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ORIGINAL ARTICLE

Serum LncRNA PANDAR may Act as a Novel Serum Biomarker of Diabetic Nephropathy in Patients with Type 2 Diabetes

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SUMMARY

Background: **The current study aims to investigate the expression and significance of lncRNA PANDAR in the serum of patients with type II diabetes mellitus (T2DM) and diabetic nephropathy (DN).**
Methods: **The expression of PANDAR in 77 T2DM patients, 60 DN patients, and 60 healthy controls was detected by RT-PCR. Pearson's correlation assay was carried out to analyze the correlation between serum lncRNA PANDAR and clinical indicators. Receiver operator characteristic (ROC) analysis was carried out to analyze the diagnostic value of PANDAR in T2DM and DN patients.**
Results: **The expression of PANDAR in T2DM and DN patients was significantly higher than that in the control group. Moreover, the expression of PANDAR in DN patients with massive proteinuria was significantly higher than that in DN patients with microalbuminuria. Further study showed that the expression of PANDAR was positively correlated with the level of proteinuria ($r = 0.690$, $p < 0.001$), and negatively correlated with the glomerular filtration rate ($r = -0.780$, $p < 0.001$). In T2DM and DN patients, the area under ROC curve (AUC) of PANDAR as serum marker was 0.861 (95% CI: 0.786 - 0.935, $p < 0.001$), between DN patients and T2DM patients, while the AUC of PANDAR as a marker in diabetic nephropathy was 0.914 (95% CI: 0.828 - 0.980, $p < 0.001$) between DN patients and healthy controls.**
Conclusions: **In summary, the high expression of PANDAR is related to the development of DN in T2DM patients, and it is expected to be a biomarker for predicting the prognosis of DN patients.**
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KEY WORDS

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INTRODUCTION

Diabetic nephropathy (DN), as end-stage renal disease, is one of the main causes of death in diabetic patients [1]. Early diagnosis is helpful to carry out targeted preventive treatment for high-risk patients, so as to delay the progress of the disease and improve the quality of life of patients [2]. Urinary albumin/creatinine (UACR) or urinary albumin excretion rate (UAER) are widely accepted as early diagnostic indicators of DN, but the efficacy of albuminuria as a diagnostic marker of DN is limited by many factors [3]. Therefore, it is urgent to

find a biomarker with higher sensitivity and specificity than albuminuria for early prediction and diagnosis of DN [4].

As a kind of functional RNA with more than 200 nucleotides in length, long-chain non coding RNA (LncRNA) can regulate gene expression at multiple levels [5]. It is found that abnormal expression of lncRNA plays an important role in DN and other diseases. For instance, lncRNA TCF7 is shown to trigger endoplasmic reticulum stress via interacting with miR-200c in DN patients [6]. In addition, lncRNA Rpph1 is shown to enhance the inflammation and proliferation of mesangial cells in DN patients via regulating the expression of Gal-3 [7], even though the function and mechanism of lncRNA in the process of DN is largely unclear.

The noncoding RNA PANDAR (promoter of CDKN1A antisense DNA damage activated RNA) is located about 4.5 kb upstream of the CDKN1A (p21) transcription starting site on chromosome 6 [8,9]. In the case of DNA damage, it is involved in p53 dependent pathway mediated antiapoptotic effect [10]. However, the patterns and biological function of lncRNA PANDAR in DN patients is still unclear. The purpose of this study is to explore the role and significance of lncRNA PANDA in DN patients, thereby evaluating its potential as a new biological marker for the treatment and prognosis of DN.

MATERIALS AND METHODS

Patient samples

From January 2017 to December 2018, 137 patients with diabetes, including 77 patients with T2DM (no history of DN, no albuminuria) and 60 patients with T2DM and DN (30 microalbuminuria, 30 macroalbuminuria) were collected in The First Hospital Affiliated to Nanjing University of Chinese Medicine. In contrast, 60 healthy persons without diabetes who were diagnosed in our physical examination center were collected as the control group. The peripheral blood was collected from all subjects and the details were shown in Table 1. The collected blood samples were placed at 4°C for 30 minutes, then centrifuged at 3,000 rpm at room temperature for 15 minutes. The upper serum was retained and placed in the refrigerator at - 80°C for standby. The study was approved by the ethics committee of Nanjing University of Chinese Medicine.

Real-time PCR

Total RNA was isolated from the whole blood samples (5 mL, collected in tubes containing EDTA) using RNAVzol LS (Vigorous Biotechnology Beijing Co., Ltd, Beijing, China) according to the manufacturer's protocol. The concentration and the purity of RNA samples were determined by measuring the optical density (OD) 260/280. RNA was reverse-transcribed into cDNA using TaqMan™ MicroRNA Reverse Transcription Kit (Thermo Fisher Scientific, Inc., Waltham, MA, USA). qPCR was performed using 2 x SYBR Green PCR Master Mix (Tarkara, Dalian City, China) according to the instructions. The primers used in the present study were listed as follows: PANDAR upstream primer: 5'-CTGTTAAGGTGGCGCATTG-3', PANDAR downstream primer 5'-GGGGGCTCATACTGGCTT GAT-3', GAPDH upstream primer 5'-CGCTCTCTC CCCCCTGTTC-3', GAPDH downstream primer 5'-ATCCGTGCACTCCGACCTTCAC-3'.

PCR reaction conditions: 95°C for 10 minutes; (95°C for 15 seconds, 60°C for 30 seconds, 72°C for 30 seconds) x 40 cycles. The expression level of lncRNA PANDAR was determined with the $2^{-\Delta\Delta Cq}$ method [11].

Statistical analysis

SPSS 17.0 statistical software was used for data analysis. The data are represented as the mean ± standard deviation (SD). The two-tailed unpaired Student's *t*-tests were used for comparisons of two groups. The one-way ANOVA multiple comparison test (SPSS 20.0) followed by Tukey's post hoc test were used for comparisons of two more groups. ROC analysis was used to evaluate the potential of lncRNA PANDAR as a biomarker of DN. Pearson's correlation assay was used to analyze the correlation between lncRNA PANDAR and proteinuria and estimated glomerular filtration rate (eGFR). $p < 0.05$ was statistically significant.

RESULTS

Comparison of general data

There was no significant difference in age, gender, blood pressure, course of T2DM, and BMI among the three groups. Triglyceride (TG) in the DN group was significantly higher than that in T2DM group and control group ($p < 0.05$), but there was no significant difference in TG between T2DM group and control group ($p > 0.05$). Besides, proteinuria and serum creatinine of the DN group was significantly higher than that of T2DM and control group. The eGFR of the DN group was significantly lower than that of T2DM and control group ($p < 0.05$). The glycosylated hemoglobin (HbA1c) of the T2DM group and DN group was significantly higher than that of the control group ($p < 0.05$).

Expression of lncRNA PANDAR in T2DM, DN patients, and healthy controls

qRT-PCR was used to detect the differential expression of lncRNA PANDAR in the T2DM, DN, and healthy control groups. The results showed that compared with the healthy control group, the expression of lncRNA PANDAR in T2DM and DN patients was significantly increased, with a statistically significant difference (Figure 1). Further analysis showed that the expression of lncRNA PANDAR in the DN group was much higher than that in T2DM patients, with a significant difference (Figure 1).

Table 1. Baseline characteristics of subjects.

	T2DM (n = 77)	DN (n = 60)	Control (n = 60)
Gender (M/F)	39/38	29/31	28/32
Age (years)	60.2 ± 10.0	64.0 ± 7.4	62.8 ± 6.1
Blood pressure (mmHg)	86.0 ± 12.0	96.2 ± 10.0	85.3 ± 5.2
TG (mmol/L)	1.5 ± 0.7	2.8 ± 0.4 $^{\#}$	1.2 ± 0.5
Fasting blood glucose (mmol/L)	8.5 ± 1.7 *	8.9 ± 1.3 *	4.5 ± 0.9
Course of T2DM (years)	15.5 ± 5.0	18.0 ± 6.7	
BMI (kg/m^2)	25.7 ± 4.8	26.9 ± 5.5	24.9 ± 3.6
Proteinuria (mg/24 hour)	6.7 ± 3.4	1,236.7 ± 49.0 $^{\#\#\#}$	3.8 ± 1.8
Serum creatinine (mmol/L)	65.7 ± 9.0	120.8 ± 35.0 $^{\#}$	50.8 ± 11.4
eGFR [mL/(minute x 1.73 m^2)]	95.7 ± 9.0	65.0 ± 35.0 $^{\#}$	50.8 ± 11.4
HbA1c (%)	8.7 ± 3.0 *	9.4 ± 2.2 *	4.1 ± 0.6

* - p < 0.05 vs. control, $^{\#}$ - p < 0.05, $^{\#\#\#}$ - p < 0.001 vs. T2DM.

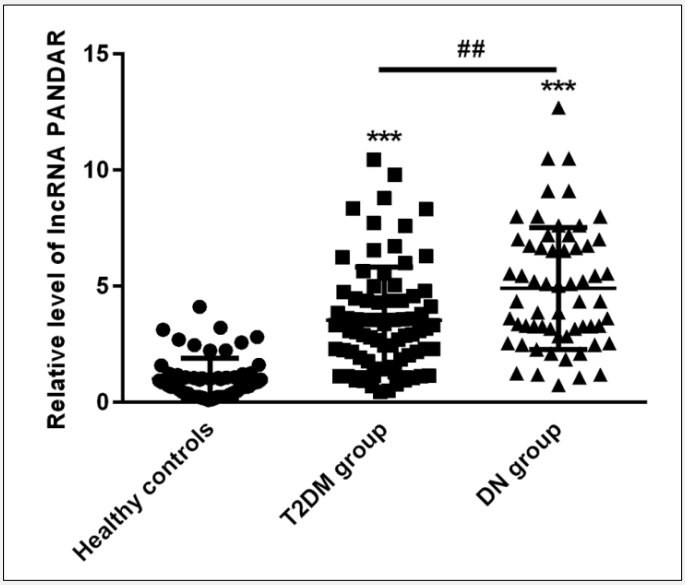

Figure 1. RT-PCR analysis showed that the expression of lncRNA PANDAR was gradually increased in DN, T2DM patients, and healthy controls.

*** - p < 0.001 vs. healthy controls, $^{\#\#}$ - p < 0.01 vs. T2DM patients.

Expression of lncRNA PANDAR in different amounts of proteinuria

Further detection of the expression of lncRNA PANDAR in patients with different degrees of proteinuria showed that the expression of lncRNA PANDAR in the serum of patients with DN combined with macroalbuminuria was significantly highest (Figure 2). In comparison, the level of lncRNA PANDAR was higher in patients with microalbuminuria than that of T2DM patients with normal albuminuria (Figure 2).

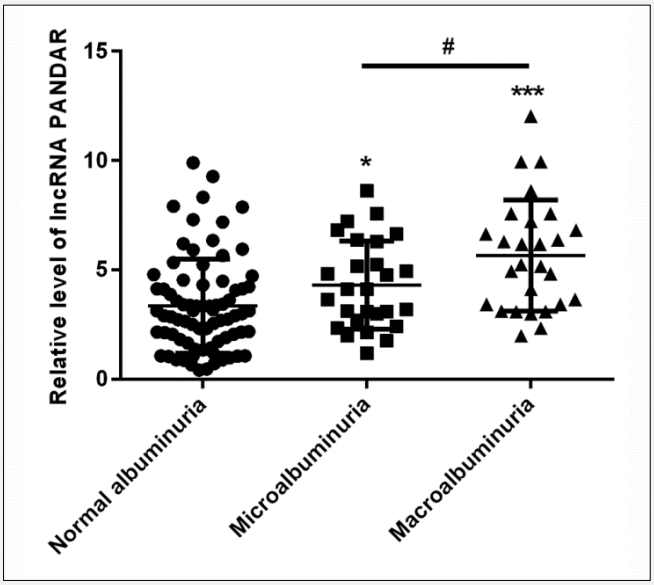

Figure 2. RT-PCR analysis showed that the level of lncRNA PANDAR in the serum of patients with DN combined with macroalbuminuria was significantly higher than that of patients with DN combined with microalbuminuria and T2DM patients with normal albuminuria.

*** - $p < 0.001$ vs. healthy controls, $^{\#}$ - $p < 0.05$ vs. T2DM patients.

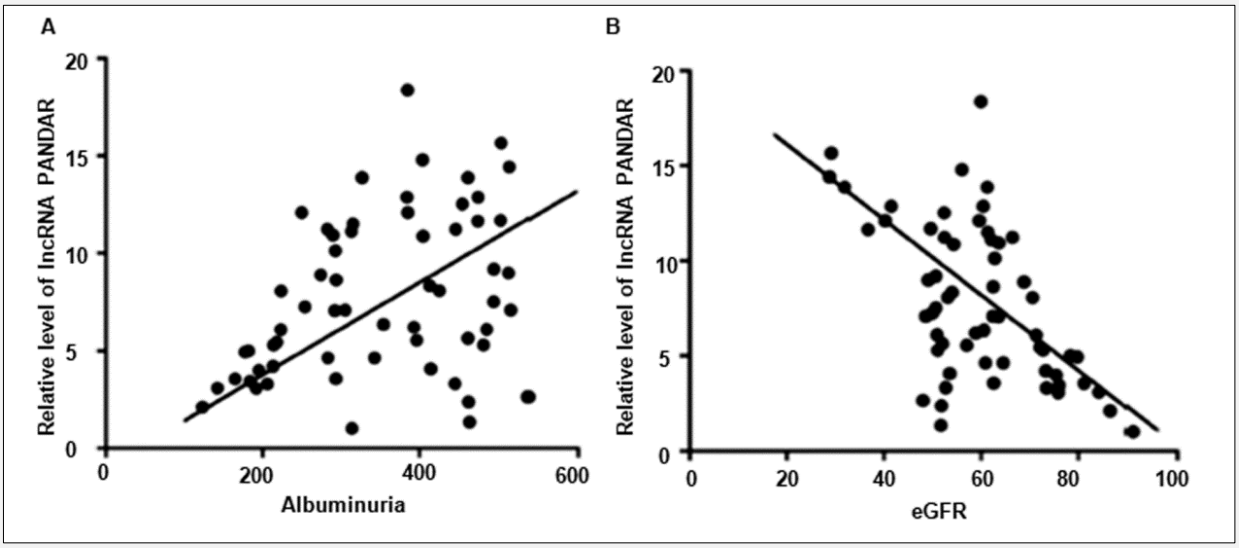

Figure 3. Pearson's correlation assay was carried out to analyze the correlation between the expression of serum lncRNA PANDAR and proteinuria and eGFR in DN patients.

The expression of serum lncRNA PANDAR was positively correlated with the expression of proteinuria (A) and negatively correlated with eGFR (B).

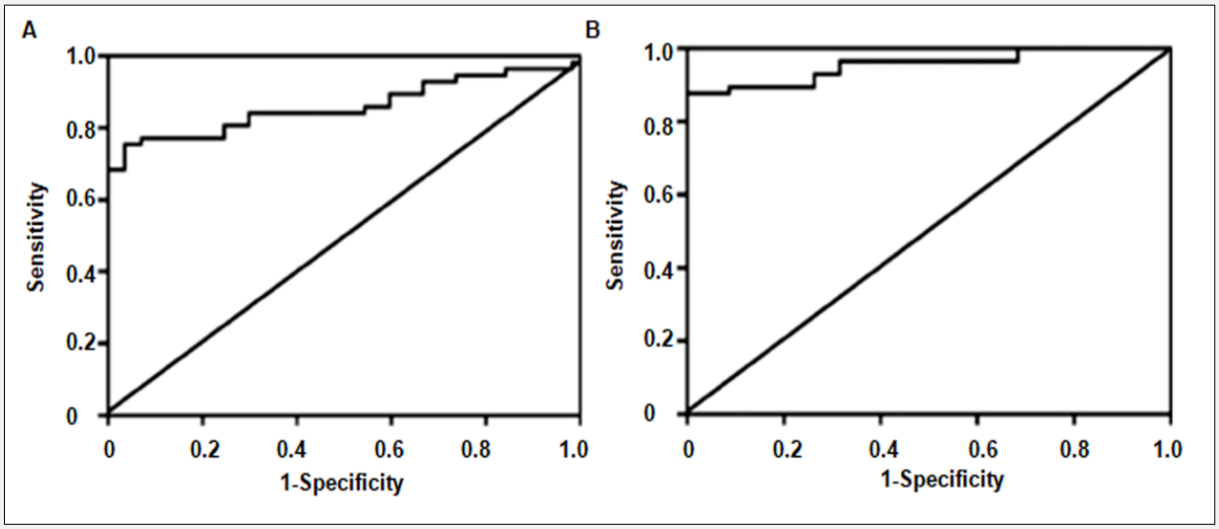

Figure 4. ROC analysis was carried out to analyze the potential of lncRNA PANDAR as a serum marker between DN patients and T2DM patients.

(A) The results showed that AUC was 0.861 (95% CI: 0.786 - 0.935, p < 0.001) between DN patients and T2DM patients. (B) The AUC of lncRNA PANDAR as serum marker between DN and healthy control group was 0.914 (95% CI: 0.828 - 0.980, p < 0.001).

Correlation between the expression of lncRNA PANDAR and proteinuria and glomerular filtration rate in DN patients

Pearson's correlation coefficient was used to analyze the correlation between the expression of serum lncRNA PANDAR and proteinuria and eGFR in DN patients. The results showed that the expression of serum lncRNA PANDAR was positively correlated with the expression of proteinuria ($r = 0.690$, $p < 0.001$, Figure 3A) and negatively correlated with eGFR ($r = -0.780$, $p < 0.001$, Figure 3B).

Diagnostic value of serum lncRNA PANDAR in DN patients

ROC analysis was carried out to analyze the potential of lncRNA PANDAR as a serum marker between DN patients and T2DM patients. The results showed that the AUC was 0.861 (95% CI: 0.786 - 0.935, $p < 0.001$, Figure 4A) between T2DM and DN patients. When the cutoff value was 2.11, the sensitivity and specificity were 87.5% and 98.7%, respectively. Furthermore, the AUC of lncRNA PANDAR as serum marker between the DN and healthy control group was 0.914 (95% CI: 0.828 - 0.980, $p < 0.001$, Figure 4B). When the cutoff value was 3.02, the sensitivity and specificity were 84.5% and 100%, respectively.

DISCUSSION

DN is the most common chronic microvascular complication in diabetic patients, and it is also the most common cause of end-stage renal disease in diabetic patients [12]. Therefore, the early diagnosis and treatment of DN is of great significance [1,13]. lncRNA is a kind of noncoding RNA with about 200 nt in length, and is easy to detect and not easily degraded by RNase [6]. Increasing evidence has shown that the differential expression of lncRNA has complex effects on the injury of islet cells and the occurrence of diabetes [14,15]. More importantly, the stable presence of lncRNA in the circulating system makes it a promising diagnostic marker and potential drug treatment target [16].

Here, we showed for the first time that compared with the control group, the expression of serum lncRNA PANDAR in T2DM and DN patients was significantly higher than that in the control group. Moreover, the expression of serum lncRNA PANDAR in DN patients was higher than that in the diabetic group and that in the normal control group. With the increase of proteinuria level, the expression of serum lncRNA PANDAR was gradually enhanced. Moreover, the expression of PANDAR was positively correlated with the level of proteinuria and negatively correlated with the glomerular filtration rate. It is suggested that the overexpression of serum lncRNA PANDAR in diabetic patients may lead to the increase of protein in urine and the de-

crease of serum albumin and renal function.

This is the reason why lncRNA PANDAR results in renal function impairment deserves further study. In the early stage of DN, the accumulation of terminal oxidation protein products can induce the increase of superoxide, caspase 3 activity, p53, and Bax, which finally result in the increase of podocyte apoptosis and urinary protein excretion [17,18]. Previous study has shown that PANDA stabilizes p53 protein in response to DNA damage and further induces cell apoptosis [19]. Hence, we propose that enhanced lncRNA PANDAR may exacerbate renal function via interacting with p53 protein and participating in podocyte apoptosis.

The AUC of PANDAR as a serum marker was 0.861 in T2DM and DN patients, and 0.914 in DN and healthy controls. When the serum lncRNA PANDAR reaches 2.11 (cutoff value), the sensitivity and specificity of the diagnosis of diabetes are 87.5% and 98.7%, while when the serum lncRNA PANDAR reaches 3.02, the specificity of the diagnosis of DN is 100%, suggesting that the expression of serum lncRNA PANDAR can be used as a non-invasive diagnostic marker of DN in T2DM patients.

There are some limitations in the current study. First, there is limited sample size. Secondly, we did not perform renal biopsies to confirm the degree of damage.

CONCLUSION

In summary, the high expression of lncRNA PANDAR is related to the development of DN in T2DM patients, and it may be a biomarker to predict the prognosis of DN in patients.

Declaration of Interest:

We declare no conflicts of interest.

References:

1. Kato M, Wang M, Chen Z, et al. An endoplasmic reticulum stress-regulated lncRNA hosting a microRNA megacluster induces early features of diabetic nephropathy. Nat Commun 2016; 7:12864 (PMID: 27686049).

2. Allison SJ. Diabetic nephropathy: A lncRNA and miRNA megacluster in diabetic nephropathy. Nat Rev Nephrol 2016;12:713 (PMID: 27748387).

3. Chen W, Peng R, Sun Y, et al. The topological key lncRNA H2k2 from the ceRNA network promotes mesangial cell proliferation in diabetic nephropathy via the miR-449a/b/Trim11/Mek signaling pathway. FASEB J 2019;33:11492-506 (PMID: 31336 052).

4. Gao J, Wang W, Wang F, Guo C. LncRNA-NR_033515 promotes proliferation, fibrogenesis and epithelial-to-mesenchymal transition by targeting miR-743b-5p in diabetic nephropathy. Biomed Pharmacother 2018;106:543-52 (PMID: 29990842).

5. Ge Y, Wang J, Wu D, et al. lncRNA NR_038323 Suppresses Renal Fibrosis in Diabetic Nephropathy by Targeting the miR-324-3p/DUSP1 Axis. Mol Ther Nucleic Acids 2019;17:741-53 (PMID: 31430717).

6. Liu H, Sun HL. LncRNA TCF7 triggered endoplasmic reticulum stress through a sponge action with miR-200c in patients with diabetic nephropathy. Eur Rev Med Pharmacol Sci 2019;23:5912-22 (PMID: 31298342).

7. Zhang P, Sun Y, Peng R, et al. Long non-coding RNA Rpph1 promotes inflammation and proliferation of mesangial cells in diabetic nephropathy via an interaction with Gal-3. Cell Death Dis 2019;10:526 (PMID: 31285427).

8. Tian L, Chen X, Lun L, et al. LncRNA PANDAR is a Novel Prognostic Biomarker in Patients with Cancer: a Meta-Analysis. Clin Lab 2019;65 (PMID: 30775883).

9. Yang L, Zhou JD, Zhang TJ, et al. Overexpression of lncRNA PANDAR predicts adverse prognosis in acute myeloid leukemia. Cancer Manag Res 2018;10:4999-5007 (PMID: 30464600).

10. Sang Y, Tang J, Li S, et al. LncRNA PANDAR regulates the G1/S transition of breast cancer cells by suppressing p16(INK4A) expression. Sci Rep 2016;6:22366 (PMID: 26927017).

11. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C (T)) Method. Methods 2001;25:402-8 (PMID: 11846609).

12. Hu M, Wang R, Li X, et al. LncRNA MALAT1 is dysregulated in diabetic nephropathy and involved in high glucose-induced podocyte injury via its interplay with beta-catenin. J Cell Mol Med 2017;21:2732-47 (PMID: 28444861).

13. Lei X, Zhang L, Li Z, Ren J. Astragaloside IV/lncRNA-TUG1/TRAF5 signaling pathway participates in podocyte apoptosis of diabetic nephropathy rats. Drug Des Devel Ther 2018;12:2785-93 (PMID: 30233141).

14. Wang J, Pan J, Li H, et al. lncRNA ZEB1-AS1 Was Suppressed by p53 for Renal Fibrosis in Diabetic Nephropathy. Mol Ther Nucleic Acids 2018;12:741-50 (PMID: 30121551).

15. Wang X, Xu Y, Zhu YC, et al. LncRNA NEAT1 promotes extracellular matrix accumulation and epithelial-to-mesenchymal transition by targeting miR-27b-3p and ZEB1 in diabetic nephropathy. J Cell Physiol 2019;234:12926-33 (PMID: 30549040).

16. Yang J, Li L, Hong S, Zhou Z, Fan W. LINK-A lncRNA activates HIF1alpha signaling and inhibits podocyte cell apoptosis in diabetic nephropathy. Exp Ther Med 2019;18:119-24 (PMID: 31 258644).

17. Ming L, Ning J, Ge Y, Zhang Y, Ruan Z. Excessive apoptosis of podocytes caused by dysregulation of microRNA-182-5p and CD2AP confers to an increased risk of diabetic nephropathy. J Cell Biochem 2019;120:16516-23 (PMID: 31131477).

18. Wang Y, He Z, Yang Q, Zhou G. XBP1 inhibits mesangial cell apoptosis in response to oxidative stress via the PTEN/AKT pathway in diabetic nephropathy. FEBS Open Bio 2019;9:1249-58 (PMID: 31077568).

19. Kotake Y, Kitagawa K, Ohhata T, et al. Long Non-coding RNA, PANDA, Contributes to the Stabilization of p53 Tumor Suppressor Protein. Anticancer Res 2016;36:1605-11 (PMID: 27069137).