Angiotensin Type 1 Receptor Blocker Restores Podocyte Potential to Promote Glomerular Endothelial Cell Growth

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Both podocytes and glomerular endothelial cells (GEN) are postulated to play important roles in the progression and potential regression of glomerulosclerosis. Inhibition of angiotensin is crucial in treatment of chronic kidney disease, presumably via effects on BP and extracellular matrix. This study aimed to investigate how angiotensin inhibition altered the interactions between podocytes and GEN. The effects of supernatants from primary cultured mouse podocytes, before or after sublethal injury by puromycin aminonucleoside, in the presence or absence of angiotensin type 1 receptor blocker (ARB), on GEN sprouting and growth were assessed. Supernatant from normal podocytes significantly increased GEN sprouting, whereas puromycin aminonucleoside–injured podocyte supernatant decreased these GEN responses. These effects were linked to decreased vascular endothelial growth factor A (VEGF-A) and angiopoietin-1 (Ang-1) protein from injured podocytes. This downregulation of VEGF-A and Ang-1 protein was reversed when injured podocytes were treated with ARB. Inhibition of VEGF-A or Ang-1 prevented this restored response by ARB. Activation of intracellular kinases (p38, extracellular signal–regulated kinase, and AKT) was suppressed in GEN that were treated with medium from injured podocytes but restored by medium from ARB-treated injured podocytes. Therefore, injured podocytes are ineffective in promoting GEN sprouting, and this effect is reversed by ARB treatment of the injured podocyte. These data support the idea that ARB effects on podocytes may mediate capillary remodeling in vivo.

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isolated by sieving. Glomeruli then were suspended in DMEM/Ham’s F12 (2:1) that contained 0.2-μm-filtered 3T3-L1 supernatant, 5% heat-inactivated FBS, ITS solution, and 100 U/ml penicillin-streptomycin; plated onto collagen type I–coated flasks; and incubated at 37°C in room air with 5% CO₂. After 5 d, cell colonies began to sprout around the glomeruli. These cells showed an epithelial morphology with a polyhedral shape when confluence was reached and were characterized as podocytes by detection of the podocyte-specific markers, synaptopodin and nephrin, by immunofluorescence staining.

Podocytes were either kept untreated or treated with puromycin aminonucleoside (PAN; 100 μg/ml) for 24 h to achieve a sublethal podocyte injury model (20) in the absence or presence of the angiotensin type 1 (AT1) receptor blocker (ARB) losartan (10⁻⁴ M) or wortmannin (Sigma, St. Louis, MO), an inhibitor of phosphatidylinositol-3 kinase (PI3-K; 100 nM, dissolved in DMSO) (21). This dose resulted in only minimal apoptosis or necrosis in our previous studies (22), confirmed here by 94 ± 1% viability by Trypan blue staining after PAN treatment.

Twenty-four-hour podocyte-conditioned medium (either containing 2% FCS or 2% FCS control media or serum-free, the latter only for signaling experiments) was used for the experiments described next. A GEN cell line, obtained from SV40 mice as described previously (23), was grown in DMEM/Ham’s F12 medium (low-glucose DMEM, 6 mM) in a 3:1 ratio, with 10% FBS, 2 mM l-glutamine, and 10 mM HEPES at 37°C in humidified air with 5% CO₂. Cells showed CD31 expression, confirming endothelial cell phenotype.

**GEN Tube Formation Assay**

For studying the effects of podocytes on GEN sprouting, 10⁵ GEN were plated in complete medium onto 96-well plates that were coated with 10 μg/ml collagen type I (BD, Franklin Lakes, NJ). After 12 h, attached GEN were treated with either fresh cell medium or 24-h podocyte-conditioned medium (all containing 2% FCS) as specified above. In some experiments, GEN were plated as indicated above and treated with either fresh cell medium or medium that was derived from podocytes that were cultured with PAN+ARB (see Cell Culture section for details) with or without anti-mouse VEGF receptor 2 (VEGFR2) and/or anti-mouse Tie2 antibodies (10 μg/ml final concentrations; both from eBioscience, San Diego, CA). After 2 d, GEN were imaged using an inverted microscope coupled to a digital camera (Axiovert 135; Carl Zeiss MicroImaging, Thornwood, NY). For quantification of capillary-like network formation, the cellular node that was defined as a cellular junction linking at least three cells was counted from digital images. Ten images were analyzed for each treatment. All images were coded, and assessment and quantitation performed in a blinded manner. Two independent experiments, each in duplicate, were performed.

**GEN Proliferation**

GEN (5000 cells/well) were plated onto 96-well plates and cultured with regular endothelium medium or podocyte-conditioned media (all containing 2% FCS) for 48 h. For determination of the effect of podocyte-secreted angiogenic factors on GEN proliferation, GEN were plated as indicated above and cultured with either fresh cell medium or medium that was derived from podocytes that were cultured with PAN+ARB (see Cell Culture section for details), with or without anti-mouse VEGFR2 or anti-mouse Tie2 antibodies (10 μg/ml). Additional experiments were performed with added angiotensin II (10⁻⁷ M) for 48 h, with or without podocyte-conditioned medium, to determine whether angiotensin induced podocyte injury patterns similar to PAN. After 48 h, [³H]-thymidine (NEN Life Science Products, Boston, MA; 1 μCi/well) then was added to wells, and cells were incubated for an additional 48 h. Cells then were processed as described previously (24). Two independent experiments were performed in triplicate.

![Figure 1](Image)

**Figure 1.** Renin-angiotensin system (RAS) in injured podocytes. Angiotensin type 1 (AT1) receptor mRNA was increased in response to puromycin aminonucleoside (PAN) and numerically increased in response to angiotensin II by nonquantitative PCR.

**GEN Migration Assay**

Cell migration was assayed in transwell plates that were fitted with 8-μm membrane filters (Corning Costar, Corning, NY). The lower wells were coated overnight at 4°C with a solution of collagen type I (10 μg/ml) and then incubated for 1 h at 37°C with a solution of BSA (3%) to inhibit nonspecific cell migration. For analysis of cell migration, 500 μl of fresh medium or 24-h podocyte-conditioned medium (all serum free) was added to the lower wells, and serum-starved endothelial cells (5 × 10⁴ cells in 300 μl serum-free medium that contained 0.1% BSA) were added to the upper well. After 4 h of incubation at 37°C, cell migration was determined as described previously (25). Two independent experiments, each in duplicate, were performed.

**PCR**

To assess whether the RAS was activated in this podocyte injury model, we performed reverse transcription–PCR (RT-PCR) and real-time PCR. Total RNA was extracted from podocytes that were exposed to normal podocyte medium as above, PAN as above, or angiotensin II
(10^{-7} \text{ M}) and reverse-transcribed to single-stranded cDNA. Semiquantitative RT-PCR and real-time PCR were performed to determine gene expressions in podocytes. For PCR, cDNA was amplified using 20 pmol of specific primers for mouse AT1, angiotensinogen, and β-actin (26,27). Primer sequences and PCR conditions were as follows: AT1a sense 5'-GAC CAA CTC AAC CCA GAA AAG C-3' and antisense 5'-CGA AGC GAT CTT ACA TAG GTG-3' for 30 cycles with annealing temperature 58°C, yielding a 339-bp product; angiotensinogen sense 5'-GCA GTC CTG TGC AGA GTG CCA GCT-3' and antisense 5'-CCC CCT CCT GTT CGC CAT CTA CGA-3' for 30 cycles with annealing temperature 58°C, yielding a 312-bp product; and β-actin sense 5'-GAA CCC TAA GGC CAA CCG TGA AAA CCA G-3' and antisense 5'-TGA TCT TCA TGG TGC TAG GAG CCA GAG CAG-3' for 30 cycles with annealing temperature 55°C, yielding a 700-bp product. The abundance of each specific mRNA was normalized to β-actin mRNA. Replicate independent experiments were performed. Quantitative real-time PCR was performed for AT1, VEGF-A, and Ang-1 and normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as described previously (n = 3 independent experiments) (28). Primers were as follows: AT1 forward 5'-CCA TTG TCC ACC CGA TGA A-3' and reverse 5'-TGA CTT TGG CCA GCA GCA-3'; VEGF-A forward 5'-GGA GAT CCT TCG AGA GCT ATG-3' and reverse 5'-GGC GAT TTA GCA GCA GAT ATG AGA A-3'; Ang-1 forward 5'-CCA TGG TGG AGA TAG GAA CCA G-3' and reverse 5'-TGA AAC CTG GGA TGT GTG ATT T-3'; and GAPDH forward 5'-CCT GCA CCA CCA ACT GCT TA-3' and reverse 5'-TCA TGA GCC CTT CCA CAA-3' (29,30).

**Western Blot**

Twenty-four-hour podocyte-conditioned serum-free medium was collected, and protein levels were measured using BCA protein assay kit (Pierce Biotech, Rockford, IL). Thirty micrograms of total protein was separated on 10% SDS-PAGE and transferred to a nitrocellulose membrane. Immunoblotting of conditioned medium was performed with either a rabbit anti-mouse VEGF-A or a mouse anti-mouse Ang-1 antibody (both from Santa Cruz Biotechnology, Santa Cruz, CA) followed by horseradish peroxidase–conjugated anti-rabbit or anti-mouse secondary antibody (both from Amersham Pharmacia Biotech, Piscataway, NJ). Immunoreactive proteins were visualized using a peroxidase-conjugated goat anti-rabbit and an ECL kit (Amersham Pharmacia Biotech).

For determination of the effect of podocyte-conditioned medium on activation of p38, extracellular signal–regulated kinase (ERK), and AKT, GEN were incubated for 15 min with podocyte-conditioned serum-free medium; washed twice with PBS; scraped; suspended in 50 mM HEPES (pH 7.5), 150 mM NaCl, and 1% Triton X-100; and centrifuged for 10 min at 14,000 rpm. Serum-free conditions were used for these experiments to be able to assess subtle changes in cell signaling that could not be detected if cells already were stimulated by serum. In some experiments, GEN also were treated with wortmannin as described above. Fifty-microgram cell lysates were separated on 10% SDS-PAGE and transferred to a nitrocellulose membrane. Immunoblotting of cell lysates was performed with rabbit anti–phospho-p38, –phos-
ANOVA followed by Fisher least significant difference test, as appropriate.

GAPDH

did not confirm a significant change of AT1 mRNA with PAN

ever, further examination of gene expression by real-time PCR

Figure 3. Quantification of GEN branching. Branching of GEN

that were incubated as shown in Figure 2 was quantified as

described in Materials and Methods. Treatment with medium

from normal podocytes without PAN [Sup/PAN(–)] significantly

increased whereas treatment with medium from PAN-

injured podocytes [Sup/PAN(+)] decreased GEN branching

compared with cells that incubated in regular medium (NC).

Addition of ARB to PAN-injured podocytes [ARB+Sup/

PAN(+)] rescued GEN branching. Addition of ARB to normal

podocytes [ARB+Sup/PAN(–)] enhanced GEN sprouting when

compared with NC levels. Addition of wortmannin

prevented induction of GEN branching that was observed with

ARB+Sup/PAN(+) medium [ARB+Sup/PAN(+) + WM].

Treatment with ARB or PAN alone did not affect GEN branch-

ing. Similarly, addition of wortmannin to Sup/PAN(+) did not

affect GEN branching further [Sup/PAN(+) + WM]. Results are

from two independent experiments, each in duplicate.

pho-ERK, or –phospho-AKT antibodies (Cell Signaling Technology, Beverly, MA), followed by horseradish peroxidase–conjugated anti-

rabbit antibody. Immunoreactive proteins were visualized as indicated above.

Total ERK, p38, and AKT content was verified by stripping the

membranes in 50 mM Tris HCl (pH 6.5) that contained 2% SDS and

0.4% β-mercaptoethanol for 1 h at 55°C and reprobing with a rabbit

anti-ERK, anti-p38, or anti-AKT antibody (Cell Signaling Technology).

Statistical Analyses

Data are presented as means ± SEM. P values were calculated by

ANOVA followed by Fisher least significant difference test, as appro-

priate. P < 0.05 was considered to be significant.

Results

ARB Restored Injured Podocyte Effects on GEN Growth

We first investigated the effects of podocyte injury on activa-

tion of the RAS in our model system. By nonquantitative PCR, angiotensinogen and AT1 mRNA were found to be ex-

pressed in these cultured podocytes, with a suggestion that

AT1 receptor mRNA was increased by PAN injury, with lesser

numeric increase in response to angiotensin II (Figure 1). How-

ever, further examination of gene expression by real-time PCR
did not confirm a significant change of AT1 mRNA with PAN

(podocyte baseline 3.1 ± 0.6, podocyte+PAN 2.6 ± 1.0, copies/

10^6 GAPDH; NS; n = 3). These differences may reflect differ-

ences as a result of varying molecules for normalization

(GAPDH versus β-actin). Addition of angiotensin II did result in

numeric downregulation of AT1 mRNA (podocyte+angioten-

sin II 1.6 ± 0.2).

We next assessed the effects of such PAN-injured podocytes

on GEN sprouting by culturing GEN with 24-h conditioned

medium from untreated or PAN-treated podocytes. Medium

from untreated podocytes significantly increased GEN sprout-

ing compared with GEN that were cultured under basal con-

ditions with only standard endothelium medium as described

in the Materials and Methods section (9.2 ± 7.5 versus 4.4 ± 2.2

branches/high-power field; P < 0.05). In contrast, medium

from PAN-injured podocytes resulted in significantly de-

creased GEN sprouting, even below basal levels (2.2 ± 0.9; P <

0.05). It is interesting that medium from PAN-injured podo-

cytes that were treated with ARB rescued GEN sprouting when

compared with medium that was derived from injured podo-

cytes only (6.5 ± 4.5; P < 0.05). PAN per se, added to GEN in

standard medium, did not affect GEN sprouting, supporting

the data that PAN is specifically toxic to epithelial cells (3.8 ±

2.3; NS). Addition of ARB to basal medium did not significantly

affect GEN sprouting (4.1 ± 1.0; NS; Figures 2 and 3). It is

interesting that ARB and normal podocyte media together nu-

merically enhanced GEN sprouting (Figures 2E and 3).

We next tested whether the decreased GEN sprouting that

was observed with medium from PAN-treated podocytes was

due to decreased GEN proliferation and/or migration. Medium

from normal podocytes significantly increased GEN prolifera-

tion and migration compared with GEN that were grown under

basal conditions (proliferation 118,699 versus 77,167 ±

4860 cpm [P < 0.001]; migration 36.9 ± 3.4 versus 24.4 ± 3.3

cells/field [P < 0.001]). In contrast, medium from PAN-treated

podocytes resulted in significantly decreased GEN proliferation

and migration with levels even less than baseline (52,337 ±

13,692 and 16.4 ± 3.1, respectively; both P < 0.001 versus

untreated). Importantly, as observed for sprouting, ARB re-

stored the injured podocytes' ability to promote GEN prolif-
ARB Treatment Enhances Secretion of Angiogenic Factors by Podocytes

Podocytes can affect GEN functions by producing angiogenic factors, including VEGF-A and Ang-1 (14,15). We therefore analyzed the levels of secreted VEGF-A and Ang-1 in 24-h podocyte-conditioned medium. As expected, both VEGF-A and Ang-1 protein were significantly decreased in conditioned medium from PAN-treated versus untreated podocytes (VEGF-A 0.79 ± 0.07 versus 1.92 ± 0.23 [P < 0.05]; Ang-1 1.85 ± 0.21 versus 3.00 ± 0.02 [P < 0.05]). It is interesting that addition of ARB to PAN-treated podocytes upregulated VEGF-A (2.5-fold) and Ang-1 secretion (1.5-fold) in conditioned medium (Figure 6), restoring protein levels to those seen in noninjured podocytes. Treatment of noninjured podocytes with ARB did not alter VEGF-A or Ang-1 secretion compared with basal levels. It is interesting that real-time PCR did not show significant changes in VEGF mRNA among groups, whereas Ang-1 mRNA decreased in response to PAN and ARB (fold changes versus baseline podocytes: VEGF-A/GAPDH podocyte baseline 1.00 ± 0, podocyte+PAN 1.06 ± 0.3, podocyte+PAN+ARB 1.12 ± 0.2 [NS; n = 3]; Ang-1/GAPDH podocyte baseline 1.00 ± 0, podocyte+PAN 0.57 ± 0.1, podocyte+PAN+ARB 0.29 ± 0.1 [P < 0.05; n = 3]). These data indicate that the observed changes in protein levels do not reflect changes in mRNA.

ARB-Induced Angiogenic Factors by Podocytes Promote GEN Proliferation and Branching

As shown in Figure 6, addition of ARB to PAN-treated podocytes stimulated the secretion of both VEGF and Ang-1. However, angiotensin II alone or with normal podocyte-conditioned medium did not significantly change GEN proliferation or branching, although there was a numeric trend for more proliferation with less formed branching (Figure 7). For determination of whether these factors were involved directly in the stimulation of GEN proliferation and branching, GEN were cultured with conditioned medium from PAN+ARB-treated podocytes in the presence or absence of anti-mouse VEGFR2 and Tie2 antibody. Proliferation and branching then were analyzed as described above. Addition of anti-VEGFR2 and/or
molecules p38, ERK, and the AKT (31). We therefore investigated the phosphorylation of these intracellular signaling molecules in GEN that were exposed to 24-h podocyte-conditioned medium. Incubation for 15 min of GEN that had been serum-starved for 24 h with medium from noninjured podocytes led to p38, ERK, and AKT phosphorylation. This activation was not seen when GEN were incubated with medium from PAN-treated podocytes. Medium that was derived from injured podocytes that were treated with ARB restored p38, ERK, and AKT phosphorylation back to levels that were observed with medium from noninjured podocytes (Figure 8, A through C). PI3-K is the major angiogenic pathway that is activated by both Ang-1 (32) and VEGF (33–35). We therefore determined whether activation of this pathway indeed was involved in GEN function. To do this, we pretreated GEN with the PI3-K inhibitor wortmannin (100 nM) for 1 h to prevent PI3-K activation. Of note, wortmannin is a nonselective inhibitor of the PI3-K family and may have effects on multiple PI3-K and even other kinases (36). Wortmannin preferentially inhibits p85 autophosphorylation and consequent PI3-K activation. Because p85 is the subunit expressed by most of the PI3-K isoforms, wortmannin usually is used as a broad-spectrum PI3-K inhibitor. Inhibition of other kinases (e.g., mitogen-activated protein kinase [MAPK], or myosin light chain kinase) usually results from doses higher than those that were used in this study. Addition of wortmannin not only abolished the ability of medium from ARB-treated injured podocyte to induce AKT phosphorylation (Figure 8D) but also prevented ARB-induced branching formation when compared with GEN that were cultured in the presence of medium from ARB-treated injured podocyte alone (Figures 2 and 3).

Discussion

Recently, the concept has been proposed and evidence has been provided, that regression of glomerulosclerosis can be achieved in various experimental settings by inhibition of angiotensin (1,5,6,37). However, the cellular and molecular mechanisms of regression that are achieved by angiotensin inhibition remain unclear. Importantly, angiotensin promotes matrix synthesis by its direct hemodynamic and matrix synthesis effects and induction of other profibrotic molecules (e.g., TGF-β) and also inhibits matrix degradation by directly inducing tissue inhibitor of metalloprotease-1 and plasminogen activator inhibitor-1 via the AT1 receptor (1,38). Inhibitors of the RAS have particular efficacy in treatment of glomerulosclerosis, thought to reflect this multiplicity of effects on matrix accumulation and BP (5,39). In vivo studies indeed have shown that high-dose ARB even have the potential to induce regression of existing sclerosis (1–6). Our study adds to the spectrum of ARB effects in chronic kidney disease, indicating that ARB can promote regeneration of capillary loops via effects on podocytes.

The podocyte-derived factors VEGF-A and Ang-1 promote endothelial cell proliferation; increase resistance to apoptosis; change proteolytic balance, cytoskeletal reorganization, and migration; and, finally, promote differentiation and formation of a new vascular lumen (31). VEGF-A is one of the major proangiogenic growth factors that are altered in progressive

Activation of Distinct Signaling Pathways in GEN Cultured with Podocyte-Conditioned Medium

We next investigated potential molecular mechanisms through which podocyte-secreted angiogenic factors might regulate GEN branching, proliferation, and migration. VEGF-A was shown previously to activate the intracellular signaling

anti-Tie2 antibody significantly decreased GEN proliferation and branching compared with cells that were treated with PAN+ARB conditioned medium (Figure 7). In contrast, addition of anti-VEGFR2 and/or anti-Tie2 to regular endothelial cell medium (which does not contain either one of the two angiogenic factors) did not alter GEN proliferation or branching (Figure 7). Therefore, ARB stimulates secretion of VEGF-A and Ang-1 by podocytes, which in turn promote GEN functions, including proliferation and branching.

Figure 7. Angiogenic factors in podocyte-conditioned medium promote GEN proliferation and branching. GEN were treated with either fresh cell medium or medium that was derived from podocytes that were cultured with PAN+ARB. In separate identically treated GEN, anti-mouse VEGF receptor 2 (VEGFR2) and/or anti-mouse Tie2 antibodies (10 μg/ml) were added. The PAN+ARB-conditioned medium promoted GEN proliferation (A) and branching (B). Addition of anti-VEGFR2 and/or anti-Tie2 antibodies to this PAN+ARB-conditioned medium significantly reduced GEN proliferation (A) and branching (B). Neither ARB nor PAN alone altered GEN proliferation (A) or branching (B). Anti-VEGFR2 and anti-Tie2 antibodies had no effect when added alone to unconditioned medium. Results are from two independent experiments, each in duplicate.

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renal disease (40). Podocyte VEGF-A is decreased in the sclerotic glomeruli, and tubular VEGF-A is lost in chronic fibrosing interstitial disease (16,41). Conversely, elegant in vivo experiments have shown that treatment with exogenous VEGF-A decreases sclerosing injuries, restoring glomerular and peritubular capillaries (42). Receptors for VEGF-A and Ang-1 are present on GEN, governing their proliferation, migration, and sprouting (43,44). We hypothesized that injured podocytes were impaired in effecting these key GEN angiogenic responses. In this study, we investigated potential mechanisms whereby angiotensin inhibition affects podocyte–GEN interaction that could decrease sclerosis. We used PAN as a podocyte injury model because PAN results in focal segmental glomerulosclerosis with podocyte-specific injury in vitro and in vivo.

Figure 8. Western blot analysis of activated extracellular signal–regulated kinase (ERK), AKT, and p38. Serum-starved endothelial cells were kept untreated (serum-free) or treated for 15 min as indicated, and levels of phosphorylated p38, ERK, and AKT were analyzed by Western blot. Activation of p38 (A) was evident only in GEN treated with Sup/PAN(−) ARB + Sup/PAN(+), or ARB + Sup/PAN(−) podocyte-conditioned media. (B) ERK phosphorylation was suppressed in GEN treated by media from PAN-injured podocytes [Sup/PAN(+)] compared with treatment from normal podocyte medium [Sup/PAN(−)] to levels comparable to those seen with serum-free medium. However, activity of ERK in GEN was restored by treating injured podocytes with ARB [ARB + Sup/PAN(+)]. (C) AKT phosphorylation was suppressed in GEN treated by medium from PAN-injured podocytes [Sup/PAN(+)] compared with treatment from normal podocyte medium [Sup/PAN(−)] to levels comparable to those seen with serum-free medium. However, activity of AKT was restored by treating podocytes with ARB [ARB + Sup/PAN(+)]. (D) The phosphatidylinositol-3 kinase inhibitor wortmannin (WM) prevented AKT phosphorylation by ARB treatment of injured podocytes [ARB + Sup/PAN(+)]. WM alone or added to injured podocyte medium did not affect AKT phosphorylation compared with controls (serum-free and Sup/PAN, respectively). Results are from two independent experiments, each in duplicate.
We observed, as expected, that injured podocytes were ineffective in mediating GEN angiogenesis, proliferation, and migration. These effects were linked to significantly decreased VEGF-A and Ang-1 protein in the supernatant from PAN-injured podocytes. Conversely, ARB treatment of injured podocytes dramatically increased VEGF-A and Ang-1 protein. In parallel, ARB treatment of podocytes restored their ability to promote endothelial cell sprouting, proliferation, and migration.

Inhibition of these angiogenic factors prevented this GEN response, supporting that these key soluble podocyte-derived factors are important contributors to GEN angiogenesis. Of note, although both angiotensinogen and AT1 are expressed by these cultured podocytes, we were not able to demonstrate increased angiotensin II or AT1 in our PAN injury model system. However, AT1 activation may occur by alternative means, such as dimerization; therefore, these findings neither prove nor exclude AT1 activation (46). Clinical data have indicated remarkable efficacy of angiotensin-converting enzyme inhibitor or ARB in decreasing BP, proteinuria, and sclerosis, even in the absence of measurable systemic increases in the RAS. Our experimental data show that angiotensin inhibition modulates podocyte injury responses and secretion of key angiogenic factors and thereby affects GEN remodeling after injury. Whether these effects are mediated specifically by the AT1 receptor or indirectly via effects on activation of the AT2 receptor or other mechanisms remains to be proved.

VEGF-mediated angiogenesis is mediated through multiple signal pathways, including p38 MAPK, MAPK/ERK, and PI3-K/AKT (47–51). Endothelial cell migration is an early key step in the initiation of angiogenesis (52–54). VEGF-A inhibits apoptosis of endothelial cells and promotes endothelial cell sprouting by activating AKT via the PI3-K-dependent pathway (33,55,56). In this study, we therefore further investigated whether these signaling cascades were affected by ARB treatment of podocytes and impact on GEN sprouting, a key marker of angiogenesis. We observed that p38, ERK, and AKT phosphorylation was suppressed in GEN treated by medium from injured versus normal podocytes. However, importantly, ARB treatment of injured podocytes led to restored activities of p38, ERK, and AKT in GEN. Furthermore, when PI3-K/AKT phosphorylation was inhibited by wortmannin, the GEN branching response to ARB-treated podocytes was prevented, further supporting a key role of this pathway in the podocyte modulation of GEN responses. These data collectively support that ARB modulation of podocyte VEGF signaling pathways is important for optimal GEN responses and growth.

**Conclusion**

Injured podocytes are ineffective in mediating GEN angiogenesis, proliferation, and migration, linked to decreased VEGF-A and Ang-1. ARB treatment of podocytes restored their ability to promote endothelial cell sprouting, proliferation, and migration, which was associated with activation of p38 MAPK, ERK, and AKT. These data support that ARB may modulate sclerosis, not only by effects on BP and extracellular matrix but also in part by effects on podocytes that promote capillary growth.

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