TRPC6 Up-Regulation in Ang II-Induced Podocyte Apoptosis Might Result from ERK Activation and NF-κB Translocation

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Angiotensin II (Ang II) has been recognized as an apoptosis inducer in podocytes, but the mechanism of apoptosis induced by Ang II is unclear. Transient receptor potential cation channel 6 (TRPC6) is a calcium channel located in podocyte membrane. The present study evaluated the alteration of TRPC6 expression and the Ca^{2+} influx involved in Ang II-induced podocyte apoptosis. The possible pathways related to TRPC6 in Ang II-induced podocyte apoptosis were also investigated. The apoptosis of mouse podocytes (MPC5) was induced by Ang II. The protein level of TRPC6 was increased markedly in response to Ang II stimulation, and the intracellular Ca^{2+} concentration was elevated. By transfection with TRPC6 siRNA, Ang II-induced podocyte apoptosis and the transient Ca^{2+} influx were inhibited. Treated with extracellular signal-regulated kinase (ERK) pathway specific inhibitor U0126 or nuclear factor-κB (NF-κB) pathway specific inhibitor ammonium pyrrolidinedithiocarbamate (PDTC) and Ang II, respectively in podocytes, not only was the TRPC6 up-regulation reduced, but the podocyte apoptosis was also decreased. Moreover, the translocation of NF-κB in nucleus resulted from Ang II was reduced by treatment with U0126. In conclusion, the enhancement expression of TRPC6 as well as the increased Ca^{2+} influx mediated by TRPC6 channels contributed to the podocyte apoptosis. The activation of ERK pathway and subsequent translocation of NF-κB was possibly necessary for the up-regulation TRPC6 induced by Ang II. Exp Biol Med 234:1029–1036, 2009

Key words: podocyte apoptosis; angiotensin II; TRPC6; calcium; ERK; NF-κB

Introduction

As an important risk factor in the progression of renal diseases, Ang II can induce a large number of non-hemodynamic effects, including induction of oxygen radicals, cytokines, and stimulation of cell apoptosis, proliferation and hypertrophy (1–3). It has been showed that Ang II could induce podocyte apoptosis both *in vivo* and *in vitro* (4, 5). Podocytes are the most differentiated cells within the glomeruli. The slit diaphragm (SD) complex is a special structure in podocyte foot processes, which is crucially involved in the maintenance of the normal structure and function of podocytes (6–9). It was also demonstrated that Ang II had an effect on the mRNA expression of the SD complex, including nephrin and podocin, *via* Ang II receptor type I (AT1R) (10).

A new SD molecule-transient receptor potential cation channel 6 (TRPC6) was reported. Its mutation caused proteinuria or nephritic syndrome clinically and focal segmental glomerulosclerosis (FSGS) pathologically in New Zealand kindred families (11). TRPC6 is localized in many types of cells and can regulate intracellular Ca^{2+} (12). It was demonstrated that too much intracellular Ca^{2+} could lead to cells apoptosis. In Winn's study, one of the mutant TRPC6 proteins was expressed much more along cells membranes than the wild-type TRPC6 protein (11). Ang II enhanced sustained Ca^{2+} influx *via* the mutant TRPC6 in HEK293 cells, which might lead to cell injury (11). However, it is still unclear whether or not TRPC6 was up-regulated during the podocyte apoptosis induced by Ang II, and whether the apoptosis resulted from the elevation of Ca^{2+} influx through TRPC6. Therefore, we raised the hypothesis that Ang II-induced podocyte apoptosis occurred through the enhancement of both TRPC6 expression and Ca^{2+} influx.

The family of mitogen activated protein kinases (MAPK), consisting of extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), and p38, contributes to the cell survival, proliferation, migration, hypertrophy, apoptosis and regulation of intracellular Ca^{2+} (13). It has been shown that MAPK signaling took part in kidney diseases (14). Nuclear factor-κB (NF-κB) is one of the transcription factors activated by Ang II. The persistent

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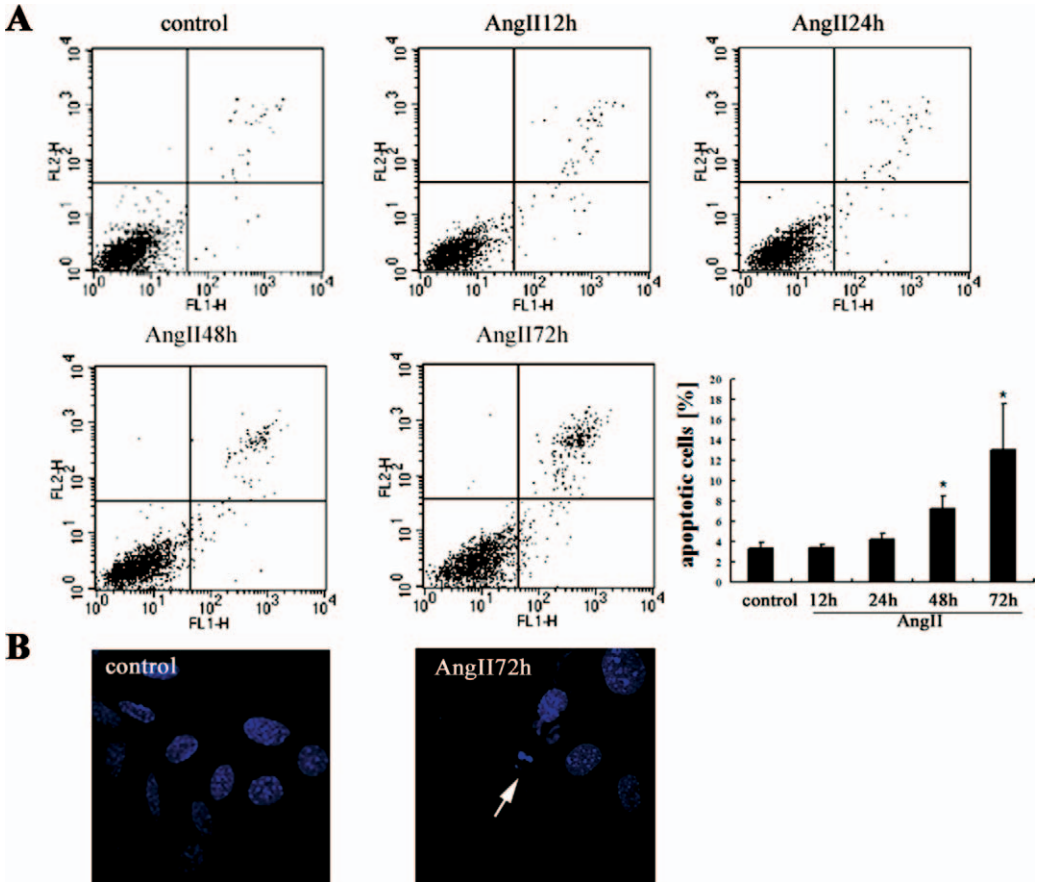

Figure 1. Effect of Ang II on podocyte apoptosis. (A) The FL1-H and FL2-H represented the Annexin V and PI staining, respectively. The podocytes were treated with Ang II (10^{-7} M) for 12 h, 24 h, 48 h and 72 h. The apoptosis was increased at 48 h and 72 h significantly. * $P < 0.05$ vs. control ($n = 4$ individual experiments). (B) The podocytes nuclei were stained by Hoechst. The left photo showed the normal nucleus, which was rounded. The arrow in the right photo showed the apoptotic nucleus. Magnification, ×720. A color version of the figure is available in the online journal.

activation of NF-κB was observed in podocytes (15). Furthermore, the activation of MAPK and NF-κB is also required for some of the gene expression. Therefore, we hypothesized that the MAPK and NF-κB pathways might be involved in the up-regulation of TRPC6 during podocyte apoptosis induced by Ang II, and the Ca^{2+} influx mediated by TRPC6 might be associated with the apoptosis.

Materials and Methods

Podocytes Culture and Treatment. Conditionally immortalized mouse podocyte clone (MPC5) (a kind gift from Prof. Peter Mundel) was cultured at 33°C in RPMI1640 medium (Gibco, Gaithersburg, MD, USA) containing 10% fetal bovine serum (Gibco), 100 U/ml of penicillin-streptomycin and 10 U/ml of recombinant mouse γ-interferon (PeproTech, London, UK). To induce differentiation, podocytes were cultured at 37°C without γ-interferon and induced to apoptosis with 10^{-7} M Ang II (Sigma, USA) in low-serum medium (2% fetal bovine serum). The differentiated inhibitors, including U0126 (2 μ*M*), SP600125 (2 μ*M*) and ammonium pyrrolidinedithiocarbamate (PDTC) (4 μ*M*) (Sigma, USA) were administered 30 mins before applying Ang II. The differentiated podocyte was divided into the following groups: control group, Ang II group, siTRPC6 + Ang II group, U0126 group, U0126 + Ang II group, SP600125 group, SP600125 + Ang II group, PDTC group, and PDTC + Ang II group.

Apoptosis Analysis. Podocytes were collected at 12 h, 24 h, 48 h and 72 h after Ang II treatment. Cells were washed twice with cold phosphate-buffered saline (PBS) and centrifuged at 1000 *g* for 5 mins. Subsequently, cell pellets were resuspended in 200 μl binding buffer with 10 μl Annexin V (1 μg/ml), and then incubated for 15 mins in the dark at room temperature (RT). Finally, cells were analyzed by flow cytometry after adding 300 μl binding buffer with 5 μl Propidium Iodide (PI) (50 μg /ml).

Immunofluorescence Staining. Podocytes cultured on the cover slips were washed with PBS and fixed for 20 mins in 2% paraformaldehyde and 4% sucrose in PBS at RT. After being permeabilized and blocked with 0.3% Triton-100 and 5% bovine serum albumin in PBS for 30 mins, cells were incubated with rabbit anti-TRPC6 (Chemicon, Temecula, CA, USA) or mouse anti-NF-κB p65 (Santa Cruz, USA) antibody overnight at 4°C. Excessive primary

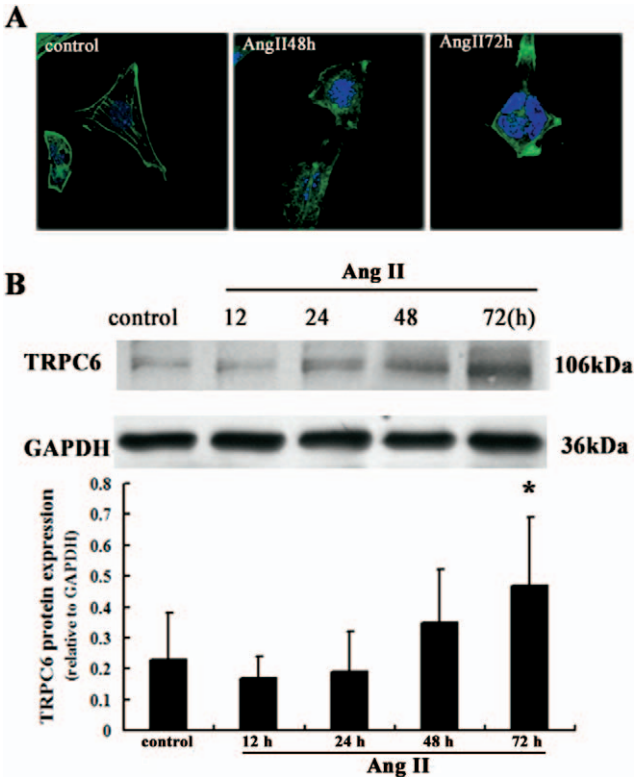

Figure 2. Effects of Ang II on the distribution and protein expression of TRPC6. (A) The nuclei were stained in blue with Hoechst; the expression of TRPC6 was shown in green. The green staining of TRPC6 was shown mainly along the cell membrane. Magnification, ×1200. (B) Western blot analysis showed that the protein level of TRPC6 was increased at 72 h. * $P < 0.05$ vs. control ($n = 6$ individual experiments). A color version of the figure is available in the online journal.

antibody was removed by washing with PBS, followed by incubation with FITC-conjugated goat anti-rabbit or -mouse IgG for 1 h at RT, and Hoechst was directly used to stain the nuclei of podocytes. Finally, the cover slips were mounted on glass slides with 15% Mowiol, and viewed by using confocal microscopy (Nikon TE 300 microscope).

Western Blotting. Podocytes were lysed in lysis buffer (50 mM Tris-HCl [pH 7.4], 150 mM NaCl, 1% Nonidet P-40, 0.1% sodium dodecyl sulfate [SDS], 0.5% deoxycholic acid sodium salt [DOC]). Nuclear proteins were extracted for analyzing NF-κB p65 according to the protocol of kit supplied by SunBio-Tech Company (Beijing, China). Equal amounts protein loadings were separated by SDS-PAGE on 8% or 10% gels and electrophoretically transferred to a nitrocellulose membrane. Nonspecific binding sites were blocked with 5% not-fat milk powder in PBS and 0.05% Tween 20 for 1 h at RT. Membranes were incubated in one of the following primary antibodies at appropriate concentrations: rabbit anti-TRPC6, rabbit anti-ERK1/2, mouse anti-phospho-ERK1/2, mouse anti-JNK, mouse anti-phospho-JNK, mouse anti-p38, mouse anti-phospho-p38, mouse anti-GAPDH (KangChen, Bio-Tech, China), mouse anti-NF-κB p65, rabbit anti-IκB-α, mouse anti-β-actin and mouse anti-Histone H1 (Santa Cruz, USA) overnight at 4°C. Finally, membranes were incubated with horseradish peroxidase-conjugated anti-rabbit or -mouse IgG antibody (Santa Cruz) for 1 h at RT. An enhanced chemiluminescence reagent (Santa Cruz) was used to detect the bound antibodies. The specific protein bands were scanned, and then the equal total protein loading and nuclear protein loading was tested by GAPDH or β-actin and Histone H1, respectively.

Knock Down TRPC6 by siRNA. The specific annealed siRNA targeted to TRPC6 and negative control siRNA were purchased from Ambion Company (USA). The sequences of TRPC6 siRNA were 5′-GGUUAUGUACG-GAUUGUGGtt-3′ (sense), and 5′-CCACAAUCCGUA-CAUAACCtt-3′ (antisense), respectively. Transfections were performed according to the instructions of the supplier (Ambion). Cells were trypsinized firstly, and diluted to 1×10^5 cells per ml medium. siPORTNeoFX and siRNA diluted in RPMI1640 were mixed gently and incubated for 10 mins at RT. Then, the transfection complexes were dropped into the cells. The medium was changed after 12 h. Podocytes were treated with Ang II after transfection with TRPC6 siRNA for 16 h.

Calcium Flux Studies. Podocytes were treated with Ang II for 72 h. Cells were trypsinized and diluted to 1×10^5 cells per ml medium. Then cells were collected and loaded with the 10 μ*M* Fluo-3am calcium indicator (Invitrogen, USA) in Ca^{2+} free PBS for 30 mins at 37°C. After the baseline of intracellular Ca^{2+} was recorded, the receptor-operated channels were activated by addition of 100 μ*M* 1-oleoyl-2-acetyl-sn-glycerol (OAG) (Sigma, USA) for 1 min, followed by changing the extracellular buffer to 2 m*M* Ca^{2+} ($CaCl_2$). The intracellular Ca^{2+} was detected for 10 time points at a 1 min interval by using FLX 800 spectrophotofluorometer (BioTek) with a filter of 480 nm excitation and 510 nm emission wavelength (16, 17).

Statistical Analysis. The data was presented as the mean ± SD. Differences were evaluated by using one-way ANOVA test. The level of statistical significance was $P < 0.05$. Statistical analyses were performed by SPSS version 10.0.

Results

Effect of Ang II on Podocyte Apoptosis. The positive staining Annexin V indicated the early apoptotic cells, and the double staining of Annexin V and PI indicated the late apoptotic cells. The podocyte apoptosis was increased significantly in the Ang II group at 48 h (7.27 ± 1.27%) and 72 h (13.03 ± 4.53% vs. 3.37 ± 0.57% in the control group, $P < 0.05$, $n = 4$) (Fig. 1A). The apoptotic nucleus was observed in the Ang II group at 72 h (Fig. 1B). Nuclear condensation and fragmentation were shown. Because the apoptotic effect of Ang II on podocytes was evident at least at 10^{-7} M, 10^{-7} M Ang II was applied as an apoptosis stimulus in the following experiments.

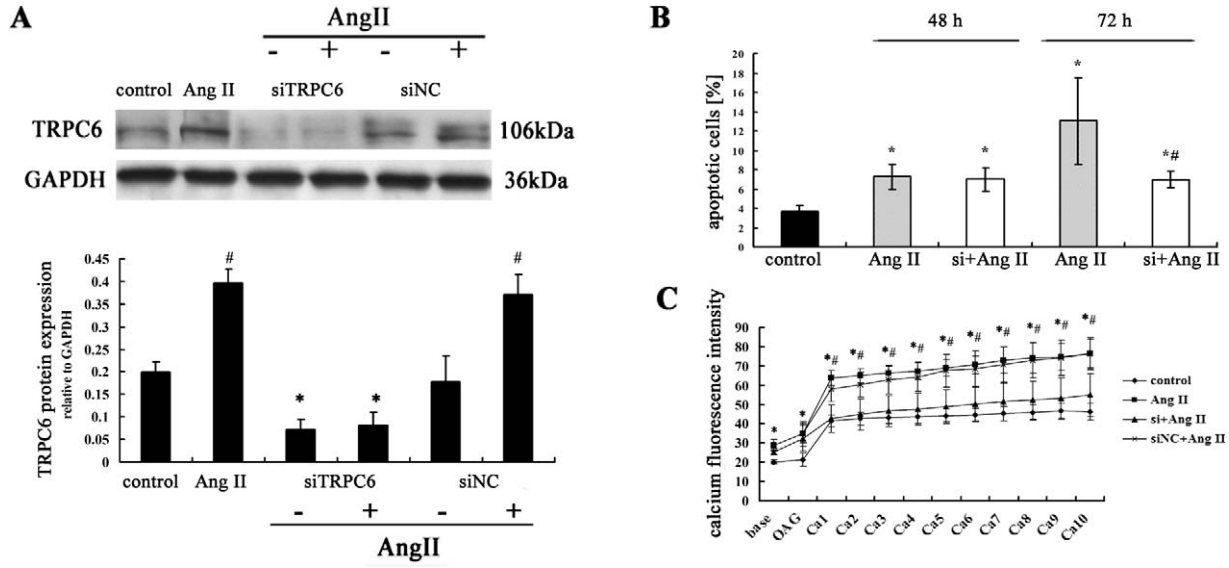

Figure 3. Inhibition of TRPC6 expression attenuated podocytes apoptosis and Ca^{2+} influx in podocytes. (A) siNC represented podocytes that were transfected with negative control small RNA. The protein level of TRPC6 was decreased by knocking down TRPC6. * $P < 0.05$ vs. Control; # $P < 0.05$ Ang II vs. control, siNC + Ang II vs. control ($n = 4$ individual experiments). (B) si + Ang II represented the siTRPC6 + Ang II group. The podocyte apoptosis was decreased by inhibiting TRPC6 expression. * $P < 0.05$ vs. Control; # $P < 0.05$ si + Ang II 72 h vs. Ang II 72 h ($n = 3$ individual experiments). (C) The baseline of intracellular Ca^{2+} level in podocytes was elevated at 72 h. * $P < 0.05$ Ang II vs. control, siNC + Ang II vs. control ($n = 4$ individual experiments). Channels were activated by OAG, followed by changing the extracellular buffer to 2 m*M* $CaCl_2$ to distinguish Ca^{2+} influx. Differences in calcium fluorescence intensity were statistically significant at the time points indicated. * $P < 0.05$ Ang II vs. control, siNC + Ang II vs. control; # $P < 0.05$ si + Ang II vs. Ang II ($n = 4$ individual experiments).

Effects of Ang II on the Distribution and Protein Expression of TRPC6. The distribution of TRPC6 in podocytes was observed by immunofluorescence staining. In both the control group and the Ang II group, podocytes exhibited a marked staining of TRPC6, which localized mainly along the cells membranes (Fig. 2A).

Compared with the control group, the protein expression of TRPC6 in the Ang II group was increased markedly at 72 h (0.47 ± 0.22 in the Ang II group vs. 0.23 ± 0.15 in the control group, $P < 0.05$, $n = 6$) (Fig. 2B).

Effects of Ang II on Intracellular Ca^{2+} in Podocytes. A significant increase in the basal intracellular Ca^{2+} level was detected in the Ang II group compared to the control group (20.27 ± 1.06 vs. 28.95 ± 2.83, $P < 0.05$, $n = 4$) (Fig. 3C). Furthermore, after the stimulation of TRPC6 activator OAG and extracellular Ca^{2+}, the intracellular Ca^{2+} was also much higher at each time point (Ca1–10) than in the control (Ang II vs. control, $P < 0.05$, $n = 4$) (Fig. 3C).

Inhibition of TRPC6 Expression Attenuated Podocytes Apoptosis and Ca^{2+} Influx in Podocytes. We applied siRNA against TRPC6 to inhibit endogenously expressed TRPC6 proteins selectively. The protein level of TRPC6 was decreased by 64% (0.071 ± 0.023 in siTRPC6 group vs. 0.20 ± 0.02 in control group, $P < 0.05$, $n = 4$). And the negative control siRNA had no effect on the TRPC6 protein expression in podocytes (0.18 ± 0.05 in siNC group vs. 0.20 ± 0.02 in control group) (Fig. 3A). The podocyte apoptosis in the siTRPC6 + Ang II group was decreased compared with the Ang II group at 72 h (6.97 ± 0.84% vs. 13.03 ± 4.53%, $P < 0.05$, $n = 3$) (Fig. 3B). Compared with the Ang II group, although the decrease of the basal intracellular Ca^{2+} did not reach statistical significance, the intracellular Ca^{2+} was much lower at Ca1–10 time points in siTRPC6 + Ang II group by activating TRPC6 with OAG and extracellular Ca^{2+} (siTRPC6 + Ang II vs. Ang II, $P < 0.05$, $n = 4$) (Fig. 3C). There was no difference in the intracellular Ca^{2+} between the Ang II group and the siNC (negative control) + Ang II group.

Ang II-Induced ERK Activation Involvement in TRPC6 Up-Regulation and Podocyte Apoptosis. Phosphorylated ERK1/2 was increased markedly at 15 mins, 45 mins and 60 mins after Ang II treatment (vs. control (0 m), $P < 0.05$, $n = 5$), and the initial effect persisted up to 24 h (vs. control (0 h), $P < 0.05$, $n = 5$) (Fig. 4A). A transient increase in the phosphorylation of JNK was observed at 30 mins, but the phosphorylation of p38 wasn't detected.

We pretreated podocytes using the ERK pathway inhibitor U0126 (2 μ*M*) or JNK pathway inhibitor SP600125 (2 μ*M*) prior to Ang II stimulation and then examined TRPC6 protein expression level. The protein expression of TRPC6 was decreased in U0126 + Ang II group dramatically compared with the Ang II group (0.42 ± 0.12 vs. 0.76 ± 0.14, $P < 0.05$, $n = 4$) (Fig. 4B). But SP600125 had no effect on TRPC6 protein up-regulation. At the same time, the podocyte apoptosis was also decreased markedly (6.02 ± 0.77% in the U0126 + Ang

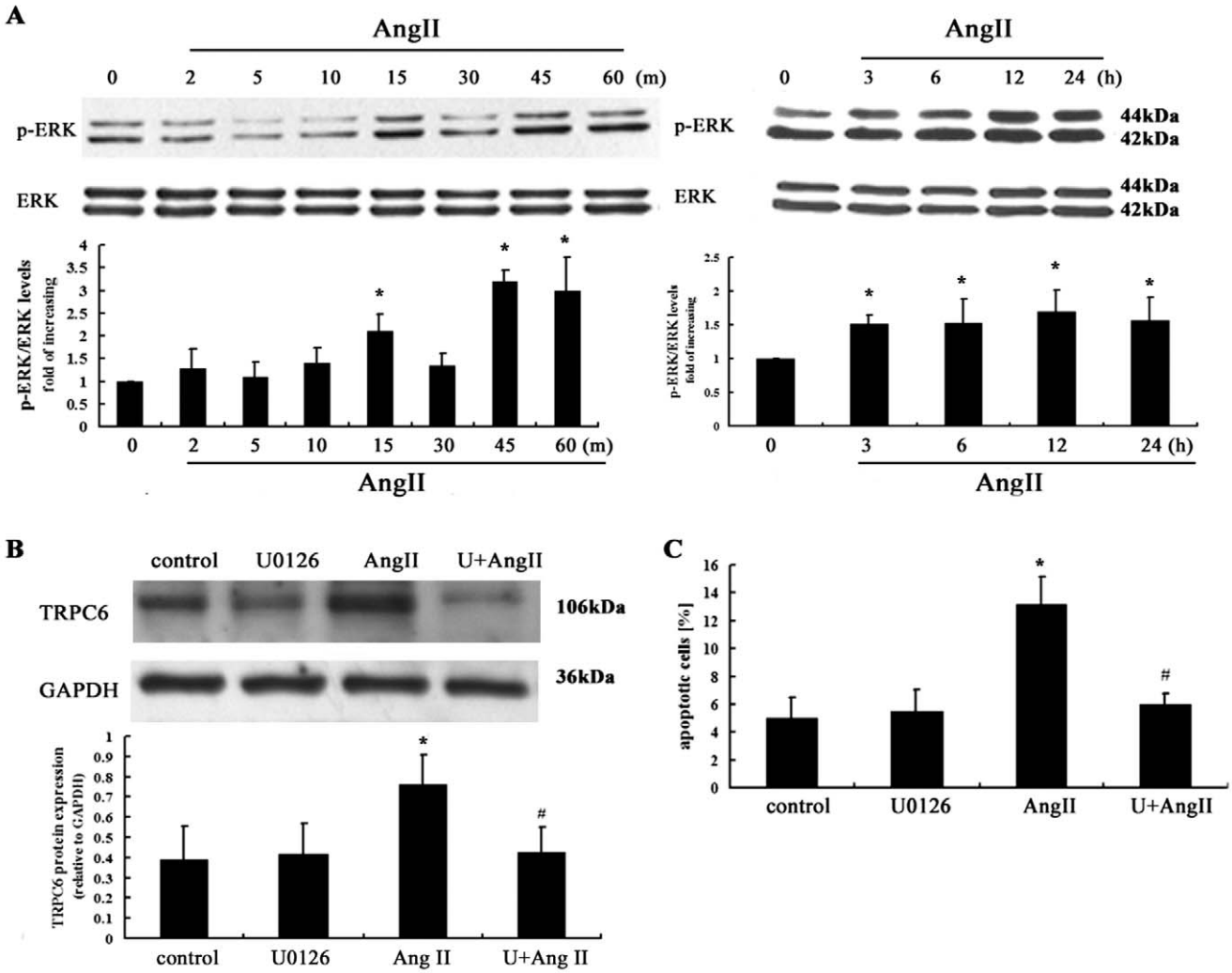

Figure 4. Ang II-induced ERK activation involvement in TRPC6 up-regulation and podocyte apoptosis. (A) The activation of ERK pathway was presented by the relative p-ERK and ERK levels. The figure showed that the activation of ERK pathway induced by Ang II persisted up to 24 h. * $P < 0.05$ vs. control (0 m or 0 h) ($n = 5$ individual experiments). (B) U + Ang II represented the U0126 + Ang II group. After treatment with ERK pathway inhibitor U0126 (2 μ*M*) before 30 mins with Ang II, the up-regulation of TRPC6 was decreased significantly. * $P < 0.05$ vs. control; # $P < 0.05$ U + Ang II vs. Ang II ($n = 4$ individual experiments). (C) The apoptosis was reduced by inhibiting ERK pathway activation. * $P < 0.05$ vs. control; # $P < 0.05$ U + Ang II vs. Ang II ($n = 3$ individual experiments).

II group vs. 13.12 ± 2.02% in the Ang II group, $P < 0.05$, $n = 3$) (Fig. 4C).

Ang II-Induced NF-κB Translocation Involvement in TRPC6 Up-Regulation and Podocyte Apoptosis. The immunoreactive NF-κB p65 green staining was mainly located into cytoplasm in control group. In the Ang II group, the green staining was increased in the nucleus at 12 h (Fig. 5A). The western blotting analysis showed the expression of NF-κB p65 in the nucleus was increased significantly (vs. control (0 h), $P < 0.05$, $n = 5$) (Fig. 5B). The NF-κB pathway inhibitor PDTC (4 μ*M*) was applied for 30 mins before stimulation with Ang II. The western blotting results demonstrated that the protein expression of TRPC6 was decreased in the PDTC + Ang II group significantly compared with the Ang II group (0.40 ± 0.05 vs. 0.69 ± 0.06, $P < 0.05$, $n = 4$) (Fig. 5C), and the marked decrease of apoptosis was also detected (9.49 ± 2.00% in the PDTC + Ang II group vs. 16.04 ± 3.92% in the Ang II group, $P < 0.05$, $n = 3$) (Fig. 5D).

To investigate whether Ang II induced NF-κB translocation through the ERK pathway, podocytes were pretreated with U0126 for 30 mins. The results showed that the expression of NF-κB in nucleus was reduced significantly in the U0126 + Ang II group at 12 h (0.33 ± 0.08 vs. 0.56 ± 0.11 in the Ang II group, $P < 0.05$, $n = 5$) (Fig. 5E). And there was no difference in the IκB-α expression among the control group and the Ang II groups ($n = 4$) (Fig. 5F).

Discussion

As a new SD molecule, TRPC6 is an important molecule for keeping the structure and function of

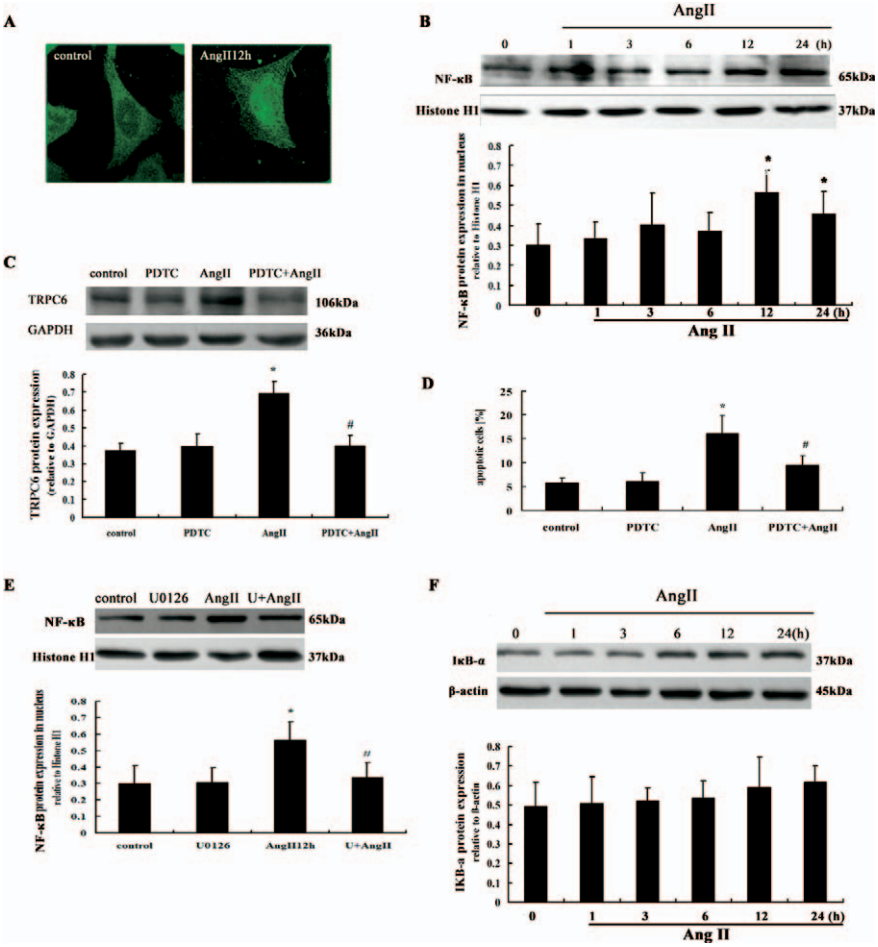

Figure 5. Ang II-induced NF-κB translocation involvement in TRPC6 up-regulation and podocyte apoptosis. (A) The expression of NF-κB p65 was shown in green staining. The marked translocation of NF-κB p65 was observed at 12 h. Magnification, ×1200. (B) The nuclear proteins were extracted from the control and the Ang II group, respectively. The western blot analysis showed that the expression NF-κB was increased significantly at 12 h and 24 h. * $P < 0.05$ vs. control (0 h) ($n = 5$ individual experiments). (C) After treatment with NF-κB inhibitor PDTC (4 μ*M*) before 30 mins with Ang II, the up-regulation of TRPC6 was reduced significantly. * $P < 0.05$ vs. control; # $P < 0.05$ PDTC + Ang II vs. Ang II ($n = 4$ individual experiments). (D) The apoptosis was reduced by inhibiting NF-κB translocation. * $P < 0.05$ vs. control # $P < 0.05$ PDTC + Ang II vs. Ang II ($n = 3$ individual experiments). (E) The expression of NF-κB in nucleus was decreased significantly by inhibiting the activation of ERK pathway. * $P < 0.05$ vs. control; # $P < 0.05$ U + Ang II vs. Ang II ($n = 5$ individual experiments). (F) There was no difference among the control (0 h) and the Ang II groups in IκB-α expression. A color version of the figure is available in the online journal.

podocytes as well as regulation of signaling transduction in podocytes and interacting with other SD molecules, such as nephrin, podocin and CD2AP (18–20). In the present study, the protein level of TRPC6 was increased up to two-fold in podocyte apoptosis induced by Ang II. The study showed that not only was an increase of basal intracellular Ca^{2+} level observed, but also the continuous Ca^{2+} influx stimulated transiently by OAG, an activator of TRPC6, was also increased. Therefore, we speculated that TRPC6 and Ca^{2+} influx mediated by TRPC6 were involved in the Ang II-induced podocyte apoptosis.

In this study, the podocyte apoptosis was decreased by inhibiting the expression of TRPC6 by means of siRNA. After inhibiting the TRPC6 expression, although the basal intracellular Ca^{2+} in podocytes was not decreased to statistical difference, the continuous Ca^{2+} influx stimulated transiently by OAG was reduced. It is well-known that the calcium overload resulting from the transient calcium change is an important event for cell apoptosis. These findings further suggest that the up-regulation of TRPC6 resulted from Ang II, and also that the increase of Ca^{2+} influx *via* TRPC6 plays a key role in podocyte apoptosis. The elevation of TRPC6 was also observed in other injury models. TRPC6 expression in cultured podocytes exposed to complement showed a significant increase. In the glomeruli of PAN-injected rats, the TRPC6 levels were increased markedly (17). In addition, the up-regulation of TRPC6 in podocytes has also been detected in some acquired kidney diseases, such as minimal change disease, membranous glomerulonephritis and FSGS (17). The Ca^{2+} influx mediated by the TRPC family correlated with cell proliferation and apoptosis. As the family members of TRPC, the overexpression of TRPC3 and the increased Ca^{2+} influx *via* TRPC3 resulted in apoptosis in mouse cardio-

myocytes (21). Similarly, TRPC7 could act as a Ca^{2+} channel leading to myocardial apoptosis induced by Ang II (22). All these results indicate that TRPC6 is a crucial molecule in apoptosis, and works as a Ca^{2+} influx pathway, which is an event of podocyte apoptosis. However, in the present study, the increased apoptosis was detected earlier than the significant up-regulation of TRPC6, and by silencing the TRPC6 gene expression, the Ca^{2+} influx attenuated, but the apoptosis was not suppressed completely. These results suggest that there might be other pathways involved in the apoptosis.

The signaling pathway for Ang II-induced TRPC6 up-regulation was also studied in this study. Our study showed the activation of ERK1/2 persisted up to 24 h and the transient activation of JNK was observed. By inhibiting the ERK pathway, the Ang II-induced TRPC6 up-regulation was suppressed, and the podocyte apoptosis was also attenuated. However, inhibiting the JNK pathway showed no effect on the expression of TRPC6 and podocyte apoptosis. These results suggest that the activation of ERK pathway is involved in the up-regulation TRPC6 induced by Ang II.

NF-κB, consisting of p65 and p50 subunits, is activated in response to numerous stimuli and then translocates into the nucleus by the degradation of the inhibitor protein IκB. It was also proved by other investigators that NF-κB could bind to the κB element presenting in many gene promoters, and enhance gene transcription (23). In this study, more and more NF-κB translocated into nuclei of podocytes stimulated with Ang II. When the translocation of NF-κB was inhibited by PDTC, an inhibitor of NF-κB pathway, the Ang II-induced TRPC6 up-regulation was reduced, and the apoptosis was attenuated in our study. These findings provide support that the NF-κB translocation is essential for Ang II-induced TRPC6 up-regulation. There were some evidences from other study groups that NF-κB could regulate the expression of the TRPC family. Takahashi *et al*. (24) reported that Ang II could up-regulate TRPC1 through the activation of NF-κB in vascular smooth muscle cells (VSMCs). Moreover, Martinka's study also demonstrated that persistent NF-κB activation was observed in podocytes in a mouse model of HIV-associated nephropathy (15). These results suggest that the activation of NF-κB might be an important as well as an earlier response for podocyte injury. Therefore, we consider that Ang II and subsequent NF-κB translocation facilitate podocyte apoptosis *via* up-regulating TRPC6 expression.

We detected the correlation between ERK pathway and the nuclear pathway of NF-κB. In our study, the translocation of NF-κB was suppressed after inhibiting the activation of ERK pathway. Interestingly, the expression of IκB wasn't decreased. These results suggest that in podocytes treated with Ang II, the activation of ERK pathway results in NF-κB translocation, which is not attributed to the degradation of IκB possibly. In fact, the activation of non-classical pathway was also reported by others. Zhang *et al*. (25) reported that mitogen-activated protein kinase-1 (MEK1) inhibition suppressed Ang II-induced NF-κB DNA-binding activity. Knockdown of ERK by siRNA in VSMC was found to suppress the activation of NF-κB and the phosphorylation of p65 induced by Ang II.

In conclusion, our results suggest that the up-regulation of TRPC6 is involved in Ang II-induced mouse podocyte apoptosis. The increased Ca^{2+} influx in podocytes is induced through TRPC6 channel in podocyte apoptosis. The activation of ERK pathway and subsequent translocation of NF-κB might lead to TRPC6 up-regulation induced by Ang II in mouse podocytes. TRPC6 might be intended to be a target for pharmacological modification in order to maintain the function of podocytes in the future.

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