Biochemical Pharmacology 116 (2016) 140–152

Contents lists available at ScienceDirect

Biochemical Pharmacology

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

CKIP-1 ameliorates high glucose-induced expression of fibronectin and intercellular cell adhesion molecule-1 by activating the Nrf2/ARE pathway in glomerular mesangial cells

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ARTICLE INFO

Article history:
Received 22 June 2016
Accepted 28 July 2016
Available online 29 July 2016

Keywords:
CKIP-1
Diabetic nephropathy
Nrf2/ARE pathway
Oxidative stress
Keap1

ABSTRACT

Glucose and lipid metabolism disorders as well as oxidative stress (OSS) play important roles in diabetic nephropathy (DN). Glucose and lipid metabolic dysfunctions are the basic pathological changes of chronic microvascular complications of diabetes mellitus, such as DN. OSS can lead to the accumulation of extracellular matrix and inflammatory factors which will accelerate the progress of DN. Casein kinase 2 interacting protein-1 (CKIP-1) mediates adipogenesis, cell proliferation and inflammation under many circumstances. However, whether CKIP-1 is involved in the development of DN remains unknown. Here, we show that CKIP-1 is a novel regulator of resisting the development of DN and the underlying molecular mechanism is related to activating the nuclear factor E2-related factor 2 (Nrf2)/antioxidant response element (ARE) antioxidative stress pathway. The following findings were obtained: (1) The treatment of glomerular mesangial cells (GMCs) with high glucose (HG) decreased CKIP-1 levels in a time-dependent manner; (2) CKIP-1 overexpression dramatically reduced fibronectin (FN) and intercellular adhesionmolecule-1 (ICAM-1) expression. Depletion of CKIP-1 further induced the production of FN and ICAM-1; (3) CKIP-1 promoted the nuclear accumulation, DNA binding, and transcriptional activity of Nrf2. Moreover, CKIP-1 upregulated the expression of Nrf2 downstream genes, heme oxygenase (HO-1) and superoxide dismutase 1 (SOD1); and ultimately decreased the levels of reactive oxygen species (ROS). The molecular mechanisms clarify that the advantageous effect of CKIP-1 on DN are well connected with the activation of the Nrf2/ARE antioxidative stress pathway.

1. Introduction

Diabetic nephropathy (DN), a major microvascular complication of diabetes, has become the chief cause of end-stage renal disease [1,2]. The main pathological feature of DN is characterized by renal fibrosis, including glomerulosclerosis and tubulointerstitial fibrosis [1,2]. As intrinsic cells of glomerulus, glomerular mesangial cells (GMCs) produce fibronectin (FN), an important component of extracellular matrix, and secrete intercellular adhesionmolecule-1 (ICAM-1). Overproduction of FN and excessive secretion of ICAM-1 trigger the thickening of glomerular and tubular basement membranes, which accelerate the pathogenesis of glomerulosclerosis and tubulointerstitial fibrosis, and ultimately progress to DN [3–5]. Therefore, the inhibition of expression of FN, ICAM-1 can effectively decrease the extracellular matrix and inflammatory factors accumulation and postpone or prevent glomerulosclerosis and tubulointerstitial fibrosis. These effects considerably contribute to the prevention of the initiation and development of DN.

The pathological mechanisms of DN remain unclear but they are believed to be ascribed to the integrated effects of multiple factors, such as glucose and lipid metabolic dysfunction, changes of

Abbreviations: ARE, antioxidant response element; CKIP-1, Casein Kinase 2-Interacting Protein-1; DN, diabetic nephropathy; FN, fibronectin; HO-1, heme oxygenase 1; ICAM-1, intercellular adhesionmolecule-1; Keap1, kelch like ECH-associated protein 1; MnSOD, manganese superoxide dismutase; NAD^+, nicotinamide adenosine dinucleotide $^+$; Nrf2, nuclear factor E2-related factor 2; OSS, oxidative stress; ROS, reactive oxygen species; SOD, superoxide dismutase; HG, high glucose; NG, normal glucose.

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http://dx.doi.org/10.1016/j.bcp.2016.07.019
0006-2952/

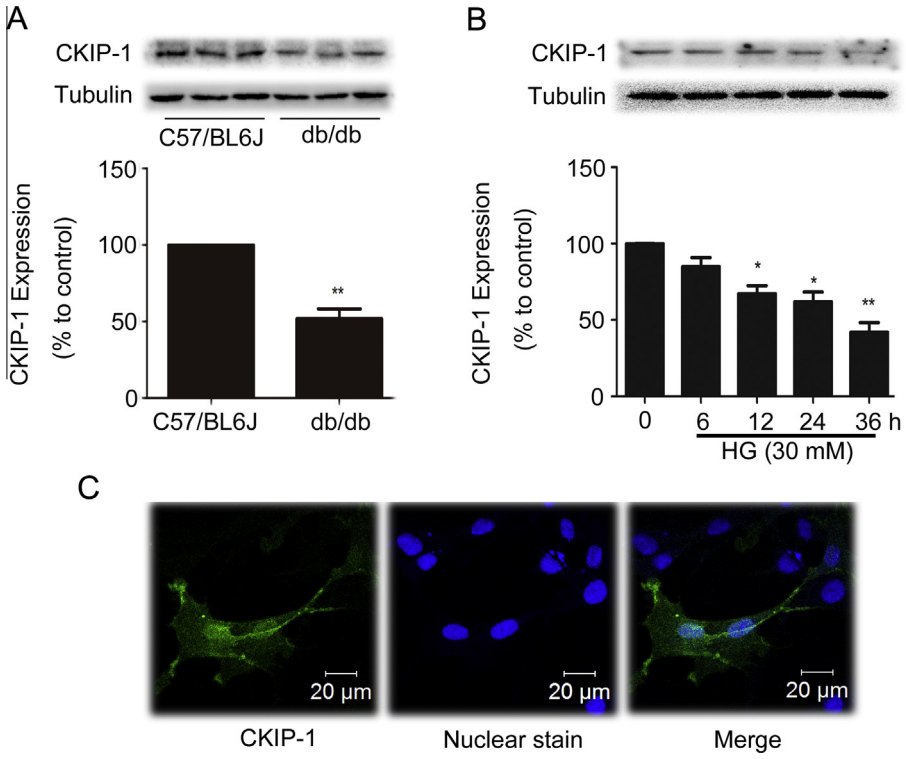

Fig. 1. CKIP-1 protein expression dramatically decreased in both the kidney tissues of db/db mice and GMCs cultured in high glucose. The expression of CKIP-1 was assessed by Western blot in C57BL/6J and db/db mouse kidney tissues (n = 6) (A). $^{**}P < 0.01$ vs C57BL/6J. After treatment of GMCs with 30 mM HG, the CKIP-1 levels were detected by Western blot assay (B). $^{*}P < 0.05$, $^{**}P < 0.01$ vs 0 min. The subcellular distribution of CKIP-1 in GMCs under normal conditions was detected by immunofluorescent staining when GMCs were transfected with CKIP-1 plasmid, Bar: 20 μm (C). Independent experiments are performed at least three times with similar results.

renal hemodynamics, increased nonenzymatic glycation of proteins and oxidative stress (OSS) [6]. Reactive oxygen species (ROS) levels boost in renal cells cultured under high glucose (HG) and in the kidney tissues of diabetic mice [7,8]. Excessive production of ROS in the kidney can lead to continuous OSS and reduce the expression of antioxidative enzymes, such as catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GSH-px) in rat GMCs [9–11]. ROS can also activate a large number of cytokines and promote the generation of FN and ICAM-1, which ultimately initiates and participates in the development of diabetic renal fibrosis [12–15]. Thus, inhibition of the expression of FN and ICAM-1 induced by HG through OSS is beneficial to prophylaxis and treatment of DN.

Currently, it is generally acknowledged that the nuclear factor E2-related factor 2 (Nrf2)/antioxidant response element (ARE) pathway, as an antioxidative stress regulator, can protect cells from OSS injury by regulating the gene expression of antioxidants [16]. When suffering oxidative and electrophilic stimulation, Nrf2 disassociates from its cytosolic inhibitor kelch like ECH-associated protein 1 (keap1) and then translocates to the nucleus where it interacts with antioxidant response element (ARE) to mediate the transcription of its target genes, including heme oxygenase-1 (HO-1), SOD1, glutathione peroxidase, and NAD(P)H quinine oxidoreductase 1 [17–19]. Both Nrf2-knockout (KO) and keap1-knockdown (KD) mice have been reported unable to improve their resistance to insulin insult, which indicates the importance of the Nrf2/ARE antioxidative stress pathway in diabetes [20]. Multiple studies have shown that activating the Nrf2/ARE pathway can protect cells from OSS insult with decreasing expression of ICAM-1 and FN, and then play a vital role in preventing the OSS-induced initiation and development of diabetic renal fibrosis [21–24]. Moreover, Nrf2/ARE pathway also plays critical roles in the development of diabetic cardiomyopathy and cardiac injury [25,26]. Actually, diabetic nephropathy is usually associated with an increased risk of cardiovascular complications disease [27]. Therefore, it is important to find potential targets which could activate the Nrf2/ARE pathway.

CKIP-1 was originally identified as a specific interacting protein of the casein kinase 2 (CK2) α subunit (but not α′ or β subunit) [28]. The pleckstrin homology (PH) domain of CKIP-1 at the N-terminus is necessary for the protein's plasma membrane localization and the self-inhibition domain of CKIP-1 at the C-terminus is also important for its nucleus localization [29]. As a scaffold protein, CKIP-1 plays a significant role in mediating interactions with various proteins, such as CK2α, α-subunit of capping protein (CPα), PI3K/AKT, and caspase in multiple signaling pathways, controlling cell growth, apoptosis, differentiation, cytoskeleton and bone formation [30–36]. CKIP-1-deficient mice showed an increase in body weight, white adipose tissue gains, and a ~3-fold higher circulating leptin level than their WT counterparts when fed on a high-fat diet [37]. The disturbance of adipogenesis and the increase level of leptin can induce insulin resistance, and progress to type 2 diabetes mellitus [38,39]. Studies also demonstrated the potential involvement of CKIP-1 in regulating cell proliferation and inflammation [40–42]. Interestingly, OSS can also lead to cell proliferation and inflammation. Given the critical involvement of CKIP-1 in regulating adipogenesis, cell proliferation and inflammation, CKIP-1 is postulated to protect against DN and renal fibrosis. On account of the importance of OSS in diabetes, we are also interested in the involvement of CKIP-1 in regulating the Nrf2/ARE antioxidative stress pathway.

Given the above-mentioned notes, the study aimed to demonstrate whether CKIP-1 could inhibit HG-induced overproduction of FN and ICAM-1, and explored the mechanism underlying CKIP-1-mediated protection against DN with special focus on the Nrf2/ARE antioxidative stress pathway.