Diabetic Nephropathy

0019
Triptolide, an Extracted Phytomedicine Attenuates Glomerular Sclerosis in Diabetic Nephropathy Rats via Regulation of Akt/AMPK/mTOR and TGF-beta1/Smad Signaling Activities, Compared with Rapamycin

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Objectives: Triptolide (TP), a natural extract from Triteriperygium wilfordii has been applied extensively for treating glomerular sclerosis (GS) in patients with early diabetic nephropathy (DN) in China. Activation of mTOR plays a critical role in pathologic forms of hypertrophy and proliferation in kidneys under hyperglycemia other than classical TGF-beta1/Smad pathway. Hyperglycemia increases mTOR activity by combined actions of Akt activation and AMPK inhibition. This study aimed to investigate effects and mechanisms of TP on GS, compared with rapamycin through regulating Akt/AMPK/mTOR or TGF-beta1/Smad signaling activities.

Methods: Rats were randomly divided into 4 groups, sham-operated group, TP-treated group, vehicle-given group and rapamycin-treated group, and sacrificed at week 8 after induction of DN by 2 consecutive intraperitoneal injections of streptozotocin (STZ) with an interval of 1 week following nephrectomy. Daily oral administration of TP, rapamycin and saline were started after the second injection of STZ until the sacrifice. Proteinuria, UAlb, BG, biochemical indicators, renal pathological changes, as well as key protein expressions in Akt/AMPK/mTOR and TGF-beta1/Smad signaling pathways were examined, respectively. An experiment in glomerular mesangial cells was performed to examine effects of TP on cellular proliferation and collagen synthesis.

Results: Akt/AMPK/mTOR and TGF-beta1/Smad pathways were concurrently activated in kidneys. TP, similar to rapamycin, regulated protein expressions of p-Akt, p-mTOR, p-p70S6K, p-Akt, p-Smad2/3 and TGF-beta1 in kidneys, and ameliorated proteinuria, mesangial matrix expansion, alpha-SMA expression and collagen deposition in glomeruli, without lowering hyperglycemia. Addition-ally, retardation in glomerular sclerosis development was observed. In HBZY-1 cell line, TP, analogous to rapamycin, decreased high-glucose-induced cell proliferation and expressions of TGF-beta1, p-Smad2/3 and collagen IV.

Conclusion: Activated Akt/AMPK/mTOR and TGF-beta1/Smad pathways jointly contribute to glomerular injury. TP, as a natural regulator, effectively attenuate GS by potential molecular mechanisms involving reduction of mesangial matrix and suppression of Akt and mTOR activation, as well as regulation of TGF-beta1/Smad signaling activity.

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0020
Total Flavone Glycosides of Flos Abelmoschus Manihot Ameliorates Renal Fibrosis in Diabetic Nephropathy Rats via Inhibiting Oxidative Stress and p38MAPK Signaling Activity Compared with Alpha-Lipoic Acid

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Objectives: Total flavone glycosides of flos abelmoschus manihot (TFA) has been widely used for treating renal fibrosis in patients with diabetic nephropathy (DN) in China. However, therapeutic mechanisms remain unclear. Oxidative stress (OS) is a determinant during renal fibrotic progress under hyperglycemia. As one of the regulative approaches of OS, p38MAPK signaling pathway plays a pivotal role. This study thereby aimed to investigate effects and mechanisms in vivo of TFA on renal fibrosis, compared with alpha-lipoic acid (ALA) as an antioxidant in clinic, through attenuating OS-related injury and p38MAPK signaling activity.

Methods: Rats were randomly divided into 5 groups, sham-operated group, vehicle-given group, low dose of TFA-treated group, high dose of TFA-treated group and ALA-treated group. TFA, ALA and saline were daily administrated for 8 weeks after induction of DN by streptozotocin (STZ) with unilateral nephrectomy. The DN rats’ general state, biochemical indicators, renal pathological changes, OS-related markers, as well as key protein expressions in p38MAPK signaling pathway, fibrogenic cytokines and inflammatory factors were examined, respectively.

Results: DN model rats exhibited typical renal fibrosis, OS-related features and increases in expressions of p-p38MAPK, TGF-β1 and TNF-α. UAlb, BUN, UA, Alb, TG, TC and OS-related markers including MDA, T-SDO, GSH-Px, 8-OHdG and NOX4 in serum or kidneys were ameliorated in treated groups, especially in high dose of TFA-treated group. Of note, TFA synchronously inhibited p38MAPK signaling activity and TGF-β1 and TNF-α protein overexpressions, whereas, ALA only suppressed TNF-α protein overexpression in kidneys.

Conclusion: By means of the DN model rats, we demonstrated that OS promotes renal fibrosis and p38MAPK signaling activity. TFA, as a natural regulator in vivo, can improve OS-related renal damage via regulating protein overexpressions of p38MAPK, TGF-β1 and TNF-α, which is different from ALA.

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0023
Deposition of Complement 3 Influences the Prognosis of Nodular Sclerosis of Diabetic Nephropathy

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Objective: To study the influence of the deposition of complement 3 on the prognosis of nodular sclerosis of diabetic nephropathy.

Methods: Clinical and pathological materials were collected from 89 patients who suffered from nodular sclerosis of diabetic nephropathy proved by renal biopsy and took Angiotensin-Converting Enzyme Inhibitors or
Angiotensin Receptor Blocker normally. Based on whether the renal biopsy specimens were deposited by complement 3, the patients were divided into two groups. This study selected eGFR< 45 ml/min/1.73 m² or dialysis as endpoints. At the end of follow-up, the difference of serum creatinine and 2 year renal survival rates were compared.

**Results:** The group with complement 3 deposited has higher 24-hour proteinuria (3.4 ± 1.6 vs 4.5 ± 2.0, P = 0.039), however, clinical data of patients at baseline showed no differences in systolic and diastolic blood pressures, BMI, FBG, HbA1c, Albumin and scr between the two groups. More than sixty presents subjects (87%) received renin angiotensin system blocker. After two years of follow-up, 29.2%(21/78) patients progress to eGFR < 45 ml/min/1.73 m². And the cumulative progression rate by two years was 38.7%(12/31) for patients in the C3 positive group and 19.1%(9/47) for patients in the C3 negative group. The survival of stable renal function during the study was similar between two groups (P = 0.052). In the subgroup analysis of patients without nephrotic syndrome, patients in the C3 positive group were less likely to progress to eGFR < 45 ml/min/1.73 m² than those in the C3 negative group (P = 0.007). In a multivariate model, we found C3 positive (HR, 2.726; 95% CI, 1.108-6.703) was a risk factor for progressive renal function.

**Conclusion:** The deposition of complement 3 has a certain degree influence on the prognosis of nodular sclerosis of diabetic nephropathy.

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**0036**

**Urinary Tubular Biomarkers Predict Renal Injurious Progress in Early Diabetic Nephropathy Patients**

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**Objectives:** Diabetic nephropathy (DN) is a dominant cause of end-stage renal disease in China. The early diagnosis of DN has focused on measurement ofUAib excretion rate, but it is not sensitive marker for DN patients with inchoate injuries in glomeruli and renal tubules. This study thus aimed to evaluate clinical significance of urinary tubular biomarkers in predicting development of DN patients at early stage.

**Methods:**

The study was performed on 92 diabetes mellitus with different levels ofUAib and certain range of Scr (<106 μmol/L). According to albumin-to-creatinine ratio (ACR) in urine, all patients were categorized into 3 groups, normoalbuminuria (A) group, microalbuminuria (B) group and macroalbuminuria (C) group. In addition to UAib, Scr and ACR, levels of tubular biomarkers including UNAG, URBP and UcysC in urine were tested respectively before renal protective drugs intervention.

**Results:** Compared with A group, levels of UNAG, URBP and UcysC in B and C groups were significantly different (P < 0.01). Along with UAib, stepwise increase in levels of UNAG, URBP and UcysC were detected respectively in B and C groups. Moreover, in univariate analysis, there was immediate relevance between UAib, ACR and tubular biomarkers including UNAG (r = 0.706, P < 0.01; r = 0.808, P < 0.001), URBP (r = 0.687, P < 0.01; r = 0.701, P < 0.001) and UcysC (r = 0.727, P < 0.01; r = 0.790, P < 0.001) in all groups. In addition, we found that UNAG was positively correlated with URBP (r = 0.652, P = 0) and UcysC (r = 0.785, P = 0). Urinary tubular biomarkers including UNAG, URBP and UcysC were two predictive factors of increased UcysC.

**Conclusion:** At early stage of DN, increased levels of UNAG, URBP and UcysC are independently associated with UAib, and these urinary tubular biomarkers similar to UAib may be widely used as practical targets in clinic in detecting and managing DN, and predicting renal tubular damaged progression.

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**0037**

**A Novel Therapy for Type-2 Diabetic Nephropathy by Targeting Smad3-dependent IncRNA_5318**

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**Objective:** Increasing evidence shows that non-coding RNAs play an important role in kidney disease. Recently, we identified Smad3-dependent IncRNAs related to renal fibrosis by high-throughput RNA-sequencing. In this study, we investigated the role and therapeutic potential of IncRNA_5318 in type-2 diabetic nephropathy (T2DN).

**Methods:** Expression of IncRNA_5318 in the diabetic kidney of db/db mice was examined by real-time PCR. The role and therapeutic effect of IncRNA_5318 on T2DN were determined by knocking down IncRNA_5318 from the diabetic kidney using an ultrasound-microbubble-mediated shRNA