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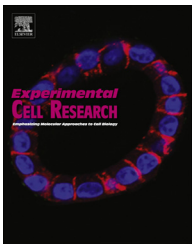

Research Article

High glucose increases Cdk5 activity in podocytes via transforming growth factor-β1 signaling pathway

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ABSTRACT

Podocytes are highly specialized and terminally differentiated glomerular cells that play a vital role in the development and progression of diabetic nephropathy (DN). Cyclin-dependent kinase 5 (Cdk5), who is an atypical but essential member of the Cdk family of proline-directed serine/threonine kinases, has been shown as a key regulator of podocyte differentiation, proliferation and morphology. Our previous studies demonstrated that the expression of Cdk5 was significantly increased in podocytes of diabetic rats, and was closely related with podocyte injury of DN. However, the mechanisms of how expression and activity of Cdk5 are regulated under the high glucose environment have not yet been fully elucidated. In this study, we showed that high glucose up-regulated the expression of Cdk5 and its co-activator p35 with a concomitant increase in Cdk5 kinase activity in conditionally immortalized mouse podocytes in vitro. When exposed to 30 mM glucose, transforming growth factor-β1 (TGF-β1) was activated. Most importantly, we found that SB431542, the Tgfbr1 inhibitor, significantly decreased the expression of Cdk5 and p35 and Cdk5 kinase activity in high glucose-treated podocytes. Moreover, high glucose increased the expression of early growth response-1 (Egr-1) via TGF-β1-ERK1/2 pathway in podocytes and inhibition of Egr-1 by siRNA decreased p35 expression and Cdk5 kinase activity. Furthermore, inhibition of Cdk5 kinase activity effectively alleviated podocyte apoptosis induced by high glucose or TGF-β1. Thus, the TGF-β1-ERK1/2-Egr-1 signaling pathway may regulate the p35 expression and Cdk5 kinase activity in high glucose-treated podocytes, which contributes to podocyte injury of DN.

Introduction

Diabetic nephropathy (DN) is one of the most serious microvascular complications of diabetes and the leading cause of end-stage renal failure [1]. DN is characterized by specific renal morphological and functional alterations. Glomerular visceral epithelial cells, namely podocytes, are terminally differentiated cells overlying the outer aspect of the glomerular basement membrane of renal glomeruli

Abbreviations: Cdk, Cyclin-dependent kinase; DN, Diabetic nephropathy; Egr, Early growth response; ERK, Extracellular signal-regulated kinase; MEK, Mitogen-activated protein kinase/ERK kinase; siRNA, Small interference RNA; TGF-β1, Transforming growth factor-β1.

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and play a vital role in renal physiology. Among the characteristic findings of DN, podocytes are involved in the development of glomerular hypertrophy, podocytopenia, glomerulosclerosis, and foot process effacement [2,3]. Because podocyte depletion may be a key initiating lesion in these processes, it is considered important to determine the underlying molecular and cellular mechanisms.

Cyclin-dependent kinases (Cdks) play essential roles in the regulation of cell division cycle. Cyclin-dependent kinase 5 (Cdk5) is a proline-directed serine/threonine kinase that belongs to the family of Cdks. Distinct from other members of the Cdk family, the activation of Cdk5 does not require binding to cyclins, but rather, association with its regulator to perform kinase activity [4]. One major regulating partner for Cdk5 is p35, which was first reported in postmitotic neurons. The crucial role of the Cdk5-p35 complex is to support the development of the central nervous system [5]. However, numerous extraneuronal functions of Cdk5-p35 have been discovered in recent years, in addition to the roles in the central nervous system [6]. For example, in the kidney, the glomerular expression of Cdk5 is limited to podocytes. Moreover, Cdk5 is a key regulator of podocyte differentiation, proliferation and morphology [7]. Recent studies have shown that the absence of p35 confers increased susceptibility of podocytes to apoptosis in disease [8]. Our previous work has shown that the expression of Cdk5 was increased in a time-dependent manner and roscovitine; a Cdk5 inhibitor, significantly ameliorated podocyte injuries in diabetic rats [9]. Furthermore, in cultured podocytes in vitro, high glucose increased the expression of Cdk5, and knockdown of Cdk5 attenuated podocyte apoptosis induced by high glucose stimulation [10]. These findings suggest that Cdk5 plays an important role in multiple mechanisms involved in podocyte injuries of DN. However, the mechanisms of how expression and activity of Cdk5 are regulated under the high glucose environment have not yet been elucidated.

Transforming growth factor-β1 (TGF-β1) is an important member of a superfamily of multifunctional growth factors involved in many cellular processes including cell proliferation, differentiation, migration, and apoptosis. TGF-β1 has been proposed as a major mediator of matrix accumulation in diabetic kidney and high glucose-exposed mesangial cells and podocytes, leading to the development of DN [11,12]. Moreover, increased expression of TGF-β1 in podocytes coincides with the onset of apoptosis and albuminuria in diabetes [13]. Correspondingly, the increase of the TGF-β1 levels has been recognized as a marker of DN [14,15]. Because TGF-β1 has been shown to act as a mediator of Cdk5 activity in sensory neurons [16], we want to know under the high glucose environment, whether TGF-β1 could act as an upstream regulator of Cdk5 in podocytes, affecting the development and progression of DN.

In this study, we investigated that the role of TGF-β1 on Cdk5 expression and activity in high glucose-treated podocytes. We found that high glucose and TGF-β1 up-regulated the expression of Cdk5 and its co-activator p35 with a concomitant increase in Cdk5 kinase activity. SB431542, the Tgfbr1 inhibitor, significantly decreased the expression of Cdk5 and p35 and Cdk5 kinase activity. Furthermore, the ERK1/2-Egr-1 signaling pathway was involved in the regulation of TGF-β1 on p35 and Cdk5 in high glucose-cultured podocytes. More importantly, inhibition of Cdk5 kinase activity was demonstrated to be effective in alleviating podocyte apoptosis induced by high glucose or TGF-β1.

Materials and methods

Antibodies and reagents

Primary antibodies recognizing Cdk5 and Egr-1 were purchased from Epitomics Company (CA, USA). Antibody to p35 was purchased from Santa Cruz Biotechnology (CA, USA). Antibodies to TGF-β1, Smad-2, phospho-Smad-2 and β-actin were purchased from Signalway Antibody Company (Maryland, USA). Antibodies against p44/42 MAPK (ERK1/2) and phospho-p44/42 MAPK (ERK1/2, Thr202/Tyr204) were obtained from Cell Signaling Technology (MA, USA). Tgfbr1 inhibitor SB431542, the MEK inhibitor U0126 and Cdk5 kinase activity inhibitor roscovitine were purchased from Sigma Aldrich (Dorset, UK). Recombinant murine IFN-γ was purchased from Peprotech Company (NJ, USA).

Conditionally immortalized mouse podocytes in culture

Conditionally immortalized mouse podocytes purchased from the Cell Culture Center (PUMC, CAMS, Beijing, China) were cultured as previously described [17]. To induce proliferation, cells were grown on collagen I-coated plastic culture bottles (BD Biosciences, Bedford, MA), at 33 °C in Dulbecco's modified Eagle's medium (DMEM, Gibco BRL, Gaithersburg, MD, USA) supplemented with 10% fetal bovine serum (FBS, Gibco BRL, USA), 100 U/ml penicillin (Invitrogen, USA), and 100 μg/ml streptomycin (Invitrogen, USA), to which recombinant mouse IFN-γ 10 U/ml (Pepro Tech, USA) was added (growth permissive conditions). To induce quiescence and the differentiated phenotype, podocytes were grown at 37 °C and deprived of IFN-γ (growth restrictive conditions) in DMEM supplemented with 10% FBS, penicillin, and streptomycin. All studies were performed on days 10 to 14 for cells grown under restrictive conditions.

RNA interference analysis

Conditionally immortalized mouse podocytes grown in 6-well plates for 24 h in DMEM with 10% FBS were transfected with siRNA against Smad2 (Smad2 siRNA, sc-38375, Santa Cruz, USA), Egr-1 (Egr-1 siRNA, sc-35267, Santa Cruz), or control siRNA (sc-37007, Santa Cruz) with Lipofectamine RNAi MAX (Invitrogen, USA) as per the manufacturer's protocol. After 24 h transfection, the cells were treated with high glucose and then were analyzed.

Immunocytochemistry

When high glucose treated for 12 h, podocytes were fixed with 4% paraformaldehyde at room temperature for 15 min. After pretreatment with 0.1% Triton X-100 for 10 min at 37 °C, cells were blocked with goat serum for 30 min at 37 °C. Then, the cells were incubated with rabbit anti-Cdk5 (1:200) antibody overnight at 4 °C. After three washes with PBS, cells were incubated with a polymer helper and polyperoxidase-anti-mouse/rabbit IgG at 37 °C for 30 min, and the cells were then stained with diaminobenzidine. A negative control was performed by replacing the primary antibody with PBS buffer.