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Short Communication

TGF-β Mediates the Role of P53 and microRNA-Related Signaling Pathways in the Pathogenesis of Diabetic Nephropathy in Rats

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Authors' Contribution
YM and JM contributed equally to this work. They participated in conceiving the design of the study and collecting and reviewing the data and coordination of project. QZ and HZ participated in doing literature review, collecting the data and analysis and in preparing the manuscript. SM helped in critical revision and finalizing the manuscript. All authors read, revised, and approved the final manuscript.

Key words
TGF-β, P53, microRNA-related signaling pathways, Diabetic nephropathy

ABSTRACT

The objective of this study was to study the relationship between TGF-β-mediated P53 and microRNA-related signaling pathways and the occurrence and development of diabetic nephropathy. The sample of rats was randomly assigned to a control group and an experimental group, and a rat model of diabetic nephropathy was established. After the establishment, HE staining, immunohistochemical staining and real-time quantitative PCR were performed to compare the groups. The situation after the successful model creation; observe the two groups of TGF-β1, P53, microRNA103 and microRNA105 mRNA expression determination and TGF-β1 and P53 protein expression levels. The success rate of the experimental rat model was 100%; the expression levels of TGF-β1, P53, and microRNA105 mRNA in the experimental group were significantly higher than those in the control group, and the difference was statistically significant ($P<0.05$), but the microRNA103 content of the groups was compared. The difference was not statistically significant ($P>0.05$); after modeling, the expression levels of TGF-β1 and P53 protein in the experimental group were significantly higher than those in the control group ($P<0.05$). It was concluded that TGF-β-mediated P53 and microRNA-related signaling pathways can coordinate with each other and jointly promote the occurrence and development of diabetic nephropathy.

Diabetic nephropathy (DN) occurs due to failure to treat diabetes effectively and timely (Dong *et al*., 2022). In severe cases, it progresses to end-stage renal disease (ESRD), which is a major risk factor for the eventual progression to organ damage and death in diabetic patients (Zhang, 2018). DN is the main cause of renal failure. It has no clinical manifestations in the early stage, which leads to the development of ESRD disease in some patients at the time of diagnosis. When irreversible lesions occur in the kidney, it can seriously affect the quality of patients' daily life (Xu *et al*., 2019). The most clinically important comorbidity that threatens the lives of diabetic patients is T2DN, with a prevalence of about 20-40%. The T2DN population is majority abdominal obese, but there are fewer studies on abdominal obesity and T2DN (Liu *et al*., 2020). The pathogenesis of T2DN is not yet clear, and the main clinical approach is to reduce the damage to the kidney by lowering blood glucose (Umanath and Lewis, 2018). Therefore, it is beneficial to study the pathogenesis of T2DN to provide new research ideas for clinical practice. In this paper, SD rats were selected as experimental subjects to analyze the effect of TGF-β-mediated P53 and microRNA-related signaling pathways on T2DN patients as well as the related mechanisms. It is the purpose of our study to study the role of P53 and microRNA-related signaling pathways for diabetic rats. Furthermore, we included TGF-β as a mediating variable. We used a rat model of diabetic nephropathy to study this effect.

Materials and methods

Forty Sprague-Dawley (SD) male rats, clean grade, 250-300 g, 8 weeks old, were selected and provided by

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the Department of Laboratory Animal Science, Faculty of Medicine, Peking University (animal license number: SCXK (Beijing) 2011-0012). Before the experiment, the rats were housed in a quiet environment with good ventilation, appropriate temperature, proper light, and free access to water and food. All experimental steps performed on rats during the whole experiment were in compliance with the relevant regulations for animal experimentation management (Umanath and Lewis, 2018). All rats were kept under the same environment for 7 d and then randomly divided into control and experimental groups, with 10 rats in the control group and 30 rats in the experimental group.

All rats were modeled at the beginning of the study. Streptozotocin (2%) was injected at a dose of 40 mg/kg, by tail vein injection. Control rats were injected with sodium citrate buffer at a specific dose of 40 mg/kg using tail vein injection.

Tail vein blood (2ml) was collected from both groups 3 d and 7 d after the completion of drug injection while they were fasting serum immediately after centrifugation was taken for the blood glucose levels. If the blood glucose level of the rats exceeded 216.7 mmol/L on both occasions, both rat models were proved to be successful.

For histological and immunohistochemical staining for the kidneys were fixed for paraffin embedding, and then 4 μm thick section were cut and stained for H.E according to the routine histological protocol. For immunohistochemical staining the paraffin sections were dewaxed twice using xylene and dehydration using gradient alcohol according to the established method.

For real-time quantitative PCR assay, the preserved kidney tissues were removed and thawed. When the grinding was more adequate, the tissue was removed and transferred to a clean centrifuge tube, shaken gently to mix, placed at room temperature for 5 min, and then 0.2 ml of chloroform was put into it, shaken vigorously and placed back at room temperature for the same time. Finally, the obtained RNA was transferred into a clean PCR tube and 1ul of RNA was extracted to determine the concentration of nucleic acids using a UV spectrophotometer.

The PCR reaction system was 20μl with different components and the PCR reactions were cycled at different temperatures. After the reaction, the final product was stored in a refrigerator at 4°C. *HphI* cleavage (CYP2D6*10) and *Bcg I* cleavage (β1-AR) of PCR products in a 20μl reaction system was digested in a water bath at 37°C for 1 hour and stored at 4°C.

All data were analyzed with statistical package for social sciences (SPSS 18.0). The count data were expressed as the number and frequencies, and t as well as chi-square tests were performed respectively. $p<0.05$ was considered a statistically significant difference.

Results

The success rate of the rat model in this study was 100%. The rats in the experimental group ate and watered a lot, urinated frequently and had frequent diarrhea after the end of the modeling. In addition, the rats' hair was dry and yellowish. Moreover, their body weight decreased gradually with time after modeling, their activity level reduced progressively, and their blood glucose level remained high. In the control group, the rats' hair condition was normal. The blood glucose level and body weight were within the normal range. The mental condition was good, and the feeding and drinking conditions were consistent with those before the modeling.

After the completion of modeling, the expression levels of TGF-β1, P53 and microRNA105 were significantly higher in the experimental group than in the control group ($P<0.05$), while there was no significant difference in microRNA103 content between the two groups (Table I). The expression content of TGF- β1 and P53 protein in the experimental group was significantly higher than that in the control group, and the difference was statistically significant (Table I).

Table I. Comparison of the content of various indexes and protein expression levels in the kidney tissue of study groups.

Indexes	Control group (n=10)	Experimental group (n=30)	t value	P value
Content of various indices				
TGF- β1 ($\times10^{-3}$)	19.53±5.24	53.86±10.52	0.326	0.013
P53 ($\times10^{-3}$)	10.93±4.21	58.63±9.52	0.521	0.025
microRNA103	2.52±0.18	3.04±1.01	5.624	0.635
microRNA105	4.23±1.02	6.52±1.14	0.144	0.014
Protein expression levels				
TGF- β1 ($\times10^{-3}$)	21.36±3.52	41.53±6.54	0.584	0.021
P53 ($\times10^{-3}$)	12.53±3.21	38.69±5.92	0.469	0.019

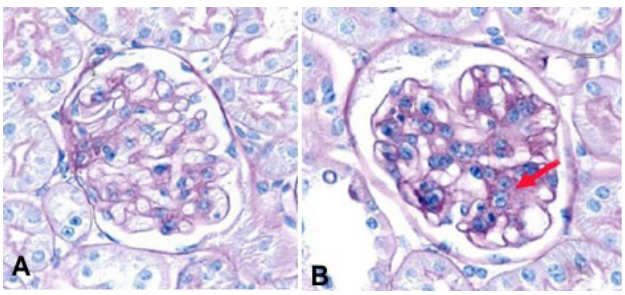

Fig. 1. Kidney histomorphology of rats in control (A) and experimental (B) groups (×400).

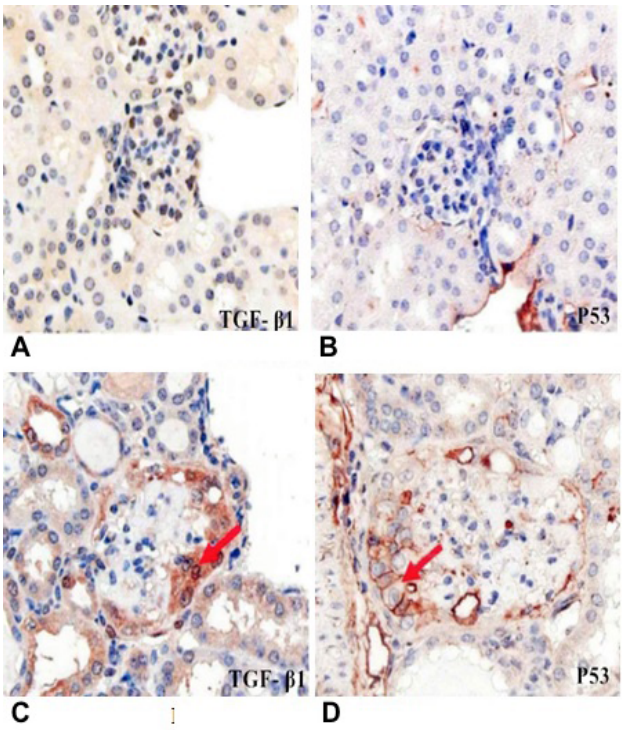

Fig. 2. Expression of TGF- β1 and P53 (A and B) in the control group and TGF- β1 and P53 (C and D) in the kidney cortex of study groups (×400).

The kidney tissue staining of both groups showed that the glomeruli of the control group were normal in structure, with no hypertrophy of the thylakoid cells and no hyperplasia of the stroma. In the experimental group, PAS staining was significantly increased, glomerular thylakoid cells were hypertrophic, and the thylakoid stroma was significantly proliferated and expanded (Fig. 1). Immunohistochemistry and PCR showed that the levels of TGF-β1 and P53 in the kidney cortex of the experimental group were significantly higher than those in the control group (Fig. 2).

Discussion

The incidence of diabetes mellitus (DM) has increased significantly in recent years as people's living standards have improved, and one quarter to one half of patients have a family history of the disease. Patients with DM can develop chronic pathologies in various organs of the body over time, which can seriously threaten their life and health. According to the 2017 edition of the Chinese guidelines for the prevention and treatment of T2DM, the prevalence of T2DM in adults aged 18 years was as high as 10.4%, and the prevalence in those aged ≥60 years was 20% (Murtagh and Hui, 2011; Diabetes Branch of Chinese Medical Association, 2018). Patients with DM have abnormalities in cardiomyocytes due to elevated blood glucose levels, resulting in reduced cardiac function followed by heart failure, which seriously affects the quality of daily life of patients with DM (Feng, 2018; Zhang *et al*., 2020). Diabetes mellitus is classified into type 1 and type 2 according to its type of islet cell lesion. Among them, T2DM, also known as adult-onset diabetes in clinical practice, accounts for more than 90% of all diabetes. The incidence of T2DN has also increased and is a serious threat to the life and health of patients. Therefore, it is important to develop an effective treatment plan to improve the quality of survival and reduce mortality for patients (Jiang *et al*., 2019; Sanchez-álamo *et al*., 2019). Diabetic nephropathy is a major factor contributing to increased mortality in patients with T2DN. Some studies have discovered that the incidence of progression to stage 5 T2DN is significantly higher in those with insulin resistance than in those without insulin resistance (Zhang *et al*., 2016). In addition, it has been found that treatment by combining glucose-lowering drugs with drugs beneficial to the kidneys is more effective, but widespread clinical dissemination has not yet begun (Wen *et al*., 2015).

The results of this study showed that the expression levels of TGF-β1, P53 and microRNA105 mRNA were significantly higher in the experimental group than in the control group after the completion of modeling, while there was no significant difference in microRNA103 content between the two groups. It was demonstrated that the expression levels of TGF-β1, P53 and microRNA105 mRNA were greatly increased after the onset of diabetic nephropathy. It has been shown that TGF-β1 can be used as a predictor of diabetic nephropathy. When its level is found to be significantly elevated, it can be concluded that the patient has diabetic nephropathy (Gao *et al*., 2018). In this study, the expression levels of TGF-β1 and P53 were remarkably higher in the experimental rats than in the control group. This result is another good evidence that the TGF-β1 and P53 protein levels in diabetic patients are elevated substantially after the onset of diabetes. It can be inferred that TGF-β1 and P53 can increase their expression levels in patients through coordinated interactions, which can promote the development of diabetic nephropathy in patients. Some studies have demonstrated that two factors, TGF- β1 and P53, can be used simultaneously as diagnostic indicators of diabetic nephropathy, but they have not been confirmed and need to be gradually involved in future studies (Liu *et al*., 2018).

Conclusion

TGF-β mediates P53 and microRNA-related signaling pathways that can coordinate with each other to promote the development and progression of diabetic nephropathy.

However, since the present study was conducted in SD male rats, not humans, and the sample size was small, it was only to provide a reliable basis for the clinical study. In addition, this study is not in-depth, and the results obtained are only a speculation based on the results of this study, and the specific results need to be explored slowly in future studies.

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IRB approval

This study was approved by the Affiliated Hospital of Qinghai University, Xining, Qinghai810000, China.

Ethical approval

The study was carried out in compliance with guidelines issued by ethical review board committee of Affiliated Hospital of Qinghai University, China. The official letter would be available on fair request to corresponding author.

Statement of conflict of interest

The authors have declared no conflict of interest.

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