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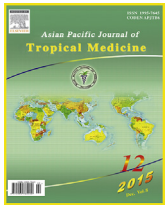

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AEG-1 participates in high glucose-induced activation of Rho kinase and epithelial–mesenchymal transition in proximal tubular epithelial cells

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ABSTRACT

Objective: To prove whether astrocyte elevated gene-1 (AEG-1) plays a role in high glucose-stimulated Rho kinase activation and epithelial–mesenchymal transition (EMT) in human renal tubular epithelial (HK-2) cells.
Methods: The protein levels of AEG-1, alpha-smooth muscle actin, E-cadherin and MYPT1 were determined by Western blot.
Results: AEG-1 protein level was upregulated in HK-2 cells stimulated with high glucose. AEG-1 siRNA downregulated Rho kinase protein expression and blocked high glucose-induced EMT.
Conclusions: Our results show that AEG-1 acts a key role in high glucose-induced activation of Rho kinase and EMT in HK-2 cells.

1. Introduction

Tubulointerstitial fibrosis is a progressive pathomechanism in most chronic renal disorders. Excessive generation of extracellular matrix from the activated interstitial myofibroblasts takes part in the pathoganism of kidney interstitial fibrosis. Accumulating studies indicate that these myofibroblasts might come from tubular epithelial cells through epithelial-to-mesenchymal transition (EMT) [1,2]. EMT is a process of losing polygonal shape and obtaining the myofibroblast phenotype [3] and is thought to participate in the process of renal tubulointerstitial fibrosis [1,2].

Astrocyte elevated gene-1 (AEG-1) was first discovered in primary human fetal astrocytes induced by HIV-1 or tumor necrosis factor-α [4]. Recent studies confirmed that AEG-1 shows an abnormally high expression in many malignant tumors [5,6] and plays a major role in cellular transformation, apoptosis inhibition, invasion, metastasis, angiogenesis and resistance to chemotherapeutic agents via activation of various signaling pathways [7–12]. Our previous report demonstrated that AEG-1 takes part in EMT process induced by TGF-beta1 [13].

Rho kinase, members of the Ras superfamily, are major effectors of small GTPase RhoA. Rho kinase act an crucial role in organization of actin cytoskeleton, cellular contraction, adhesion, migration and proliferation [14,15]. According to the previous reports, Rho kinase also takes part in the process of EMT, while the exact mechanism remains to be elucidated. Our present study is to find out whether AEG-1 participates in high glucose-induced Rho kinase upregulation and EMT in HK-2 cells.

2. Materials and methods

2.1. Cell culture

Human proximal tubular (HK-2) cell was bought from cell bank of Xiangya Central Experiment Laboratory and prepared as described previously [13]. The protein expressions of α-smooth muscle actin (SMA), E-cadherin and AEG-1 were detected by Western blot after incubation with 5.5 mM, 25 mM D-glucose or 5.5 mM D-glucose combined with 19.5 mM mannitol served as

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hyperosmosis for 48 h. In addition, HK-2 cells were incubated with high glucose for 48 h with or without AEG-1 siRNA transfection (Shanghai R&S Biotechnology, Co., Ltd).

2.2. RNA interference

AEG-1 (MTDH) siRNA oligonucleotide were synthesized by Shanghai R&S Biotechnology, Co. Ltd. No. of Gene Bank: NM_178812.3, F: 50-GACACUGGAGAUGCU AAUAUU-30, R: 50-UAUUAGCAUCUCCAGUGUCUU-30. Lipofectamine 2000 reagent (Invitrogen, Grand Island, NY, USA) was used to introduce siRNA into cells. Cells were stimulated with 5.5 mM, 25 mM D-glucose or 5.5 mM D-glucose combined with 19.5 mM mannitol for 48 h after transfected into HK-2 cells for five hours.

2.3. Western blot

Protein was prepared as described previously [13] and detected with antibodies against AEG-1 (Abcam), E-cadherin (BD Biotechnology), α-SMA (Sigma), MYPT1 (Santa Cruz Biotechnology) and beta-actin (Sigma).

2.4. Statistical test

Data were shown as means ± SD. Statistical test was conducted by ANOVA, and significance was defined as $P < 0.05$.

3. Results

3.1. AEG-1 involved in high glucose-stimulated EMT in HK-2 cells

High glucose upregulated the protein levels of AEG-1 and α-SMA, but downregulated E-cadherin protein expression when compared with the control. AEG-1 siRNA potently reversed high glucose-induced EMT (Figure 1).

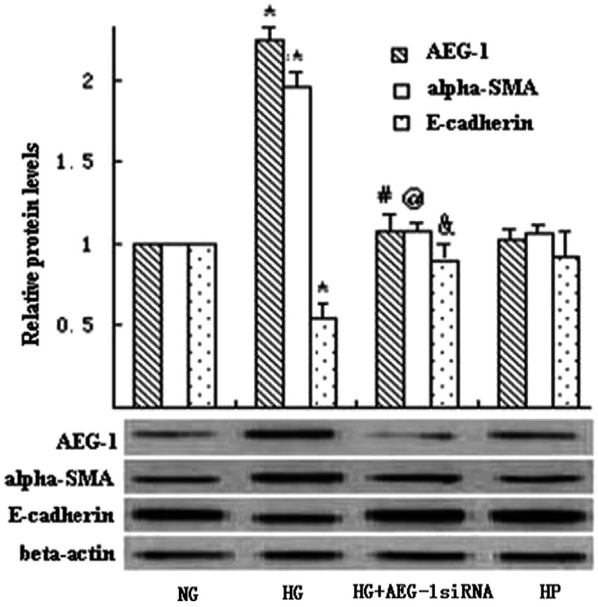

Figure 1. High glucose upregulated the expression of AEG-1 and induced EMT in HK-2 cells. AEG-1 depletion inhibited high glucose-induced EMT. $^{*}P < 0.001$ compared with the NG cells. $^{\#}P < 0.001$ compared with the HK-2 cells treated with high glucose and AEG-1 siRNA. $^{@}P = 0.001$ compared with the HK-2 cells treated with high glucose and AEG-1 siRNA. $^{\&}P < 0.01$ compared with the HK-2 cells treated with high glucose and AEG-1 siRNA. HP (5.5 mM D-glucose combined with 19.5 mM mannitol served as hyperosmosis).

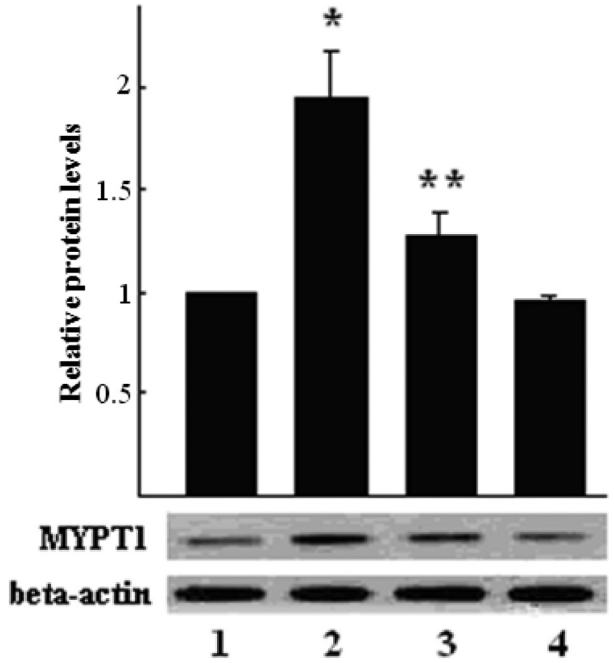

Figure 2. AEG-1 participated in high glucose-induced activation of Rho kinase.
Western blot showed that high glucose significantly increased the protein expression of MYPT1 in HK-2 cells after 48 h of treatment and it could be strikingly inhibited by AEG-1 siRNA. $^{*}P < 0.05$ compared with NG cells. $^{**}P < 0.05$ compared with HG cells. 1. NG; 2. HG; 3. HG+ AEG-1 siRNA; 4. HP (5.5 mM D-glucose combined with 19.5 mM mannitol served as hyperosmosis).

3.2. AEG-1 participation in activation of Rho kinase stimulated by high glucose

High glucose significantly increased MYPT1 protein expression in HK-2 cells, which could be markedly reversed by AEG-1 siRNA (Figure 2).

4. Discussion

Our results hold the hypothesis that AEG-1 acts a major effect in high glucose-stimulated EMT; while EMT, epithelial cell phenotypic changes include losing their polygonal morphology, expressing alpha-SMA and vimentin, and the inhibition of E-cadherin [16]. At present, our findings showed that high glucose upregulated AEG-1 and α-SMA protein levels, but downregulated the protein levels of E-cadherin when compared with the control. Knockdown of AEG-1 potently reversed high glucose-induced EMT, suggesting that AEG-1 is helpful to high glucose-stimulated EMT.

Based on the previous reports, Rho kinase is involved in cytoskeletal arrangement [15], EMT [13] and in renal injury such as renal fibrosis in unilateral ureteral obstructive kidney disease [17], and ischemic acute renal failure [18]. Nagatoya K *et al.* [19] reported that Y-27632, a specific ROCK inhibitor, blocks interstitial fibrosis in mouse kidneys with unilateral ureteral obstruction. In subtotally nephrectomized spontaneously hypertensive rats, fasudil might be a treatment optimism for renal injury in part through improving tubulointerstitial

inflammation [20]. Meanwhile, Rho kinase also regulates angiotensin II-stimulated kidney lesion [21]. In this study, we also found out that AEG-1 depletion inhibited high glucose-induced Rho kinase activation, indicating that AEG-1 is involved in high glucose-mediated upregulation of Rho kinase in proximal tubular epithelial cells.

Our results first show that tubular EMT-induced by high glucose is closely associated with Rho kinase signal pathway and AEG-1 activation. From these observations, we aim to recognize its role in AEG-1 activation stimulated by high glucose and AEG-1 may be a strategic target for therapeutic development against high glucose-induced EMT. Moreover, AEG-1 depletion does not completely block EMT process, indicating that other signal events might be involved.

In conclusion, we display that AEG-1 involves in Rho kinase activation and EMT stimulated by high glucose in HK-2 cells.

Conflicts of interest statement

The authors have no financial conflicts of interest.

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