



Protection from renal fibrosis, putative role of TRIB3 gene silencing

Wen-yuan Ding^{a,b,1}, Wen-bo Li^{a,b}, Yun Ti^{a,b}, Xiu-ping Bi^{a,b}, Hui Sun^{a,b}, Zhi-hao Wang^{a,c}, Yun Zhang^{a,b}, Wei Zhang^{a,b,*}, Ming Zhong^{a,b,*}

^a Key Laboratory of Cardiovascular Remodeling and Function Research, Chinese Ministry of Education and Chinese Ministry of Public Health, Department of Cardiology, Qilu Hospital of Shandong University, Ji'nan 250012, PR China

^b Department of Cardiology, Qilu Hospital of Shandong University, Ji'nan 250012, PR China

^c Geriatrics, Qilu Hospital of Shandong University, Ji'nan 250012, PR China

ARTICLE INFO

Article history:

Received 4 December 2013

Available online 22 December 2013

Keywords:

TRIB3

siRNA

MAPK

Renal fibrosis

ABSTRACT

Background: Renal fibrosis is thought to be the common pathway in most cases of chronic kidney disease. Recently, TRIB3 was found to play an important role in progression of cardiac fibrosis in an insulin-resistant state. We investigated whether TRIB3 might participate in the pathogenesis of renal fibrosis in insulin-resistant rats.

Methods: We randomly separated 40 male Sprague–Dawley into 4 groups for treatment ($n = 10$ each): control and high-fat diet (HFD) with TRIB3 siRNA adenovirus transfection, vehicle transfection or HFD alone. Insulin resistance markers were measured. Renal tissues were stained with hematoxylin and eosin, Masson's trichrome and periodic acid–Schiff.

Results: Rats with HFD showed insulin resistance and TRIB3 overexpression. Upregulated TRIB3 expression could induce renal fibrosis accompanied by increased phosphorylation of extracellular signal-regulated kinase (ERK). Also, TRIB3 siRNA knockdown could ameliorate renal fibrosis, which was accompanied by decreased phosphorylation of ERK.

Conclusions: TRIB3 gene silencing can attenuate renal fibrosis for beneficial effect on the development of renal fibrosis in chronic kidney disease in rat.

Crown Copyright © 2014 Published by Elsevier Inc. All rights reserved.

Introduction

Chronic kidney disease has common pathways for renal fibrosis, which is characterized by tubulointerstitial fibrosis and glomerulosclerosis (Eitner and Floege, 2003; Remuzzi and Bertani, 1998). In general, renal fibrosis is a process of excess accumulation and deposition of extracellular matrix (ECM) components (Liu, 2006). ECM is composed of a complex fibrillar collagen network, mainly collagen IV in the renal system (Sánchez-López et al., 2008). A long-term high-fat diet (HFD) can cause additional damage to kidney and induce renal fibrosis (Lu et al., 2003). However, the mechanism of HFD-induced collagen deposition in renal fibrosis remains unclear.

Insulin resistance plays a causal role in the pathogenesis of renal fibrosis. In an insulin-resistant state, TRIB3 is activated (Prudente et al., 2005). TRIB3 modifies cellular survival and metabolism and interferes with signal transduction pathways as a pseudokinase (Morse et al., 2010). TRIB3 is also a molecular switch and regulates the activation of the 3 classes of mitogen-activated protein kinases (MAPKs) (Kiss-Toth et al., 2004). ERK and p38 MAPKs have an important role in the

development of collagen fibrosis (Park et al., 2007; Saka et al., 2006). We previously demonstrated that the activation of ERK mediates the pathogenesis of collagen deposition in cardiac fibrosis in an insulin-resistant state (Ti et al., 2011). However, whether the involvement of TRIB3 in the process of renal fibrosis is by regulating ERK in an insulin-resistant state is unknown.

A long-term HFD can cause insulin resistance (Kang et al., 2011), associated with renal fibrosis. We wondered whether in an insulin-resistant state induced by long-term HFD, TRIB3 was implicated in renal fibrosis by regulating ERK. In HFD-induced insulin resistance, by activating ERK, TRIB3 may promote renal collagen deposition to cause renal fibrosis. We investigated the effect of TRIB3 on renal fibrosis in a rat model with long-term HFD.

Materials and methods

Animals

We purchased 40 male Sprague–Dawley rats (120–140 g) from the experimental animal center of Shandong University of Traditional Chinese Medicine (Ji'nan, China). The animals were housed at 22 °C with 12-h light–dark cycles. After 1 week of acclimatization, the rats were randomized into 2 groups for treatment: control and HFD. The control group received normal chow and the HFD group a diet of 34.5% fat, 17.5% protein, 48% carbohydrates (Beijing HFK Bio-Technology).

* Corresponding authors at: Department of Cardiology, Qilu Hospital of Shandong University, No.107 Wenhua West Road, Ji'nan 250012, PR China. Fax: +86 531 86169356.

E-mail addresses: zhangweisdu@gmail.com (W. Zhang), zhongmingzm@gmail.com (M. Zhong).

¹ Wen-yuan Ding contributed to this work.

The HFD group was further divided into 3 subgroups ($n = 10$ each) for injection at week 17 with TRIB3 siRNA adenovirus or vehicle or as HFD alone. Animals were injected via the jugular vein with 2.5×10^{10} plaque-forming units of an adenovirus harboring TRB3 gene (TRB3-siRNA) or a control empty virus (vehicle). Adenovirus transfer was repeated in 2 weeks. The rats were killed at the 22nd week. All protocols were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health and approved by the Ethics Committee for Animal Research of Shandong Province in China.

Blood analyses

After rats deprived of food overnight, we collected blood sample through the jugular vein. Total cholesterol (TC), triglyceride (TG), and fasting blood glucose (FBG) were analyzed using the Bayer 1650 blood chemistry analyzer (Bayer, Tarrytown, NY). Fasting insulin (FINS) levels were measured by enzyme-linked immunosorbent assay. The insulin sensitivity index (ISI) = $[\ln(\text{FBG} \times \text{fasting insulin})^{-1}]$ was calculated.

Histochemistry

Rats were killed at the end of the experiment. Excised kidney tissues were fixed in 4% paraformaldehyde and embedded in paraffin, and 4- μm sections were cut for hematoxylin and eosin and Masson's trichrome staining or 3- μm sections for periodic acid-Schiff (PAS) staining viewed under a microscope. Interstitial fibrosis was calculated by dividing the area of trichrome-stained interstitium by total cortical area. Percentage glomerulosclerosis was quantified by dividing the total area of PAS-positive staining in the glomeruli by total area of the glomeruli. The degree of interstitial fibrosis and glomerulosclerosis was analyzed by use of ImagePro Plus v5.0.

Western blot analysis

Kidney tissues were finely minced and harvested in sample buffer, resolved by SDS-PAGE, and transferred to polyvinylidene difluoride membrane (Wei et al., 2006) for incubation with antibodies for phosphorylated ERK (p-ERK), ERK (Cell Signaling Technology, Beverly, MA) or collagen IV (all 1:1000; Abcam) or TRIB3 (a 1:500; Calbiochem, La Jolla, CA), then horseradish peroxidase-conjugated secondary antibody (1:4000). Immunoreactive bands were visualized by the use of enhanced chemiluminescence reagent. Densitometry involved use of Quantity One.

Statistical analysis

Data are presented as mean \pm SEM and analyzed by use of the SPSS v16.0 (SPSS, Chicago, IL). Analysis involved one-way ANOVA or Student's *t* test. A $P < 0.05$ was considered statistically significant.

Results

TC, TG and FBG concentrations and ISI

At the end of the experiment, the levels of TC, TG, FBG and FINS were significantly higher in HF group than the control group (TC: 1.79 ± 0.03 vs. 1.00 ± 0.04 ; TG: 1.37 ± 0.26 vs. 0.71 ± 0.13 ; FBG: 10.68 ± 0.69 vs. 7.16 ± 0.50 ; FINS: 16.44 ± 0.36 vs. 13.39 ± 0.85 ; respectively, Fig. 1A–D). The ISI was significantly decreased and the IRI significantly increased in HF group (ISI: -5.15 ± 0.04 vs. -4.52 ± 0.08 ; IRI: 7.79 ± 0.30 vs. 4.28 ± 0.33 ; respectively, Fig. 1E–F). Thus, HFD rats were associated with insulin resistance.

Features of rats with HFD

Renal fibrosis appeared as tubulointerstitial fibrosis and glomerulosclerosis. Rats with HFD showed renal fibrosis, with collagen deposition in tubular compartments and glomeruli (Fig. 2A1–2; Fig. 3A1–2; Fig. 4A1–2). Tubulointerstitial fibrosis was significantly greater in HF group than the control group (CVF%: 3.90 ± 0.4 vs. 0.7 ± 0.2 ; $P < 0.05$, Fig. 3A1–2). Mesangial expansion in glomeruli seen on PAS staining was greater in HF group than the control group (Glomerulus' sclerosis index%: 12.7 ± 1.4 vs. 5.0 ± 0.6 ; $P < 0.05$, Fig. 4A1–2). Thus, we may conclude HFD could induce renal fibrosis.

TRIB3/MAPK signaling in rats with HFD

We found no notable adverse effects and no deaths in rats with TRIB3 siRNA treatment. Renal TRIB3 protein expression was significantly increased in HF group (Fig. 5A). With TRIB3 overexpression, the phosphorylation of ERK1/2 was markedly increased (Fig. 5B). The protein expression of collagen IV was elevated in HF group than the control group (Fig. 5C). Compared to vehicle treatment, TRIB3 siRNA treatment downregulated the protein expression of TRIB3 in renal tissue. What's more, collagen IV content and phosphorylation of ERK1/2 were significantly lower in HF group with TRIB3 siRNA than vehicle only (Fig. 5A–C), which suggested that the TRIB3/MAPK signaling might participate in the collagen synthesis of renal fibrosis in HFD rats.

Effect of TRIB3 silencing on metabolism and insulin sensitivity

After 4-week TRIB3 siRNA transfection, there were no significant differences between TRIB3 siRNA and vehicle treatment in TC, TG and FBG (TC: 1.67 ± 0.08 vs. 1.78 ± 0.21 ; TG: 1.45 ± 0.10 vs. 1.46 ± 0.16 ; FBG: 10.73 ± 0.67 vs. 10.74 ± 0.49 ; respectively, Fig. 1A–C). However, FINS and IRI were significantly lower with TRIB3 siRNA than vehicle treatment (FINS: 13.47 ± 0.84 vs. 15.92 ± 1.18 ; IRI: 6.31 ± 0.41 vs. 7.59 ± 0.59 ; respectively, Fig. 1D, F), and ISI was significantly elevated with TRIB3 siRNA than vehicle treatment (ISI: -4.92 ± 0.07 vs. -5.10 ± 0.07 , Fig. 1E). Thus, TRIB3 silencing might alleviate the insulin resistance.

Effect of TRIB3 silencing on renal fibrosis

Tubulointerstitial fibrosis was significantly ameliorated with TRIB3 siRNA than vehicle treatment in HF group (Fig. 2A3–4; CVF%: 1.3 ± 0.4 vs. 3.0 ± 0.60 , $P < 0.05$, Fig. 3A3–4). The same to the glomerulosclerosis (Glomerulus' sclerosis index%: 6.8 ± 0.7 vs. 12.4 ± 2.3 , $P < 0.05$, Fig. 4A3–4, E). Therefore, we may conclude that TRIB3 silencing could alleviate renal fibrosis.

Discussion

We found TRIB3 involved in the pathogenesis of renal fibrosis induced by HFD in rats. In an insulin-resistant state, upregulated TRIB3 expression promoted collagen deposition and induced renal fibrosis by activating ERK; however TRIB3 silenced with siRNA attenuated collagen synthesis and alleviated renal fibrosis by downregulating ERK.

TRIB3 is overexpressed in murine models with insulin resistance (Du et al., 2003; Koo et al., 2004). Our HFD-fed rats showed insulin resistance and increased TRIB3 level. These findings are consistent with previous studies (Du et al., 2003; Koo et al., 2004; Taniguchi et al., 2006).

In an insulin-resistant state, MAPK is fully activated (Kim et al., 2006). TRIB3 might be an important modulating protein of MAPKs (Kiss-Toth et al., 2004). TRIB3 significantly upregulated in HFD rats with insulin resistance was paralleled by increased ERK expression. HFD rats showed prominent collagen accumulation in tubular compartments and

Download English Version:

<https://daneshyari.com/en/article/2775304>

Download Persian Version:

<https://daneshyari.com/article/2775304>

[Daneshyari.com](https://daneshyari.com)