Biochemical Pharmacology 132 (2017) 102–117

Contents lists available at ScienceDirect

Biochemical Pharmacology

journal homepage: www.elsevier.com/locate/biochempharm

Protein kinase CK2α catalytic subunit ameliorates diabetic renal inflammatory fibrosis via NF-κB signaling pathway

Junying Huang [a], Zhiquan Chen [a], Jie Li [a], Qiuhong Chen [a], Jingyan Li [a], Wenyan Gong [a], Jiani Huang [a], Peiqing Liu [a,b], Heqing Huang [a,c,*]

[a] Laboratory of Pharmacology & Toxicology, School of Pharmaceutical Sciences, Sun Yat-sen University, Guangzhou 510006, China
[b] National and Local United Engineering Lab of Druggability and New Drugs Evaluation, Guangzhou 51000, China
[c] Guangdong Engineering & Technology Research Center for Disease-Model Animals, Sun Yat-sen University, Guangzhou 510006, China

ARTICLE INFO

Article history:
Received 28 December 2016
Accepted 21 February 2017
Available online 23 February 2017

Keywords:
Casein kinase 2α subunit
Diabetic nephropathy
NF-κB pathway
Renal fibrosis
TBB treatment

ABSTRACT

Activation of casein kinase 2 (CK2) is closely linked to the body disturbance of carbohydrate metabolism and inflammatory reaction. The renal chronic inflammatory reaction in the setting of diabetes is one of the important hallmarks of diabetic renal fibrosis. However, it remains unknown whether CK2 influences the process of diabetic renal fibrosis. The current study is aimed to investigate if CK2α ameliorates renal inflammatory fibrosis in diabetes via NF-κB pathway. To explore potential regulatory mechanism of CK2α, the expression and activity of CK2α, which were studied by plasmid transfection, selective inhibitor, small-interfering RNA (siRNA) and adenovirus infection in vitro or in vivo, were analyzed by means of western blotting (WB), dual luciferase reporter assay and electrophoretic mobility shift assay (EMSA). The following findings were observed: (1) Expression of CK2α was upregulated in kidneys of db/db and KKAy diabetic mice; (2) Inhibition of CK2α kinase activity or knockdown of CK2α protein expression suppressed high glucose-induced expressions of FN and ICAM-1 in glomerular mesangial cells (GMCs); (3) Inhibition of CK2α kinase activity or knockdown of CK2α protein expression not only restrained IκB degradation, but also suppressed HG-induced nuclear accumulation, transcriptional activity and DNA binding activity of NF-κB in GMCs; (4) Treatment of TBB or CK2α RNAi adenovirus infection ameliorated renal fibrosis in diabetic animals; (5) Treatment of TBB or CK2α RNAi adenovirus infection suppressed IκB degradation and NF-κB nuclear accumulation in glomeruli of diabetic animals. This study indicates the essential role of CK2α in regulating the diabetic renal pathological process of inflammatory fibrosis via NF-κB pathway, and inhibition of CK2α may serve as a promising therapeutic strategy for diabetic nephropathy.

1. Introduction

Diabetic nephropathy (DN) is a primary chronic microvascular complication of diabetes mellitus (DM) which ultimately leads to end-stage renal disease and is the leading cause of morbidity and mortality in diabetic patients [1,2]. DN is also known as diabetic glomerulosclerosis characterized by renal fibrosis. Glomerular mesangial cells are the dominating functional cells in glomeruli, which are associated with both renal physiological functions and pathological changes [3,4]. The accumulation and increasement of secretory extracellular matrix (ECM) components in GMCs such as fibronectin (FN) and intercellular adhesion molecule-1 (ICAM-1) are critically involved in glomerular basement membrane thickening and glomerular fibrosis [5,6].

Accumulating evidence suggests that glucolipid metabolism disorders, nonenzymatic glycation of proteins, oxidative stress and cytokine secretion are believed to participate in the development and progression of diabetic kidney fibrosis. However, the regulatory mechanism of DN has not been fully elucidated. In recent years, it receives serious attention that diabetic renal fibrosis disease is a kind of a renal chronic inflammatory response [7,8]. Many lines of evidence reveal that inflammation is implicated in the

Abbreviations: DN, diabetic nephropathy; DM, diabetes mellitus; CK2, casein kinase 2; CK2α, casein kinase 2α catalytic subunit; ECM, extracellular matrix; FN, fibronectin; ICAM-1, intercellular adhesion molecule-1; NG, normal glucose; HG, high glucose; FBS, fetal bovine serum; NF-κB, nuclear factor of kappa B; IκB, inhibitor of kappa B; IKK, inhibitor of kappa B kinase; GMCs, glomerular mesangial cells; SD, Sprague-Dawley; STZ, streptozotocin; CMC-Na, sodium carboxymethylcellulose; KW/BW, kidney/body weight; BUN, blood urea nitrogen; SCr, serum creatinine; FBG, fasting blood glucose; PAS, periodic acid-schiff; HE, hematoxylin-eosin; siRNA, small-interfering RNA; WB, western blotting; EMSA, electrophoretic mobility shift assay.

* Corresponding author at: Laboratory of Pharmacology & Toxicology, School of Pharmaceutical Sciences, Sun Yat-sen University, 132 Waihuan East Road, Guangzhou Higher Education Mega Center, Guangzhou 510006, China.
E-mail address: huangheq@mail.sysu.edu.cn (H. Huang).

http://dx.doi.org/10.1016/j.bcp.2017.02.016
0006-2952/

development and progression of DN. Especially, NF-κB signaling pathway regulates expression of genes joining in inflammatory response during clinical and experimental kidney injury [8–10]. In DN, the activated NF-κB translocates to nucleus from cytoplasm, and then triggers gene expression closely related to the pathogenesis of DN, including FN and ICAM-1 [11–14]. In normal condition, NF-κB is combined with IκB in the cytoplasm and acts as inactive. In response to stimulation, IκB kinase (IKK) is phosphorylated by upstream kinase and phosphorylates IκB to give rise to its ubiquitin-mediated degradation via proteasome, which promotes release of NF-κB from the anchor protein quickly and then NF-κB translocates into nucleus to improve the expression of inflammatory genes [15,16]. Under diabetic state, NF-κB is closely related to inflammatory reaction, and is believed to be involved in the pathophysiological process of diabetic renal fibrosis. It is activated by upstream protein and regulates the expression of inflammatory molecules, such as ICAM-1, which results in continuous inflammation and further boosts secretion of FN in kidneys. Therefore, further research about the molecular mechanism of diabetic renal fibrosis and inflammation shows important significance in hindering the progression of diabetic renal disease and exploring a promising target for DN treatment.

Protein kinase CK2 (formerly casein kinase Ⅱ) is a highly conserved, ubiquitous protein serine/threonine kinase that phosphorylates more than 300 substrates and plays a pivotal role in many biological and pathological processes. It exists as a tetrameric complexes consisting of two catalytic (α and/or α′) subunits and two regulatory β subunits and is present as a messenger-independent protein kinase distributing in nuclei and cytoplasm of eukaryotic cells [17]. Increasing number of studies suggest that CK2 performs as a kinase to phosphorylate substrates mainly in the shape of ααββ, and individual α catalytic subunit also shows an important kinase activity. Several studies show that α catalytic subunit is the primary functional subunit for disease treatment [18,19]. CK2 has extensive physiological functions and is an important participant in regulating numerous key biological processes such as cell proliferation, differentiation and apoptosis. Moreover, CK2 is implicated in development and progression of several diseases including cancer [20], angiogenesis [21], glomerulonephritis [22] and organogenesis [23].

In recent years, the relationship between the protein kinase CK2α and carbohydrate metabolism as well as inflammation has received serious attention. Activation of CK2α promotes blood glucose during glycometabolism [24,25], and insulin-activated insulin receptor activates CK2 and upregulates expression of CK2α, which develops the negative feedback loop of insulin release by triggering phosphorylation of PDX-1 to suppress release of insulin [26,27]. Elevated protein level and kinase activity of CK2α have been observed in inflammation and downstream inflammatory proteins are phosphorylated by activated CK2α. Lots of proteins involved in inflammatory signaling pathway interact with CK2α, such as NF-κB and AP-1 [28,29]. It is provided that renal chronic inflammatory reaction is one of important features of diabetic renal fibrosis under DM. Therefore, we are interested in whether CK2α, which is closely related to the body glucose metabolism disorder and inflammatory reaction, can affect the pathological process of renal fibrosis. In this study, we assessed the activation of NF-κB pathway, the expressions of FN and ICAM-1 influenced by CK2α in HG-induced GMCs and STZ-induced diabetic animals.

2. Materials and methods

2.1. Reagents and antibodies

D-Glucose was purchased from Amresco (Solon, OH, USA). Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), RNAiMAX transfection reagent and LTX reagent with PLUS™ reagent were purchased from Life Technologies (Grand Island, NY, USA). Antibody against pT255-CK2α, streptozotocin (STZ) and dimethyl sulphoxide (DMSO) were obtained from Sigma–Aldrich Corporation (St. Louis, MO, USA). Antibodies against NF-κB, CK2α, Tubulin and Lamin B1 were produced by Abcam (Cambridge, MA, USA). Antibodies against FN, ICAM-1 and IκBα were from Santa Cruz Biotechnology (Dallas, TX, USA). Plasmid CK2α and CK2α K68M were acquired from addgene (Cambridge, MA, USA). Horseradish peroxidase-conjugated secondary antibodies and Dual-Glo® Luciferase Assay System were obtained from Promega Corporation (Madison, WI, USA). Alexa Fluor® 488 goat anti-mouse IgG, Alexa Fluor® 488 goat anti-rabbit IgG and Alexa Fluor® 594 donkey anti-goat IgG were purchased from Thermo Fisher Scientific (Rockford, IL, USA). CK2α RNAi adenovirus with a knockdown function and the negative control adenovirus were from Hanbio (Shanghai, China). Antibody against pSer529-p65 was from Abclonal (Wuhan, Hubei, China). Nuclear extract kit was purchased from Active Motif (Carlsbad, CA, USA).

2.2. Cell culture

Primary GMCs were separated from the glomeruli of Sprague-Dawley (SD) rats (around 150 g) and identified via a specific assay as previously described [30]. The cortex fragments of kidneys were cut into pieces in ice-cold PBS and harvested by filtration on three specific meshes (sizes are 175, 147 and 74 μm orderly). In the end, the filtrable matter was collected and digested with 0.1% collagenase IV in serum-free DMEM for 15–25 min at 37 °C. The digested fragments were resuspended, seeded in culture bottle and incubated in 10% FBS DMEM at 37 °C with 5% CO_2. Before experiment, the GMCs were cultured in normal glucose (NG) DMEM with 10% FBS and used at confluence between the 5th and 12th passages. When GMCs were 80% confluent, they were rendered quiescent by incubation in serum-free medium for 24 h before treating with glucose or other stimuli.

2.3. Western blot assay

Western blot assay was performed with the standard protocol as previously described [31]. Cultured GMCs or kidney tissues were harvested and lysed for extracting proteins. Level of total proteins was acquired using RIPA with protease and phosphatase cocktail, and of nuclear and cytoplasmic proteins was acquired using a commercially available assay kit.

The collective proteins from cells or tissues were separated by 8% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and then transferred to PVDF membrane for blocking with 5% defatted milk at room temperature. Proteins were incubated at 4 °C with primary antibodies exceeding 12 h. After incubation, the membrane was washed with 0.1% Tween-20/TBS (TBST) and incubated with the second antibodies at room temperature for 1 h. The signals of protein level were visualized by ImageQuant LAS4000mini produced by GE healthcare (Waukesha, WI, USA) and analyzed using the Quantity One Protein Analysis Software produced by Bio-Rad Laboratories (Hercules, CA, USA).

2.4. Immunofluorescence

GMCs were seeded and cultured on glass coverslips. After treatment, cells were washed with cold PBS for three times, fixed with 4% paraformaldehyde for 15 min and then permeabilized with 0.1% TritonX-100 for 10 min at room temperature. After another washing for three times, cells were blocked for 30 min using 10% goat serum and incubated with primary antibodies overnight at 4 °C. Then cells were incubated with fluorescent secondary antibody