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Klotho attenuates high glucose-induced fibronectin and cell hypertrophy via the ERK1/2-p38 kinase signaling pathway in renal interstitial fibroblasts

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ABSTRACT

Although exogenous klotho attenuates renal fibrosis, it is not known if exogenous klotho attenuates diabetic nephropathy (DN). Thus, we studied the anti-fibrotic mechanisms of klotho in terms of transforming growth factor-β (TGF-β) and signaling pathways in high glucose (HG, 30 mM)-cultured renal interstitial fibroblast (NRK-49F) cells. We found that HG increased klotho mRNA and protein expression. HG also activated TGF-β Smad2/3 signaling and activated extracellular signal-regulated kinase (ERK1/2) and p38 kinase signaling. Exogenous klotho (400 pM) attenuated HG-induced TGF-β bioactivity, type II TGF-β receptor (TGF-βRII) protein expression and TGF-β Smad2/3 signaling. Klotho also attenuated HG-activated ERK1/2 and p38 kinase. Additionally, klotho and inhibitors of ERK1/2 or p38 kinase attenuated HG-induced fibronectin and cell hypertrophy. Finally, renal tubular expression of klotho decreased in the streptozotin-diabetic rats at 8 weeks. Thus, exogenous klotho attenuates HG-induced profibrotic genes, TGF-β signaling and cell hypertrophy in NRK-49F cells. Moreover, klotho attenuates HG-induced fibronectin expression and cell hypertrophy via the ERK1/2 and p38 kinase-dependent pathways.

1. Introduction

Diabetic nephropathy (DN) is characterized by renal hypertrophy and an expansion of extracellular matrices which results in renal fibrosis (Chuang and Guh, 2001). Fibroblast activation is important in the pathogenesis of diabetic renal fibrosis (Meran and Steadman, 2011). Additionally, we and others have found that high glucose (HG) activates renal fibroblasts while inducing collagen, fibronectin, transforming growth factor-β (TGF-β), extracellular signal regulated kinases (ERK1/2), p38 kinase and cell hypertrophy (Chao et al., 2010; Chuang et al., 2006; Wu and Derynck, 2009).

Fibroblast growth factor 23 (FGF23), a phosphaturic hormone induced by vitamin D, is mainly produced by bone (Hu et al., 2013; Olauson and Larsson, 2013). Klotho, a co-receptor for FGF23, is mainly expressed in the distal renal tubules (Hu et al., 2013; Olauson and Larsson, 2013). It can also be secreted into the circulation (secretory klotho) through ectcodomain shedding from the cell surface and acts independent of FGF23 (Hu et al., 2013; Olauson and Larsson, 2013). Interestingly, deletion of renal klotho increases FGF23 levels (Olauson and Larsson, 2013) whereas exogenous FGF23 increases renal klotho levels (Takenaka et al., 2013).

Abbreviations: DN, diabetic nephropathy; TGF-β, transforming growth factor-β; HG, high glucose; ERK, extracellular signal-regulated kinase; TGF-βR, TGF-β receptor; FGF23, fibroblast growth factor 23; DMEM, Dulbecco's Modified Eagle Medium; SDS, sodium dodecyl sulfate; FBS, fetal bovine serum; GFP, green fluorescence protein; RT-PCR, reverse transcriptase polymerase chain reaction; TCA, trichloroacetic acid; PBS, phosphate-buffered saline.

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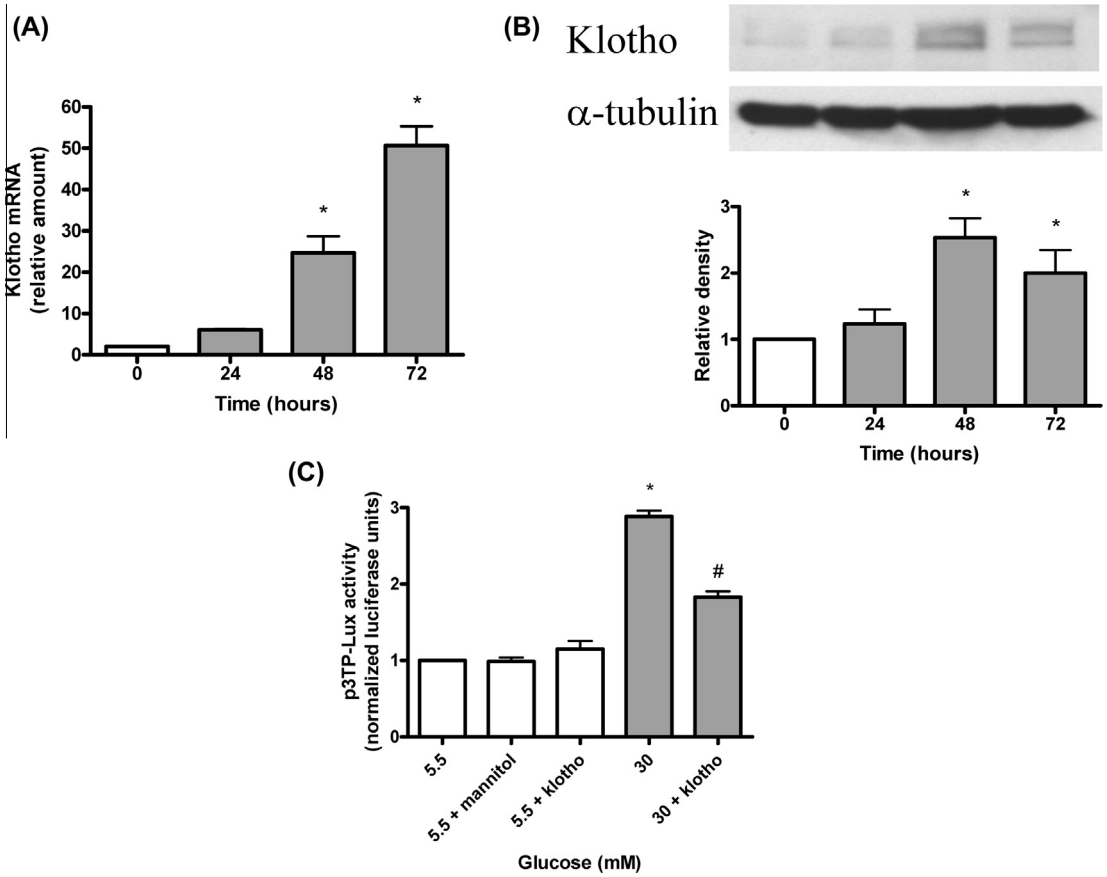

Fig. 1. Effects of high glucose (HG) on klotho mRNA and protein expression and effects of klotho on HG-induced TGF-β bioactivity in NRK-49F cells. Cells were treated with normal glucose (5.5 mM glucose, open bars) or HG (30 mM glucose, closed bars) at the indicated time points. (A) Klotho mRNA was measured by real-time PCR and the density was normalized to that of 18S rRNA. (B) Klotho protein was measured by immunoblotting and the density was normalized to that of α-tubulin. (C) Effects of HG or mannitol (24.5 mM in 5.5 mM glucose, open bar) on TGF-β bioactivity. Cells were transfected with p3TP-Lux plasmid and luciferase activity was measured. These experiments were repeated at least three times and the values were expressed as the means ± SEM. $^{*}P < 0.05$ versus lane 1. $^{\#}P < 0.05$ versus HG alone.

Renal FGF23 increases whereas renal klotho expression decreases in chronic kidney disease (Olauson and Larsson, 2013). Renal klotho expression also decreases in experimental DN (Asai et al., 2012; Ishizaka et al., 2007; Zhao et al., 2011) and deletion of klotho exacerbates experimental DN while increasing renal TGF-β signaling (Lin et al., 2013). Interestingly, deletion of klotho also aggravates renal fibrosis while increasing renal TGF-β levels (Sugiura et al., 2012) whereas exogenous klotho attenuates renal fibrosis in mice with klotho deletion or unilateral ureteral obstruction (Chen et al., 2013; Doi et al., 2011; Zhou et al., 2013). Klotho also attenuates TGF-β signaling whereas TGF-β decreases klotho expression in renal tubular cells (Sugiura et al., 2012; Zhao et al., 2011; Zhou et al., 2013). However, it is not known if exogenous klotho attenuates DN. Moreover, the molecular mechanisms of klotho's anti-fibrotic effects in DN remain unknown.

Although almost all intrinsic renal cells are involved in the pathogenesis of diabetic nephropathy (Chuang and Guh, 2001), the progression of renal insufficiency is most closely correlated to the degree of tubular atrophy and interstitial fibrosis (Meran and Steadman, 2011). Thus, our purpose is to study the role of klotho in diabetic kidney fibrosis in vitro. Because fibroblasts are central to the process of fibrosis (Meran and Steadman, 2011) while klotho decreases TGF-β-induced fibrosis markers in renal fibroblast (NRK-49F) cells (Sugiura et al., 2012), we hypothesize that exogenous klotho may be anti-fibrotic in HG-cultured NRK-49F cells. Thus, one of the specific aims of this study is to test the effects of exogenous klotho on HG-induced TGF-β, ERK1/2 or p38 kinase signaling. The other aim is to test the effects of klotho on HG-induced cell hypertrophy and expression of fibrosis-related fibronectin and collagen.

2. Materials and methods

2.1. Materials

Dulbecco's Modified Eagle Medium (DMEM), antibiotics, trypsin–EDTA, trypan blue stain, and all medium additives were obtained from GIBCO (Grand Island, NY). Antibodies to type I TGF-β receptor (TGF-βRI), TGF-βRII, phospho-ERK1/2 (p-ERK1/2), ERK1/2, p-p38 kinase, p38 kinase and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Recombinant mouse secretory klotho protein and anti-klotho antibody were purchased from R&D Systems (Minneapolis, MN). Klotho was dissolved in phosphate-buffered saline. Anti-α-tubulin antibody was obtained from Lab Vision Corporation (Fermont, CA). Antibodies to fibronectin, phospho-Smad2/3 and Smad2/3 were obtained from Millipore (Billerica, MA). PD98059 and SB203580 were purchased from Calbiochem (La Jolla, CA). Lipofetamine 2000 was obtained from Invitrogen Corp. (Grand Island, NY). Horseradish peroxidase-conjugated goat anti-rabbit or anti-mouse secondary antibody, streptavidin-peroxidase, L-[2,3,4,5-^{3}H]proline, [γ-^{32}P]ATP, and the enhanced chemiluminescence kit were obtained from Amersham