days, the cells were washed with PBS, and the cells were scraped off with cell scraping. After centrifugation, the cells were collected, and PMSF and RIPA were counted as 1:100 mixed, add appropriate amount of lysate, lyse the cells at 4 °C for 40 min, shake the cells 3 times during the lysis, centrifuge at 15 000 r/min for 4 min at 4 °C, carefully aspirate the supernatant, and perform protein quantification test to detect the protein concentration in the supernatant. The sample was mixed with 5× protein loading buffer at 4:1, and the protein was denatured by boiling at 98 °C for 8 min, and placed in a refrigerator at -20 °C. SDS-PAGE gel electrophoresis, gelatinization, membrane transfection, blocking, rabbit anti-TNF-α primary antibody (1:800 dilution) and rabbit anti-MCP-1 primary antibody (1:800 dilution) were added, and overnight at 4 °C. The PVDF membrane was washed with TBST, and the HRP-labeled goat anti-rabbit IgG secondary antibody (1:7 000 dilution) was diluted with TBST, added to the PVDF membrane, placed on a shaker at room temperature, developed after 2 h, and analyzed by Gray Image with Image J software, and GAPDH was the internal reference.

Statistical analysis

The experimental data were analyzed by SPSS 19.0 statistical software. The data of the two groups were compared by t test. The data of multiple groups were compared by one-way ANOVA.

Results

Rpph1 is highly expressed in kidney tissues of DN mice and mesangial cells cultured in high glucose

The differentially expressed genes were analyzed by second-generation sequencing, and Rpph1 levels were significantly up-regulated in kidney tissues of DN mice com-

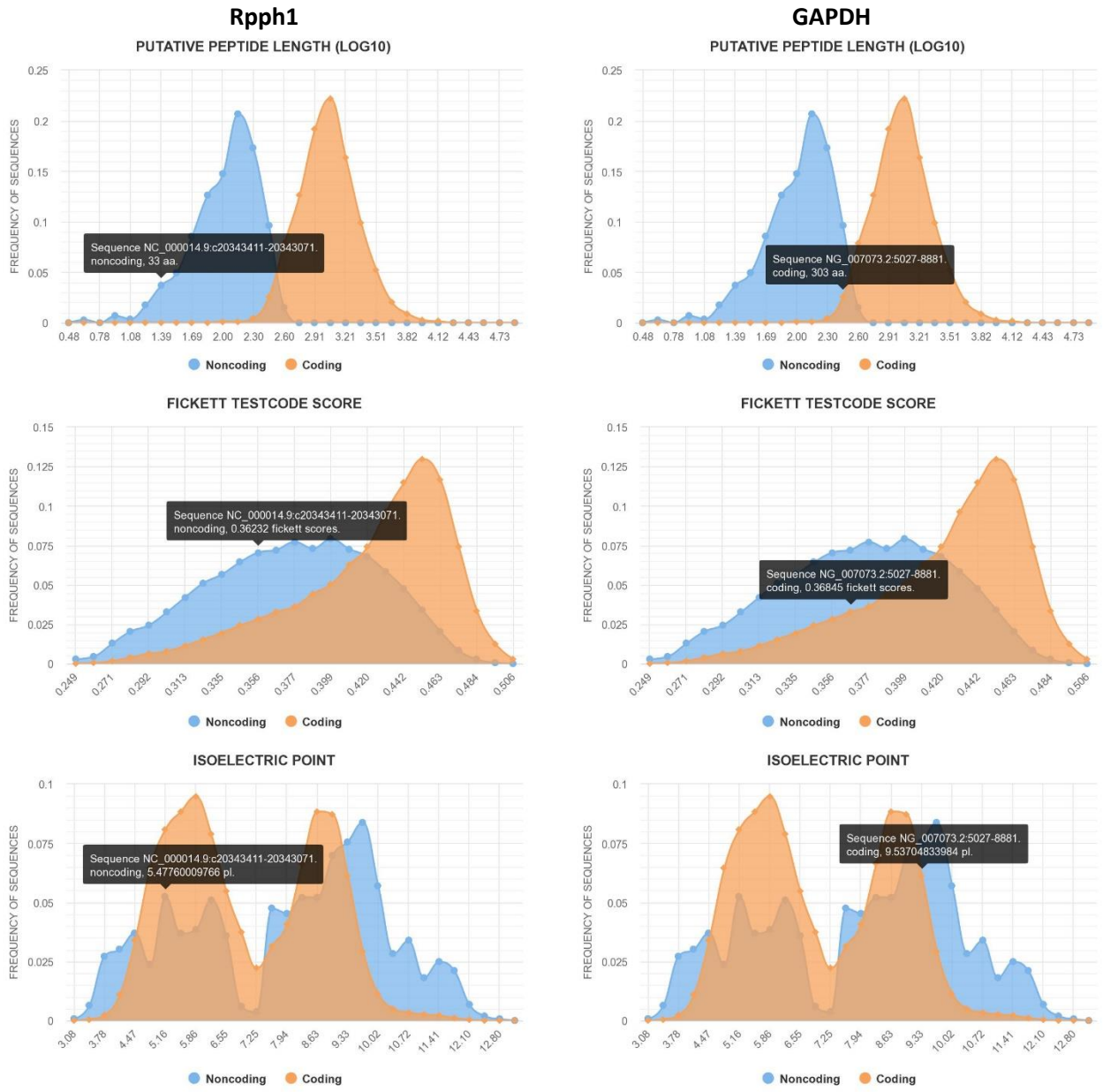

Figure 2. CPC2 analysis of the ability of Rpph1 encoding protein. Sequence of Rpph1 got Fickett score 0.36232 with a complete putative ORF 33 AA, a pI 5.47760009766, which, in total, classify it as a noncoding sequence with coding probability 0.00938689. Sequence of GAPDH got Fickett score 0.36845 with a complete putative ORF 303 AA, a pI 9.53704833984, which, in total, classify it as a coding sequence with coding probability 0.99959.

pared to kidney tissues of normal control mice. The expression of Rpph1 in DN mouse kidney tissue and normal mouse kidney tissue, as well as high glucose cultured mesangial cells and low glucose cultured mesangial cells were further examined by qRT-PCR. The expression of Rpph1 in kidney tissues of DN mice was significantly higher than that in normal mice. Similarly, the expression level of Rpph1 in mesangial cells in high glucose environment was significantly higher than that in low glucose cultured cells ($P < 0.05$, Fig. 1).

Bioinformatics analysis of non-coding Rpph1

The protein coding ability of Rpph1 was analyzed by CPC2 and ORF Finder online software. The results showed that Rpph1 had no protein coding ability, with GAPDH as control (Fig. 2) which suggest that Rpph1 is indeed a lncRNA with no protein coding ability.

Rpph1 is mainly localized in mesangial cell cytoplasm

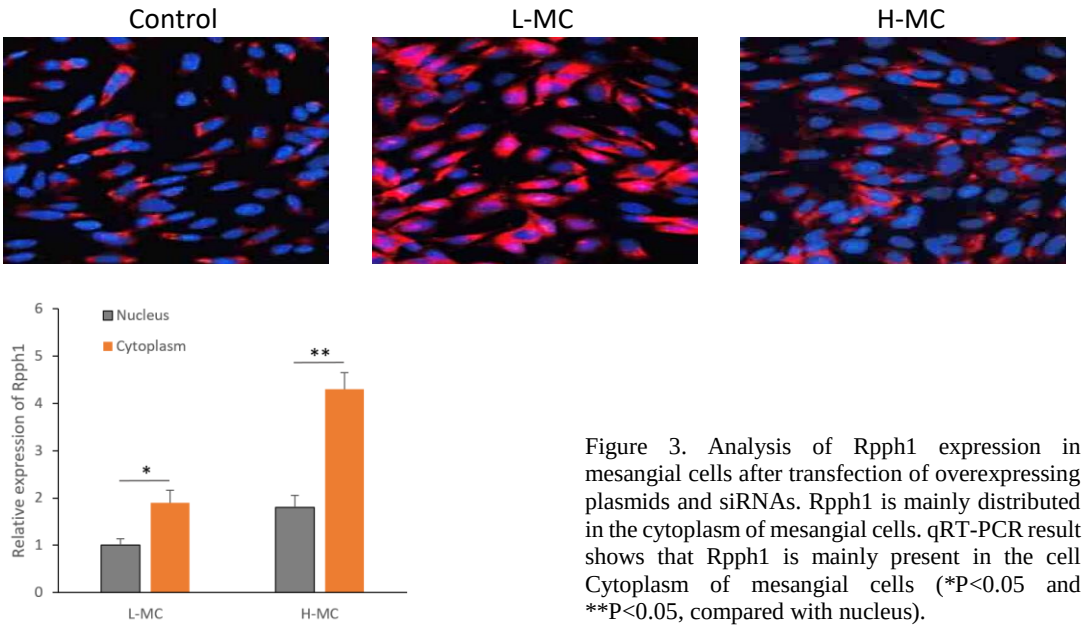

Figure 3. Analysis of Rpph1 expression in mesangial cells after transfection of overexpressing plasmids and siRNAs. Rpph1 is mainly distributed in the cytoplasm of mesangial cells. qRT-PCR result shows that Rpph1 is mainly present in the cell Cytoplasm of mesangial cells (*P<0.05 and **P<0.05, compared with nucleus).

FISH results showed that Rpph1 was distributed in the cytoplasm and nucleus of mesangial cells, but mainly in the cytoplasm. At the same time, the level of mesangial cells in high glucose environment was higher than that in low glucose cultured cells (Fig. 3A). In addition, qRT-PCR results indicated that Rpph1 was mainly present in the cytoplasmic part of mesangial cells ($P < 0.05$, Figure 3B), consistent with the results of the FISH experiment.

Transfection efficiency of Rpph1 overexpression plasmid and screening of Rpph1 siRNAs

The knockdown efficiency of three Rpph1 siRNAs by qRT-PCR is shown in Figure 4. The knockdown efficiency of siRNA-3 was optimal in mesangial cells cultured in high glucose, and the interference efficiency was over 60%. Therefore, subsequent experiments will select this siRNA for proceeding. At the same time, qRT-PCR confirmed the expression of Rpph1 after transfection of Rpph1 overexpression plasmid in low glucose cultured mesangial cells, and its expression level was significantly increased ($P < 0.05$).

Rpph1 promotes the expression of inflammatory factors in mesangial cells under high glucose conditions

The effect of transfection of Rpph1 overexpression plasmid and siRNA on the expression of inflammation-related factors TNF-α and MCP-1 was detected by Western blot. The results showed that the protein expression levels of TNF-α and MCP-1 were significantly up-regulated after transfection of Rpph1 overexpression plasmid in low glucose cells ($P < 0.05$). However, after transfection of Rpph1 siRNA in high glucose cultured mesangial cells, the protein expression levels of TNF-α and MCP-1 were significantly decreased ($P < 0.05$, Figure 5). It indicates that Rpph1 has a regulatory effect on the expression of inflammatory factors in mesangial cells and participates in DN inflammation.

Discussion

In recent years, more and more studies have found that inflammation participates in DN. Many scholars have proposed microinflammation theory, which refers to the stimulation of some organisms, endotoxin, microorganisms, receptors, adipokines, chemokines, etc. Activation of the monocyte/macrophage system, a slow and sustained microinflammation of increased secretion of inflammation-related factors, which is thought to be a disease of low-grade inflammation and innate immunity, inflammation can cause kidney damage, to DN Progress has played a crucial role [5-6]. The inflammatory response plays an important role in the progression of DN, and the abnormal expression of inflammatory factors is a typical feature of DN inflammatory response. However, due to the numerous complex factors involved in the development of DN, the regulation of DN inflammatory factors has not been determined.

lncRNA has become a research hotspot in recent years and has participated in many complex regulatory networks. lncRNA has many functions and is widely involved in biological processes such as histone modification, DNA methylation and chromatin remodeling. It can directly interact with some RNA molecules and proteins, and regulate the expression of target genes at the transcriptional and post-transcriptional levels [7]. Abnormal expression of lncRNA is

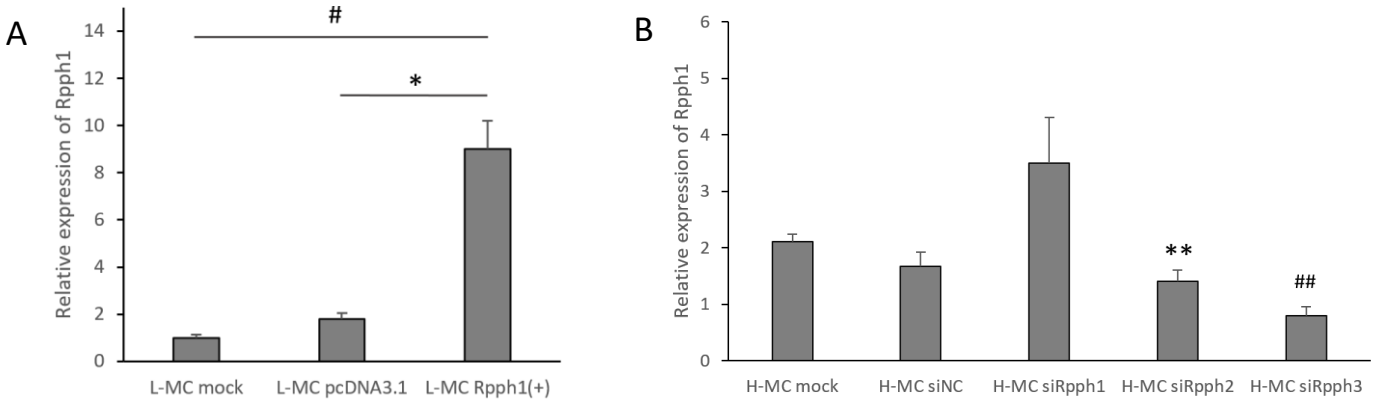

Figure 4. qRT-PCR detection of Rpph1 expression in mesangial cells after transfection of overexpressing plasmids and siRNAs. A: Rpph1 overexpression efficiency was detected in low glucose cultured mesangial cells (*P < 0.05 and $^{\#}$P < 0.05, compared with L-MC Rpph1 (+) group). B: High glucose cultured mesangial cells were tested for Rpph1-siRNA knockdown efficiency (**P < 0.05; compared with H-MC mock group; $^{\#\#}$P < 0.05, compared with H-MC siNC group).

involved in the development of a variety of diseases, including cardiovascular disease, neuropathy, cancer and metabolic diseases [8]. In addition, the abnormal expression of lncRNA plays a crucial role in the development of DN. It has been reported that lncRNA is involved in regulating renal response to hyperglycemia and promoting DN progression [9]. WANG et al [10] reported that lncRNA CYP4B1-PS1-001 was significantly down-regulated in vitro and in vivo in early diabetic, while overexpression of CYP4B1-PS1-001 inhibited mesangial cell proliferation and fibrosis. Not only that, lncRNA is also involved in the inflammatory process. CARPENTER et al [11] reported that lncRNA-Cox2 was identified as a TLR ligand-induced dynamic regulatory gene, which in turn promotes and inhibits the expression of inflammatory factors; some lncRNAs including THRIL [12], lnc13 [13] have been reported to regulate the myeloid system. Inflammatory gene expression of cells. It is suggested that lncRNA may be involved in DN inflammation, especially in the previous study, we found that lncRNA-Gm4419 is highly expressed in high glucose cultured mesangial cells and regulates DN inflammation through NF-κB signaling pathway [14]. Therefore, lncRNA may play an important role in the inflammation of diabetic nephropathy, and it is a potential novel molecular marker and therapeutic target for DN. However, the exact mechanism is still unclear.

To determine the intrinsic relationship between lncRNA and DN, lncRNAs expressing abnormalities in kidney tissues of DN mice were identified by RNA-seq, and the relevant lncRNAs were analyzed and verified. In this study, qRT-PCR was used to verify the expression of lncRNAs in kidney tissues of DN mice. Ribonuclease P RNA component H1 (Rpph1) was significantly higher in kidney tissues of DN mice than in normal control mice. The results also showed that the expression of Rpph1 in mesangial cells cultured in high and low glucose was consistent with DN and normal kidney tissues. All of the above suggest that lncRNA Rpph1 may be a factor involved in the occurrence and development of DN. Rpph1 is the RNA component of RNase ribonucleoprotein, located on mouse chromosome 14 (chr14: 50807447-50807771) and 325 bp in length. Recent studies have shown that ribonuclease is involved in the maturation of lncRNA MALAT1 [15]; Rpph1 is up-regulated in human gastric cancer tissues [16] and neocortex in patients with epilepsy [17]; ZHANG et al [18] study proved that Rpph1 knockdown It inhibited the proliferation of breast cancer cells and the occurrence of tumors, but no reports of Rpph1 and diabetic nephropathy have been reported so far. In this experiment, Rpph1 was found to have no protein coding ability by coding protein proteomic analysis software, and it was confirmed to be non-coding RNA again. However, the specific role of Rpph1 in DN requires further research.

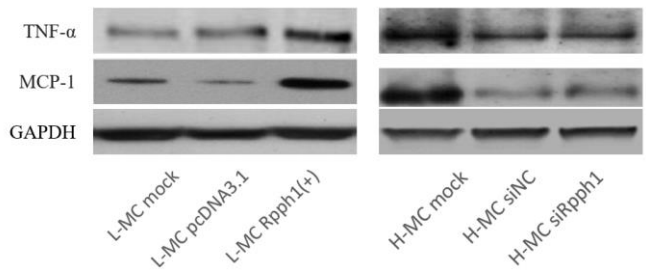

Figure 5. Effect of overexpression and knockdown of Rpph1 on the expression of TNF-α and MCP-1 in mesangial cells.

In order to explore the specific role of lncRNA-Rpph1 in DN, high glucose was used to simulate mesenchymal cells in diabetic state [19], and the distribution of Rpph1 in cells was detected by fluorescence in situ hybridization and real-time fluorescent quantitative PCR. The results suggest that Rpph1 is distributed in the nucleus and cytoplasm of mesangial cells, but mainly distributed in the cytoplasm. The distribution of lncRNA in cells is different, and its function is also different. If distributed in the cytoplasm, lncRNA mainly participates in gene regulation by inhibiting the binding of miRNA to target genes by binding to miRNAs, or by binding to specific proteins to form a complex; if distributed in the nucleus, it plays a role in affecting chromatin remodeling or transcriptional regulation [20]. Combined with the results of this study, it is suggested that the way Rpph1 works may be related to its main distribution in the cytoplasm, that is, competitive binding to miRNA or interaction with proteins to regulate gene expression. These two modes of action may be important mechanisms of Rpph1, which provides us with a direction for further research on Rpph1.

In addition, we successfully constructed Rpph1 overexpression plasmid and synthetic Rpph1 siRNA, transfected siRNA and overexpression plasmid in mesangial cells cultured in high glucose and low glucose, respectively, and detected the overexpression and knockdown efficiency of Rpph1 by qRT-PCR. Overexpression and silencing efficiency are good, which provides a good basis for the later experimental study of the function of Rpph1 in mesangial cells.

To investigate the role of Rpph1 in DN inflammation, we turned our attention to two important inflammation-related factors, TNF-α and MCP-1. The study found that TNF-α is mainly produced by macrophages, T cells, and monocytes, and can also be found in kidney-derived cells such as dendritic cells, mesangial cells, endothelial cells, and renal tubular cells. Secretion of synthetic pleiotropic inflammation-related factors [21]; TNF-α is involved in the recruitment of monocyte-macrophages and induces local inflammation by initiating a series of cytokines and increasing vascular permeability, and TNF-α on kidney cells Cytotoxicity, which can directly induce kidney injury, is an important factor causing inflammation of diabetic nephropathy [22]; CHOW et al [23] proved the pathogenic role of MCP-1 in diabetic nephropathy, knocking out MCP in diabetic model mice After -1, its kidney damage was alleviated. In diabetic nephropathy, MCP-1 is up-regulated, MCP-1 can promote the migration and activation of monocyte-macrophage, and regulate the expression of other inflammatory factors and adhesion factors, thereby promoting the development of inflammation of diabetic nephropathy [24-26]. In this study, Rpph1 was overexpressed in low glucose cultured mesangial cells and Rpph1 was silenced in high glucose cultured mesangial cells. Western blot was used to detect the expression of TNF-α and MCP-1. The results showed that the expression levels of TNF-α and MCP-1 were significantly increased after overexpression of Rpph1. Conversely, the expression of TNF-α and MCP-1 was significantly decreased after down-regulation of Rpph1 ($P < 0.01$). These results suggest that Rpph1 may participate in the occurrence and development of DN inflammation by promoting the expression of inflammatory factors TNF-α and MCP-1.

In summary, this study found that the abnormally high expression of lncRNA Rpph1 in DN mouse kidney tissue and high glucose cultured mesangial cells, and high and low expression of Rpph1 can regulate mesangial cell inflammatory factors TNF-α and MCP-1 Expression, thus participating in the occurrence and development of DN inflammation. Therefore, the role of Rpph1 in DN can provide a new direction for the prevention and treatment of DN. However, the effect of lncRNA on DN is an extremely complicated process. The specific mechanism of Rpph1 in DN inflammation needs further study.

Conflicting interests

The authors have declared that no conflict of interests exist.

References

1. Reindl J, Gröne HJ, Wolf G, et al. Uromodulin-related autosomal-dominant tubulointerstitial kidney disease-pathogenetic insights based on a case. Clin Kidney. 2018;12(2):172-179.
2. Mousum SA, Ahmed S, Gawali B, et al. Nyctanthes arbor-tristis leaf extract ameliorates hyperlipidemia- and hyperglycemia-associated nephrotoxicity by improving anti-oxidant and anti-inflammatory status in high-fat diet-streptozotocin-induced diabetic rats. Inflammopharmacology. 2018;26(6):1415-1428.
3. Helmy MW, Helmy MM, El-Mas MM.Enhanced lipoxygenase/LTD4 signaling accounts for the exaggerated hypertensive and nephrotoxic effects of cyclosporine plus indomethacin in rats.Biomed Pharmacother. 2018;102:309-316.
4. Pan J, Huang C, Chen G, et al. MicroRNA-451 blockade promotes osteoblastic differentiation and skeletal anabolic effects by promoting YWHAZ-mediated RUNX2 protein stabilization. Medchemcomm. 2018;9(8):1359-1368.
5. Reyes-Umpierrez D, Davis G, Cardona S, et al. Inflammation and Oxidative Stress in Cardiac Surgery Patients Treated to Intensive Versus Conservative Glucose Targets. J Clin Endocrinol Metab. 2017;102(1):309-315.
6. Said E, Zaitone SA, Eldosoky M, et al. Nifuroxazide, a STAT3 inhibitor, mitigates inflammatory burden and protects against diabetes-induced nephropathy in rats. Chem Biol Interact. 2018;281:111-120.
7. Sisino G, Zhou AX, Dahr N, et al. Long noncoding RNAs are dynamically regulated during β-cell mass expansion in mouse pregnancy and control β-cell proliferation in vitro. PLoS One. 2017;12(8):e0182371.

8. Lu YW, Wang DZ.Non-coding RNA in Ischemic and Non-ischemic Cardiomyopathy.Curr Cardiol Rep. 2018;20(11):115.
9. Dewanjee S, Bhattacharjee N. MicroRNA: A new generation therapeutic target in diabetic nephropathy. Biochem Pharmacol. 2018;155:32-47.
10. Leti F, Morrison E, DiStefano JK. Long noncoding RNAs in the pathogenesis of diabetic kidney disease: implications for novel therapeutic strategies. Per Med. 2017;14(3):271-278.
11. Zhou Q, Zhou L, Qian J, et al. NKILA inhibition protects retinal pigment epithelium cells from hypoxia by facilitating NFκB activation. Biochem Biophys Res Commun. 2018;503(4):3134-3141.
12. Olazagoitia-Garmendia A, Santin I, Castellanos-Rubio A. Functional implication of celiac disease associated lncRNAs in disease pathogenesis. Comput Biol Med. 2018;102:369-375.
13. Chan J, Atianand M, Jiang Z, et al. Cutting Edge: A Natural Antisense Transcript, AS-IL1α, Controls Inducible Transcription of the Proinflammatory Cytokine IL-1α. J Immunol. 2015;195(4):1359-63.
14. Yi H, Peng R, Zhang LY, et al. LincRNA-Gm4419 knockdown ameliorates NF-κB/NLRP3 inflammasome-mediated inflammation in diabetic nephropathy. Cell Death Dis. 2017;8(2):e2583.
15. Sathishkumar C, Prabu P, Mohan V, et al. Linking a role of lncRNAs (long non-coding RNAs) with insulin resistance, accelerated senescence, and inflammation in patients with type 2 diabetes. Hum Genomics. 2018;12(1):41.
16. Milione M. Prognostic factors for gastroenteropancreatic neuroendocrine neoplasms (GEP-NENs): what's better? Endocrine. 2018;59(1):1-3.
17. Begni V, Riva MA, Cattaneo A. Cellular and molecular mechanisms of the brain-derived neurotrophic factor in physiological and pathological conditions. Clin Sci (Lond). 2017;131(2):123-138.
18. Zhang Y, Tang L. Inhibition of breast cancer cell proliferation and tumorigenesis by long non-coding RNA RPPH1 down-regulation of miR-122 expression. Cancer Cell Int. 2017;17:109.
19. Tian S, Liu W, Pan Y, et al. Long non-coding RNA Linc00320 inhibits glioma cell proliferation through restraining Wnt/β-catenin signaling. Biochem Biophys Res Commun. 2019;508(2):458-464.
20. Liu J, Li Y, Tong J, et al. Long non-coding RNA-dependent mechanism to regulate heme biosynthesis and erythrocyte development. Nat Commun. 2018;9(1):4386.
21. Dong X, Swaminathan S, Bachman LA, et al. Resident dendritic cells are the predominant TNF-secreting cell in early renal ischemia-reperfusion injury. Kidney Int. 2007;71(7):619-28.
22. Borgohain MP, Lahkar M, Ahmed S, et al. Small Molecule Inhibiting Nuclear Factor-kB Ameliorates Oxidative Stress and Suppresses Renal Inflammation in Early Stage of Alloxan-Induced Diabetic Nephropathy in Rat. Basic Clin Pharmacol Toxicol. 2017;120(5):442-449.
23. Uchiyama A, Yamada K, Perera B, et al. Protective effect of MFG-E8 after cutaneous ischemia-reperfusion injury. J Invest Dermatol. 2015;135(4):1157-1165.
24. Casper J, Schmitz J, Bräsen JH, et al. Renal transplant recipients receiving loop diuretic therapy have increased urinary tract infection rate and altered medullary macrophage polarization marker expression. Kidney Int. 2018;94(5):993-1001.
25. Tsutsui H, Shimokawa T, Miura T, et al. Inhibition of α2C-adrenoceptors ameliorates cisplatin-induced acute renal failure in rats. Eur J Pharmacol. 2018;838:113-119.
26. Toda N, Mukoyama M, Yanagita M, et al. CTGF in kidney fibrosis and glomerulonephritis. Inflamm Regen. 2018;38:14.