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Original Article

The role of IQGAP1 in high glucose-induced apoptosis of podocytes and its possible mechanisms

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Abstract: The apoptosis of podocytes may contribute to the development of diabetic nephropathy (DN). MAPK signaling pathway had a vital role in apoptosis. Meanwhile, IQ domain GTPase-activating protein 1 (IQGAP1) is an important scaffolding protein regulating MAPK signaling pathway. So we aimed to investigate the role of IQGAP1 under the medium of high glucose (HG). In vitro, human podocyte cells (HPC) were divided into 5 different groups named A-E and treated with glucose with normal concentration as control, mannitol, HG, HG with benazepril and HG with u0126, respectively. Expression of IQGAP1 was measured by quantitative real-time PCR (Q-PCR), western blotting and immunofluorescence assay, respectively. Apoptosis of HPC was detected by flow cytometry. The content of ERK1/2 and p-ERK1/2 were measured by western blotting. In vitro, HG suppressed the expression of IQGAP1, increased the level of p-ERK1/2 and contributed to the apoptosis of HPC. But benazepril, an angiotensin converting enzyme inhibitor, can attenuate the suppression of IQGAP1, down-regulated the level of p-ERK1/2 and attenuated the apoptotic rate of HPC caused by HG. Meanwhile, u0126, the ERK1/2 activation inhibitor, had no effect on HG induced suppression of IQGAP1, but significantly reduced the apoptotic rate of HPC. In conclusion, HG contributed to the apoptosis of HPC accompanied with the down-regulation of IQGAP1 and the increasing content of p-ERK. Benazepril could attenuate the HG-induced apoptosis of HPC and up-regulated the expression of IQGAP1.

Keywords: High glucose, HPC, apoptosis, IQGAP1, ERK1/2 proteins, p-ERK1/2 protein

Introduction

Diabetic nephropathy (DN) is one of the major complications of diabetes and becoming the leading cause of end-stage kidney disease worldwide. DN is characterized by the expansion of extracellular matrix in the glomeruli, and finally results in proteinuria and renal failure. The underlying pathogenesis of DN remains unclear. Recent investigations reported that the apoptosis of podocytes, a type of glomerular epithelial cell, can contribute to the progress of DN [1, 2]. Furthermore, high-glucose (HG) can induce the apoptosis of podocytes, and several investigations have been conducted to explore the underlying mechanisms of this physiological phenomenon [3, 4].

IQ motif-containing GTPase activating protein 1 (IQGAP1) is a scaffolding protein containing a variety of protein-interacting domains [5-7]. It has been proved that IQGAP1 has multiple functions in regulating biological process, such as cell proliferation [8], cell-cell adhesion [9], cytoskeletal architecture [10] and selected signaling pathways [11]. Recently, a rising number of investigations demonstrated that IQGAP1 can binds with extracellular signal-regulated kinase 1/2 (ERK1/2) and modulates its activity [8, 12]. It is well known that many apoptosis process can be activated or inhibited by the regulation of ERK1/2 [13-15]. So we suspected that whether HG induces the apoptosis of podocytes through MEK-ERK1/2 pathway regulated by IQGAP1. Moreover, HG stimulated the generation of angiotensin II (AngII) [16], and researches demonstrated that AngII can induce the podocytic apoptosis under unclear mechanisms [17, 18]. So we supposed that IQGAP1 may play a vital role in regulating podocytic apoptosis associated with the regulation of AngII and ERK1/2.

In this present study, we investigated the expression changes of IQGAP1 under the treatment of HG, and explored how AngII influenced on IQGAP1 under HG, as well as how IQGAP1 regulated ERK1/2 pathways in HPC in vitro.

Material and methods

Cell culture and treatments

Conditionally, human podocytes (HPC) were a kind gift provided by Department of Medicine, Shandong University, China. HPC were incubated in RPMI 1640 medium containing 10% Fetal Bovine Serum (FBS) and γ-interferon (10 U/ml) and cultured in incubators under 33°C and with a content of 5% CO_2 for proliferation. Then cells passage culture were continued with RPMI 1640 medium only containing 10% FBS under the conditions of 37°C and 5% CO_2 containing. After incubated for 10 to 14 days, the differentiated HPC were collected for subsequent experiments. After pretreatment of the collected HPC, the HPC were divided into five groups named A-E and treated as follows: glucose (5.5 mmol/L), mannitol (24.5 mmol/L) and glucose (5.5 mmol/L), high glucose (30 mmol/L glucose), high glucose (30 mmol/L glucose) and benazepril (10 μmol/L), high glucose (30 mmol/L glucose) and u0126 (10 μmol/L), among which group A was used as control.

Quantitative real-time PCR

Total RNA were separately extracted when treated for 0 h, 24 h and 48 h using Trizol reagent (SunShineBio, Shanghai, China). To obtain the cDNA, the RNA samples were treated with a reverse transcription system (SunShineBio, Shanghai, China). Then the obtained cDNA were utilized as templates for quantitative real-time PCR (Q-PCR) analysis. Q-PCR was employed to detect gene expression levels. In this study, β-actin was used as internal standard. The primers sequences were as follows: IQGAP1, forward 5'-AGAACGTGGCTTATGAGTACCT-3' and reverse 5'-CCAGTCGCCTTGTATCTGGT-3'. β-actin, forward 5'-CAGTCGGTTGGAGCGAGCAT-3' and reverse 5'-GGACTTCCTGTAACAACGCATCT-3'. Q-PCR was performed with the 2×SYBR qPCR Mix (Promega, Beijing, China) in a 20 μL volume using a Line Gene 9600 Real-time System. The amplification procedures were as follows: pre-denaturation at 95°C for 3 min; 40 cycles at 95°C for 15 sec, 60°C for 1 min; and a single melt cycle from 60°C to 95°C.

Immunofluorescence assay

This step was conducted when HPC were treated by A-E for 0 h, 12 h, 24 h, 48 h and 72 h. Cell suspension were dropped onto a glass slide and cultured for 2 h, then 2 mL cell culture medium was added and continued to incubate for 6 h. After fixing, 50-100 μL permeabilization was added and incubated for 10 min. Then HPC were incubated with anti-IQGAP1 antibody (1:200, Abcam, Cambridge, Britain) overnight at 4°C. Subsequently, they were subjected to FITC-conjugated goat anti-rabbit IgG (1:150, JINGKEBIO, Anhui, China) for 50 min at 37°C in darkness. And finally they were treated with anti-fade fluorescence mounting medium with DAPI (HelixGen, Guangzhou, China). Cells were washed by phosphate buffered saline (PBS) in each step. All microscopic images were collected by upright fluorescence microscope (Nikon Eclipse CI, Tokyo, Japan).

Western blotting

HPC were cultured in RPMI 1640 medium with 10% FBS, penicillin and str. Total protein were extracted separately when incubated for 0 h, 24 h and 48 h under the treatment of A-E. The supernatants were collected by centrifuge at 12,000 rpm, 4°C for 20 min after HPC were treated by lysis buffer which was made up referred to Liu et al. [19]. Each sample was boiled and mixed with sodium dodecyl sulfate (SDS) loading buffer. Equal amount of samples were separated by SDS- polyacrylamide gel with a 5% spacer gel and a 12% separation gel, respectively. Then electrophoretic gels were transferred into a polyvinylidene fluoride (PVDF) membrane (Millipore, Shanghai, China). Membranes were dipped with TBS buffer (50 mmol/L Tris, 15 mmol/L NaCl) from the bottom up and block for 1 h. Then membranes were incubated by anti-ERK (Cell Signaling Technology, USA), anti-p-ERK (Cell Signaling Technology, USA) and anti-IQGAP1 antibodies (1:1000) overnight at 4°C, respectively. A horseradish peroxidase-conjugated goat anti-rabbit IgG (SunShineBio, Shanghai, China) were utilized as secondary antibody (1:1000). After incubated for 1 h, images were collected by gel imaging system (Jieda, Nanjia, China). The integrated optical density of each band was calcu-

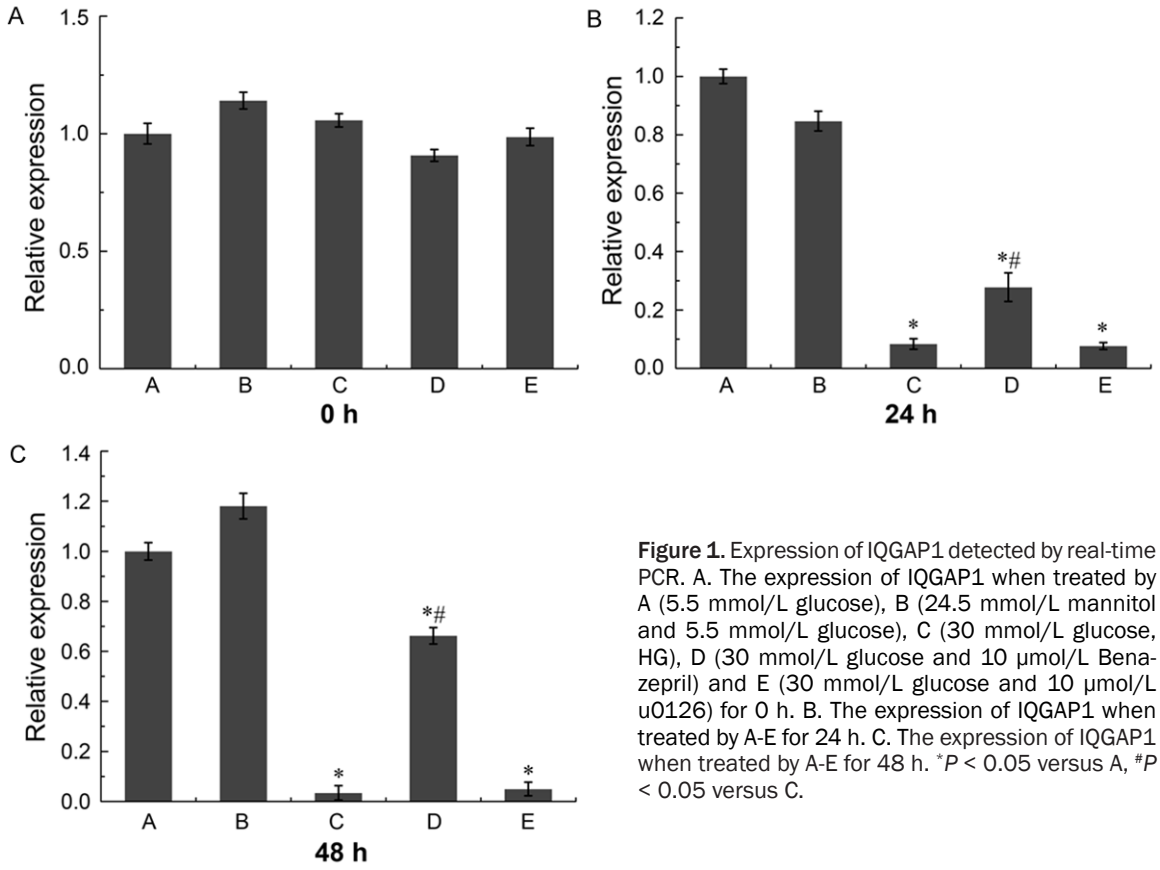

Figure 1. Expression of IQGAP1 detected by real-time PCR. A. The expression of IQGAP1 when treated by A (5.5 mmol/L glucose), B (24.5 mmol/L mannitol and 5.5 mmol/L glucose), C (30 mmol/L glucose, HG), D (30 mmol/L glucose and 10 μmol/L Benazepril) and E (30 mmol/L glucose and 10 μmol/L u0126) for 0 h. B. The expression of IQGAP1 when treated by A-E for 24 h. C. The expression of IQGAP1 when treated by A-E for 48 h. $^{*}P < 0.05$ versus A, $^{\#}P < 0.05$ versus C.

lated by software that affiliated to gel imaging system.

Measurement of apoptosis

HPC apoptosis were detected by Annexin V-FITC Apoptosis Detection Kit (KeyGEN BioTECH, Nanjing, China). The propidium iodide (PI) and fluorescein isothiocyanate (FITC) conjugated annexin V was mainly used to identify apoptotic cells under different treatments at 0 h, 24 h and 48 h. The apoptotic cells were analyzed by Becton Dickinson FACS Calibur and CELLQuest software version 3.0 (USA). Cells in the lower-left quadrant were normal living cells, the upper-right area were nonviable apoptotic cells and the lower-right domain were viable apoptotic cells. Among these, cells in upper-right and lower-right areas were identified as apoptotic.

Statistical analysis

All data were presented as mean ± SD. Comparisons among different groups were performed by one-way ANOVA. The statistical analyses were conducted by SPSS v17.0. A P value < 0.05 was considered as significant difference.

Results

Functions of HG on IQGAP1 transcripts identified by Q-PCR

In order to examine the expression changes of IQGAP1 under different treatments, Q-PCR was employed to detect the transcript levels. As shown in **Figure 1**, the expression of IQGAP1 was significantly down-regulated when treated by HG for 24 h (group C), while no significant difference was observed when treated by mannitol (group B). However, the suppression by HG was attenuated when benazepril, which can inhibit the generation of AngII, was added (group D). There was no significant difference occurred when u0126 (ERK1/2 inhibitor) was mixed with HG (group E). The expression trends of IQGAP1 treated for 48 h was consistent with 24 h, except that the inhibition of HG became stronger on IQGAP1.

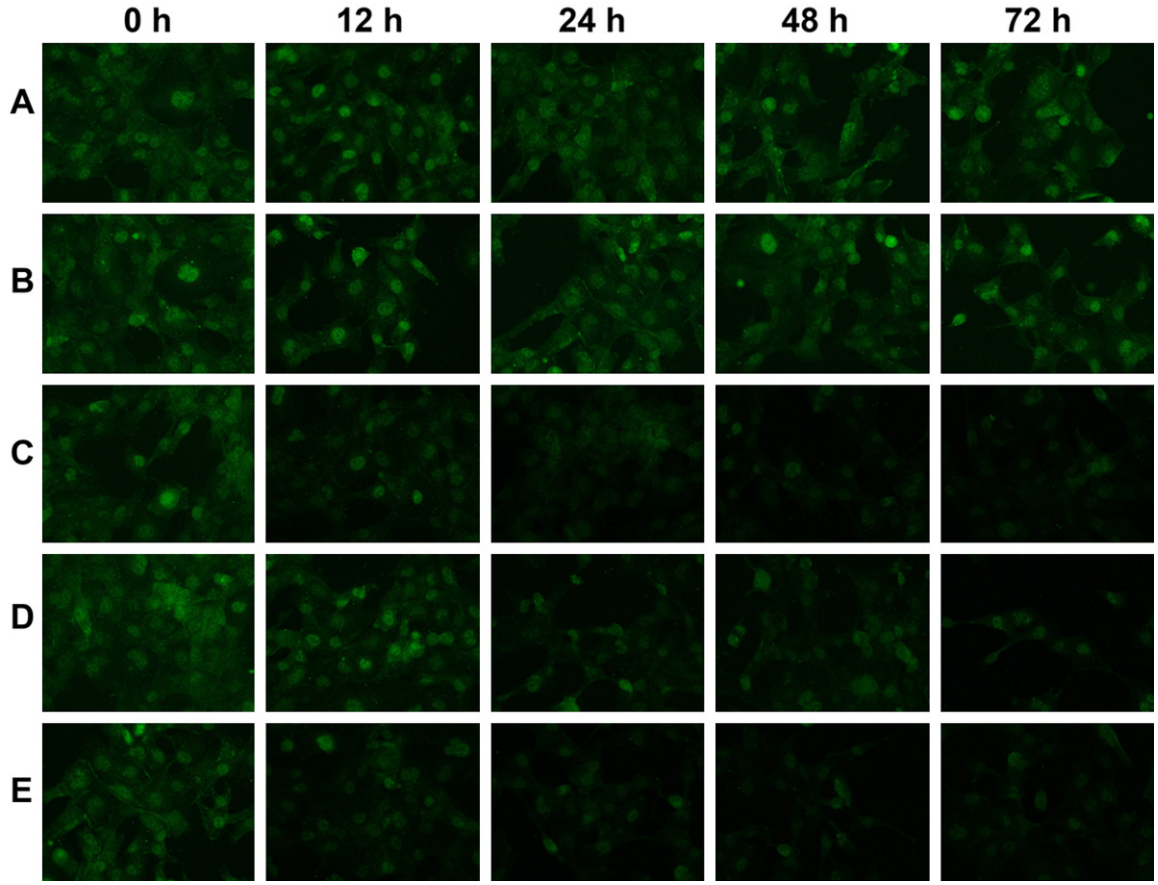

Figure 2. Distribution and expression of IQGAP1 detected by immunofluorescence assay.

Distribution and expression of IQGAP1 identified by immunofluorescence assay

We performed with the immunofluorescence assay to observe the distribution and expression changes of IQGAP1 under different treatments. As shown in **Figure 2**, IQGAP1 existed both in nucleus and cytoplasm (mainly expressed in nucleus). The fluorescence intensity significantly weakened under the treatment of HG (group C), which were consistent with the results of Q-PCR. Furthermore, the fluorescence intensity were weakened under the treatment of HG with benazepril, but was larger than HG at the corresponding time point (group D). Results under the treatment of E group were similar to that of C group. All these results were consistent with Q-PCR, which confirmed the exact of our conclusion.

Effects of HG on protein levels

To further explore the effects of HG on HPC, we detected the protein content of IQGAP1, p-ERK and ERK after treatment by A-E for 0 h, 24 h and 48 h. As shown in **Figure 3**, the protein levels of IQGAP1 were consistent with the transcripts trends of IQGAP1 under the treatment of A-E. There was no obvious variation occurred in ERK protein levels under different treatments. Notably, the protein content of p-ERK was significantly induced by HG treatment (group C) but inhibited by HG with u0126 (group E). HG with benazepril (group D) was also induced the generation of p-ERK but not as much as group C.

Effects of HG on HPC apoptosis

Finally, we examined the apoptotic of HPC under different treatments through flow cytometry. As shown in **Figure 4**, HG contributed to the occurrence of apoptosis (group C), but the process can be attenuated by benazepril (group D). In addition, apoptosis can be significantly inhibited by u0126 (group E).

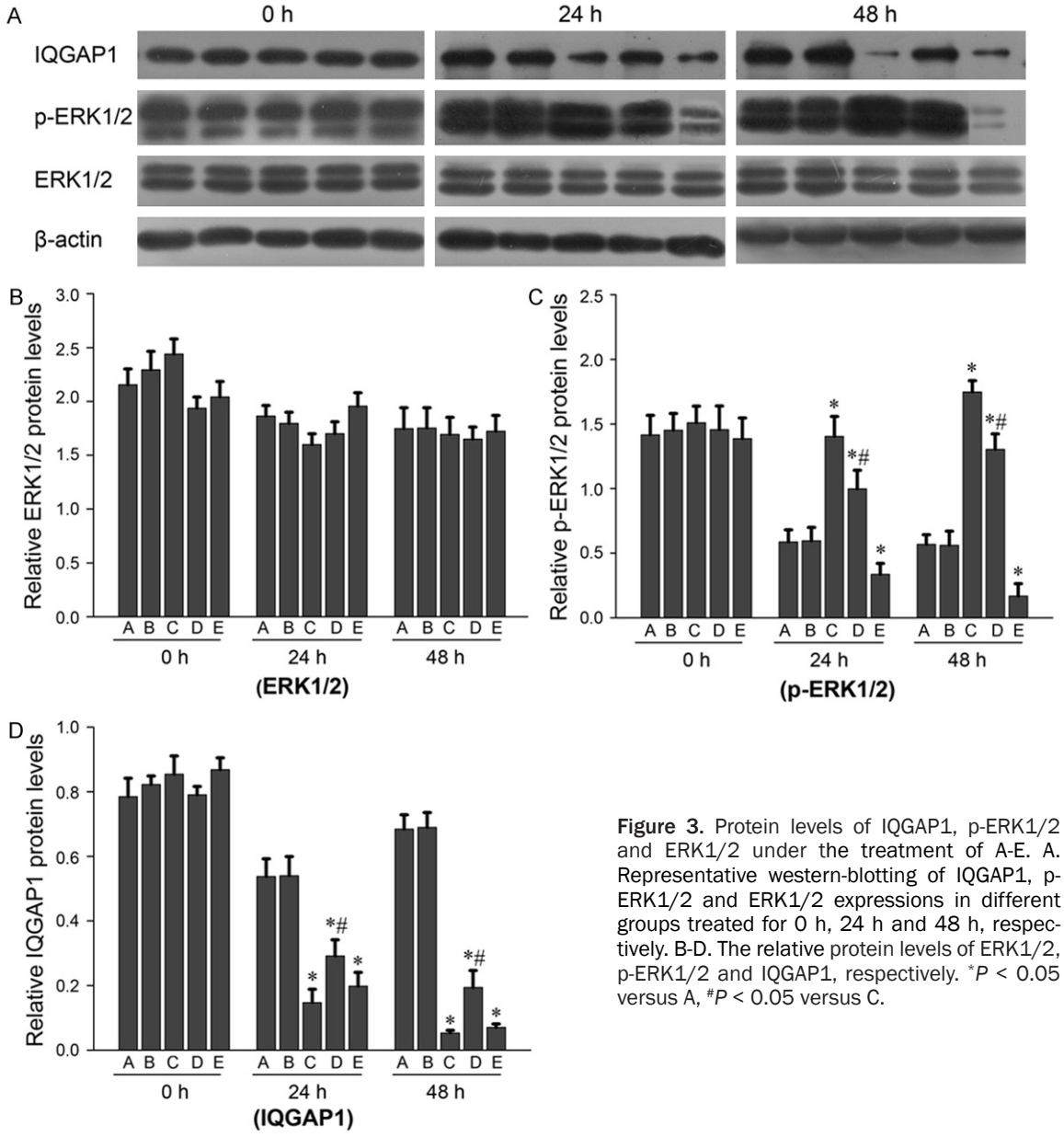

Figure 3. Protein levels of IQGAP1, p-ERK1/2 and ERK1/2 under the treatment of A-E. A. Representative western-blotting of IQGAP1, p-ERK1/2 and ERK1/2 expressions in different groups treated for 0 h, 24 h and 48 h, respectively. B-D. The relative protein levels of ERK1/2, p-ERK1/2 and IQGAP1, respectively. $^{*}P < 0.05$ versus A, $^{\#}P < 0.05$ versus C.

Discussion

In the current investigation, we came to the conclusion that HG can induce the apoptosis of HPC, meanwhile, the expression of IQGAP1 was down-regulated at both mRNA level and protein level. Besides, the protein level of p-ERK1/2 was significantly increased but no obvious changes were observed compared to normal control group. Significantly, the results caused by HG were attenuated by benazepril, including the apoptotic rate of HPC and the suppression of IQGAP1.

Our results showed that HG suppressed the expression of IQGAP1, which was consistent with a previous study described by Zhou et al. [20]. Furthermore, we found that HG-induced apoptosis was attenuated by benazepril, and the suppression of IQGAP1 was released as well. It is well known that benazepril is an inhibitor for angiotensin-converting enzyme, which can transform AngI into AngII [21]. So it seems that the block of AngII reduced the apoptosis of HPC, and increased the expression of IQGAP1. The latter conclusion was opposite to a study by Liu [19] which demonstrated that AngII stim-

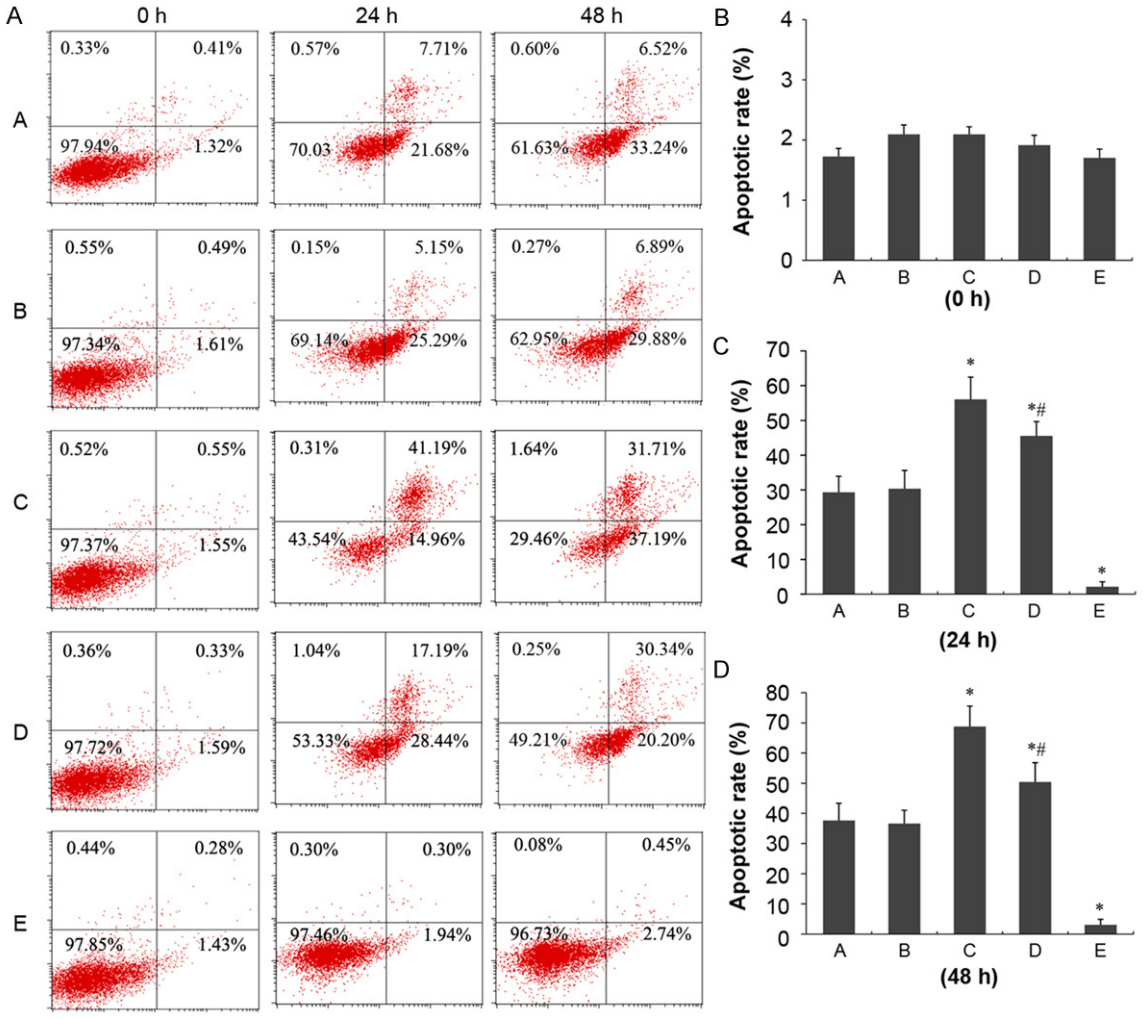

Figure 4. Flow cytometry analysis of apoptosis. A. The results of flow cytometry. Cells in upper-right and lower-right areas were identified as apoptotic. B-D. The apoptotic rate of each group when treated for 0 h, 24 h and 48 h, respectively. $^{*}P < 0.05$ versus A, $^{\#}P < 0.05$ versus C.

ulated the expression of IQGAP1 and contributed to apoptosis of podocytes. It is confused about the function of IQGAP1.

Besides the above results, Our study also observed that HG could induce the activation of ERK1/2 pathway in HPC, and the activation of ERK1/2 contributed to the development of apoptosis. Various studies have demonstrated that IQGAP1 can regulated cell physiology by binding ERK1/2 [12, 22]. And an investigation have discovered that the absence of IQGAP1 impaired the activation of ERK1/2 [23]. This was disagreed with our conclusions.

Have all data together, the behavior of IQGAP1 was perplexing. Both our study and a study by Zhou et al. [20] discovered that under HG medium, the expression of IQGAP1 was down-regulated but the level of p-ERK1/2 was increased significantly and contributed to the apoptosis of HPC finally. Just as Zhou et al. [20] described that IQGAP1 may have an important role in maintaining the content of p-ERK1/2 at proper levels under HG medium. It was consistent with the results that benazepril down-regulated the p-ERK1/2 levels and at this point the IQGAP1 was up-regulated. We proposed that there may be an unknown regulatory factor mediating HG-induced apoptosis of HPC through ERK1/2 pathway.

In conclusion, our findings revealed that HG-induced apoptosis of HPC was accompa-

nied with the decrease of IQGAP1 as well as the activation of ERK1/2 pathway. In addition, benazepril can attenuated the apoptosis of HPC under HG condition. Our discoveries provide a new direction for the exploration of the mechanisms underlying HG-induced apoptosis of HPC, and made a foundation for new therapies to DN.

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Disclosure of conflict of interest

None.

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