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p38 MAPK pathway is involved in high glucose-induced thioredoxin interacting protein induction in mouse mesangial cells

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ABSTRACT

Excessive reactive oxygen species (ROS) play a key role in the pathogenesis of diabetic nephropathy. The thioredoxin (TRX) system, a major thiol antioxidant system, regulates the reduction of intracellular ROS. Here we show that high glucose (HG) inhibits TRX ROS-scavenging function through p38 mitogen-activated protein kinase (MAPK)-mediated induction of thioredoxin interacting protein (TXNIP) in mouse mesangial cells (MMCs). Knockdown of TXNIP in MMCs reversed HG-induced reduction of TRX activity and inhibited HG-induced activation of p38 MAPK and increased synthesis of TGF-β1 and fibronectin. These data suggest that HG-induced overexpression of TXNIP in MMCs, which may be via the p38 MAPK pathway.

1. Introduction

Diabetic nephropathy (DN), as with other diabetic vascular complications, appears to be multifactorial in origin, involving a number of key pathways, including advanced glycation, activation of intracellular signaling molecules such as protein kinase C (PKC), and increased generation of reactive oxygen species (ROS) [1].

Hyperglycemia-induced production of ROS plays a crucial role in the development of DN [2]. In the kidney, a number of pathways that generate ROS such as glycolysis, specific defects in the polyol pathway, uncoupling of nitric oxide synthase, xanthine oxidase, NAD(P)H oxidase, and advanced glycation have been identified as potentially major contributors to the pathogenesis of diabetic kidney disease [3].

Thioredoxin (TRX) system (TRX, TRX reductase, and NADPH) is a ubiquitous thiol oxidoreductase system that regulates cellular reduction/oxidation (redox) status [4]. TRX interacting protein (TXNIP) also known as vitamin D3 up-regulated protein-1 or TRX binding protein-2, is the endogenous inhibitor of cellular TRX, inactivating its anti-oxidative function by binding to the redox-active cysteine residues [5]. Recent studies showed that TXNIP was found to be up-regulated by high glucose (HG) in mesangial cells, proximal tubular cells, and vascular smooth muscle cells [6–8]. The expression of TXNIP mRNA was increased in kidneys from both diabetic rats and patients with DN, and TXNIP mediated the glucose-induced impairment of TRX activity in cultured kidney cells [9].

Previous studies have demonstrated that p38 mitogen-activated protein kinase (MAPK) signal mediated the HG-induced upregulation of TXNIP expression in vascular smooth muscle cells and endothelial cells [8,10]. However, the role of p38 MAPK in HG-induced expression of TXNIP in glomerular mesangial cells is unknown. In the present study, we examined the activation of the TRX system in glucose-treated mouse mesangial cells (MMCs) and the role of p38 MAPK pathway. Meanwhile, we also investigated the effects of knockdown of TXNIP with small interference RNA on HG-induced production of ROS and activation of p38 MAPK in MMCs.

2. Materials and methods

2.1. Construction of pBAsi mU6 Neo TXNIP siRNA plasmid

Three TXNIP siRNA plasmids were constructed based on the U6 siRNA expression vector, pBAsi mU6 Neo (Takara, Mie, Japan),

Abbreviations: TXNIP, thioredoxin interacting protein; TRX, Thioredoxin; HG, high glucose; MMC, mouse mesangial cell; DN, diabetic nephropathy; MAPK, mitogen-activated protein kinase; ROS, reactive oxygen species

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which includes a mouse U6 promoter and an amp-resistance gene. The following sets of sense and antisense oligonucleotides were annealed and ligated into the vector: sense oligo 1: 5′-GATCCGGTG TGTGAAGTTACCCGAGTAGTGCTCCTGGTTGCTCGGGTAACTTCACAC ACCTTTTTTA-3′, antisense oligo 1: 5′-AGCTTAAAAAAGGTGTGTG AAGTTACCCGAGCAACCAGGAGCACTACTCGGGTAACTTCACACACC G-3′; sense oligo 2: 5′-GATCCGGGTGATGACATCTCCATCCTAGTGC TCCTGGTTGGGATGGAGATGTCATCACCCTTTTTTA-3′, antisense oligo 2: 5′-AGCTTAAAAAAGGGTGATGACATCTCCATCCCAACC AGGAG-CACTAGGATGGAGATGTCATCACCCG-3′; sense oligo 3: 5′-GATCCGA GTCATCCTTGATCTGCCCTAGTGCTCCTGGTTGGGGCAGATCAAGGAT-GACTCTTTTTTA-3′, antisense oligo 3: 5′-AGCTTAAAAAAGAGTCA TCCTTGATCTGCCCCAACCAGGAGCACTAGGGCAGATCAAGGATGACT CG-3′. To test the efficiency of the three pBAsi mU6 Neo TXNIP siRNA plasmids, MMcs cultured under HG conditions were transfected with the plasmids. Knockdown of gene expression was confirmed by RT-PCR, and TXNIP protein expression was also evaluated by Western blot. The most effective plasmid (oligo 1) was used for the study.

2.2. Cell culture and transfection

MMC (ATCC No. CRL-1927) were obtained from American Type Culture Collection (Manassas, VA). They were cultured in DMEM-F12 medium (3:1) supplemented with 5% fetal bovine serum, 2 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin in a 95% air, 5% CO_2 atmosphere. Stable transfections of MMCs with pBAsi mU6 Neo TXNIP siRNA plasmid or pBAsi mU6 Neo plasmid were performed with Lipofectamine 2000 (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. Subsequently, cells were cultured in selection medium containing 0.3 mg/ml geneticin for three weeks before single clones were isolated. The clones were further expanded in selection medium containing geneticin (0.3 mg/ml). Scrambled pBAsi mU6 Neo plasmid was used as control. MMCs were grown to 75–85% confluence, washed once with serum-free DMEM-F12 medium, and then growth-arrested in serum-free DMEM-F12 medium in normal glucose (NG, 5.6 mM) for 24 h to synchronize the cell growth. After this time period, the media were changed to fresh serum-free media containing NG, HG (30 mM), NG plus mannitol (24.4 mM) as an osmotic control, HG plus Tempol (100 nM, Sigma, St. Louis, MO) or HG plus SB203580 (10 uM, Promega, Madison, WI) stimulation at indicated time points.

2.3. Intracellular ROS detection

The intracellular formation of ROS was detected using the fluorescence probe 5-(and 6) chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate (CM-DCHF-DA, Invitrogen, Carlsbad, CA). After cultured in 6-well plates under the different experimental conditions for 0–48 h, the cells were washed, trypsinized, suspended in PBS, loaded with 10 uM DCHF-DA, and incubated at 37 °C for 30 min. The measurement of intracellular ROS was performed using a flow cytometer (BD Immunocytometry Systems, Franklin Lakes, NJ).

2.4. RT-PCR analysis

Total RNA and then cDNA were prepared from cultured cells using TRIzol reagent (Invitrogen) and RT-PCR kits (Promega). The primers used were: TXNIP, forward 5′- CAAGCCCTGACTTTACGGAG -3′, reverse 5′- ACCATCTCGTTCTCACCTGC -3′, giving 374 bp PCR

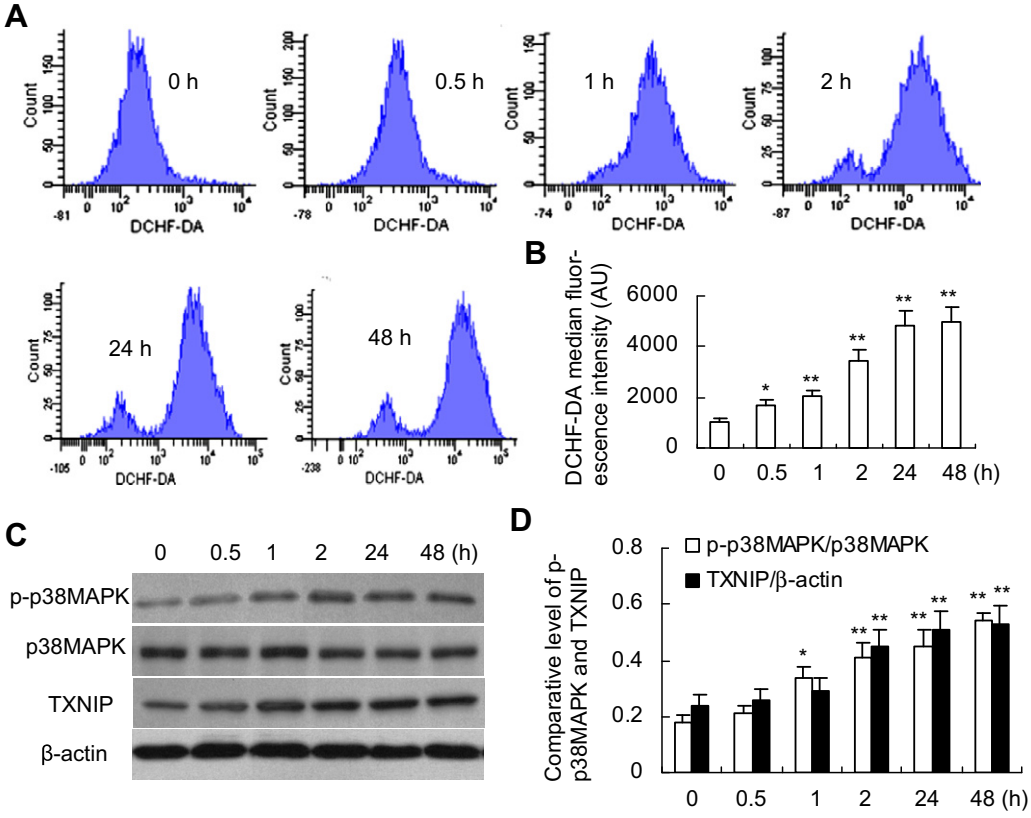

Fig. 1. Time course of the effcets of HG on ROS production, activation of p38 MAPK and expression of TXNIP. MMCs were incubated with HG (30 mM) at the indicated times (0–48 h). (A and B) Intracellular ROS was detected by flow cytometry. (C and D) The expression levels of p38 MAPK, phospho-p38 MAPK and TXNIP were analyzed by Western blot ($n = 6$). Values are expressed as means ± S.E.M. $^{**}P < 0.05$, $^{**}P < 0.01$ vs. control (0 h).