Poly(ADP-Ribose) Polymerase-1 Mediates Angiotensin II-Induced Expression of Plasminogen Activator Inhibitor-1 and Fibronectin in Rat Mesangial Cells

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Key Words

Rat mesangial cells • Angiotensin II • Poly(ADP-ribose) polymerase-1 • Plasminogen activator inhibitor-1 • Fibronectin

Abstract

Objective: To investigate the effects of poly(ADP-ribose) polymerase-1 (PARP-1) on angiotensin II (Ang II)-induced plasminogen activator inhibitor-1 (PAI-1) and fibronectin (FN) in rat mesangial cells (RMCs). **Methods:** Followed by serum starvation for 16 h, RMCs were exposed to Ang II for an indicated time to examine the protein expression of PARP-1. The cells were treated with or without Ang II for 12–24 h in the presence or absence of an inhibitor of PARP, N-(6-oxo-5,6-dihydrophenanthridin-2-yl)-N,N-dimethylacetamide hydrochloride (PJ34) or small interfering RNA (siRNA) duplexes targeting PARP-1. The mRNA and protein expressions of PARP-1, PAI-1 and FN were determined by real-time RT-PCR and Western blot, respectively. The activity of PARP-1 was examined by colorimetric assay. **Results:** Ang II did not only significantly induce PARP-1 expression and activity, but also increased PAI-1 and FN expression in RMCs. All these responses induced by Ang II were significantly inhibited by both the PARP inhibitor PJ34 and downregulating PARP-1 with the siRNA technique. **Conclusions:** Our data suggest

that PARP-1 mediates Ang II-induced PAI-1 and FN in RMCs and may thus represent a potential therapeutic target in the treatment of glomerular disease.

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Introduction

Chronic kidney disease (CKD) is common in China and worldwide [1, 2]. The estimated prevalence of CKD in south Chinese adults was more than 12.0% in 2007, based on the survey conducted by our hospital [3]. CKD is now recognized as an independent risk factor for both end-stage renal disease and cardiovascular disease [4]. Representative remodeling of the kidney in CKD includes development of glomerulosclerosis and tubulointerstitial fibrosis [5, 6]. Activation of the renin-angiotensin system, which increases the synthesis of angiotensin II (Ang II), is a common manifestation in patients with CKD. In CKD patients, systemic and internal renal concentration of renin and Ang II is frequently inappropriately high, and the latter is even much higher and plays more prominent roles in the pathogenesis of glomerulo-

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sclerosis and tubulointerstitial fibrosis, as well as many cardiovascular disorders [5]. Ang II is not only a vasoactive agent that participates in local and systemic hemodynamic regulation, but also acts as a potent cytokine that regulates cell growth, epithelial-mesothelial transition and extracellular matrix (ECM) synthesis and degradation [7, 8]. Plasminogen activator inhibitor-1 (PAI-1) is a key regulator in ECM remodeling and renal fibrosis. Increasing evidence has proven that PAI-1 takes part in the Ang II signaling pathway [9–12]. TGF- β and several other factors were also reported to participate in those processes involved in Ang II [9–12], but in vivo and in vitro research shows weak amelioration of renal diseases using inhibitors of those factors. It pushes us to explore some new mediators, which may play important roles in Ang II-induced renal diseases.

Poly(ADP-ribose) polymerase (PARPs), the second most abundant non-histone nuclear protein, is a series of poly(ADP-ribosylation) enzymes present in eukaryotes [13, 14]. PARP-1 is the most abundant and important enzyme among the family, which consists of almost 80% portion and accounts for about 90% of total cellular PARP activity [15]. When DNA is damaged, it senses and binds to DNA nick, forms homodimers and catalyzes the cleavage of nicotinamide dinucleotide (oxidized) (NAD⁺) into nicotinamide and ADP-ribose and uses the latter to synthesize branched nucleic acid-like polymers poly(ADP-ribose) covalently attached to nuclear acceptor proteins, in particular histones, several transcription factors like NF- κ B, and PARP itself, thereby regulating their activities and functions [13–15]. A hypothesis regarding PARP says that PARP is critical for many physiological events like cellular differentiation and proliferation, genome integrity, cell survival and cell death when properly activated, but causing many pathophysiological outcomes, such as inflammation, carcinogenesis and neuronal functions when improperly activated [16]. Some reports observed the overactivation of PARP-1 in diabetic complications. Du et al. [17] detected PARP-1 activation and abnormal accumulation of glucose metabolic product in aorta endothelial cells stimulated by high glucose. Szabo et al. [18] discovered in db/db diabetic mice that the activity of PARP-1 increased in the kidney, and its specific inhibitor dramatically attenuated proteinuria, glomerular mesangium expansion and podocyte injury. Furthermore, PARP-1 inhibitors ameliorate nephropathy of type 2 diabetic Lepr^{db/db} mice [19]. A recent study reported that PARP-1 gene deficiency alleviated diabetic kidney disease through decreasing mesangial expansion and fibronectin (FN) expression [20]. In addition, inhibi-

tion of PARP-1 by its inhibitor, PJ34, diminished PAI-1 and attenuated ventilator-induced lung injury [21]. These studies suggest that PAI-1 and FN are downstream of PARP-1. Therefore, we hypothesize that PARP-1 may act as a potential mediator in Ang II-induced CKD. In this study, we aim to examine whether PARP-1 regulates expression of PAI-1 and FN in Ang II-induced rat mesangial cells (RMCs).

Methods

Chemicals and Reagents

Ang II was purchased from Sigma-Aldrich (St. Louis, Mo., USA). Trypsin, ethylenediamine tetraacetic acid, fetal bovine serum, RPMI 1640 medium and TRIzolTM reagent were purchased from Invitrogen (Carlsbad, Calif., USA). Insulin was from Gan & Lee Pharmaceutical Ltd (Beijing, China). PJ34 was from Calbiochem. Cell lysis, mouse anti-rat β -actin monoclonal antibody, horseradish peroxidase-labeled secondary antibody against mouse or rabbit were purchased from Cell Signaling Technology. Rabbit anti-PARP polyclonal antibody and rabbit anti-mouse PAI-1 polyclonal antibody were purchased from Santa Cruz. The RevertAidTM First Strand cDNA reverse transcription kit was purchased from Fermentas and the HT colorimetric PARP/apoptosis assay was purchased from Trevigen Inc. (Gaithersburg, Md., USA).

Cell Culture

A rat mesangial cell line (MCs 1097), obtained from ATCC, was cultured in RPMI 1640 medium containing 15% fetal bovine serum and 0.6 U/ml insulin. Subconfluent mesangial cells were incubated with serum-free medium for 16 h to arrest and synchronize the cell growth. The cells were treated with fresh serum-free RPMI 1640 medium containing Ang II (10^{-7} M). Cells were pretreated with the PARP-1 inhibitor PJ34 (3×10^{-6} M) for 1 h followed by Ang II stimulation for 12 and 24 h, and cells were harvested at 12 h to collect total RNA and at 24 h to collect total protein for further analysis. Total RNA from RMCs was extracted using TRIzol reagent according to the manufacturer's instructions. Total protein was extracted using the method described by Rhyu et al. [22]. At the end of incubation, the cells were washed with PBS and lysed in 80 μ l of lysis buffer containing protease inhibitors for 5 min on ice. Conditioned medium and cell lysates were centrifuged at 13,000 g at 4°C for 10 min, and the concentration of cellular protein was determined by the Pierce BCA assay.

siRNA Transfection

Small interfering RNAs (siRNAs) were designed and synthesized by Genepharma Inc. (Shanghai, China). Sequences of PARP-1 siRNA duplexes were sense 5'-GGGCAAGCACAGUGUCAATT-3' and antisense 5'-UUUGACACUGUGCUUGCCCTT-3'. siRNA duplexes were transfected into cells (50–70% confluence) using Lipofectamine 2000 siRNA transfection reagent (Invitrogen). Briefly, 4 μ l Lipofectamine 2000 and 1.5 μ l PARP-1 siRNA duplexes (5×10^{-8} M) were first diluted into 300 μ l optimal medium, respectively, without serum and antibiotics,

Table 1. Primer sequences and reaction conditions used for RT-PCR

Gene	Primer sequences	Size, bp	Reaction condition
PARP-1	forward: 5'-ACGCACAATGCCTATGAC-3' reverse: 5'-CACATCTCCAAGGCGACC-3'	441	94°C 5 min, 94°C 30 s, 58°C 30 s, 72°C 45 s, 29 cycles, 72°C 10 min
PAI-1	forward: 5'-ATGGAACAAGAATGAGATCA-3' reverse: 5'-TCA AAGGGTGCTGCAATGAAC-3'	471	94°C 5 min, 94°C 30 s, 53°C 30 s, 72°C 45 s, 32 cycles, 72°C 10 min
FN	forward: 5'-CTGAAGTCACTTCTCGGGGTGC-3' reverse: 5'-GCACAACAGACCACCAAACTCG-3'	430	94°C 5 min, 94°C 30 s, 56°C 30 s, 72°C 45 s, 29 cycles, 72°C 10 min
β -Actin	forward: 5'-GGGAAATCGTGCGTGACATTA-3' reverse: 5'-TCGTCATACTCCTGCTTGCTG-3'	475	94°C 5 min, 94°C 30 s, 53°C 30 s, 72°C 45 s, 28 cycles, 72°C 10 min

Table 2. Primer sequences for real-time PCR

Gene	Primer sequences	Size, bp
PARP-1	forward: 5'-ACACGTTAGCGGAGCGGACCG-3' reverse: 5'-CGCCCGCTCTTAGCGTACTCC-3'	113
PAI-1	forward: 5'-ACCTGGGTCAAGTCGAAGCGGA-3' reverse: 5'-CGCATTGGCCCGTGCTGGTA-3'	153
FN	forward: 5'-TCAGCCATGTCCAATCCC-3' reverse: 5'-CATACCCGTAGCATCCACC-3'	135
β -Actin	forward: 5'-ACAACCTTCTTGCAGCTCCTCCG-3' reverse: 5'-GTTGTGCGACGACGAGCGCAGC-3'	89

mixed gently and incubated for 5 min at room temperature and then the two mixed gently and incubated for 30 min and added to the new mixture into cells. Negative control cells were treated with negative control vectors consisting of a scrambled siRNA. The cells were incubated for 6 h and the medium changed with RPMI 1640 containing 15% FBS overnight. The transfected mesangial cells were then incubated with serum-free media for 16 h to arrest and synchronize the cell growth. The cells were treated with fresh serum-free RPMI 1640 medium containing Ang II (10^{-7} M).

Reverse Transcription Polymerase Chain Reaction

The mRNA levels of PARP-1, PAI-1 and FN were assessed by reverse transcription polymerase chain reaction (RT-PCR). One microgram total RNA was reverse transcribed into cDNA with the Fermentas RevertAid First Strand cDNA synthesis system according to the manufacturer's instructions. Subsequently, the polymerase reaction was performed using the TaKaRa system. The forward and reverse primers and reaction condition used in this study are shown in table 1. Each sample was performed in triplicate. To confirm the amplification specification, the PCR products were subject to a melting-curve analysis and subsequent agarose gel electrophoresis. The result was normalized by β -actin and expressed as relative changes to control.

Quantitative Real-Time PCR

The total RNA was extracted from RMCs using TRIzol reagent according to the manufacturer's instructions. Total RNA (1 μ g) was reverse transcribed, followed by quantitative real-time PCR using the Bio-Rad iCycler Iq system. The PCR reactions were carried out in a total volume of 25 μ l containing 12.5 μ l of SYBR Premix Ex TaqTM (2 $\times$) (TaKaRa Biotechnology Co., Ltd, Dalian, China), 2 μ l of cDNA, 5 pmol of forward and reverse primers, and 9.5 μ l of distilled H₂O. The sequences of the primers used are listed in table 2. PCR was performed at 95°C for 30 s, and then run for 45 cycles at 95°C for 5 s and 60°C for 20 s. The relative amount of mRNA was calculated using the comparative C_t ($\Delta\Delta C_t$) [23]. The derived normalized values were the mean of three runs.

Western Blot Analysis

The protein levels of PARP-1, FN and PAI-1 protein expression were examined by the Western blot method. Samples with equal concentrations of cellular protein (20 μ g) were mixed with a 2 $\times$ sample buffer and was heated at 95°C for 5 min and separated on 8% SDS-polyacrylamide gels. After electrophoresis for 90 min, the proteins were transferred onto a nitrocellulose membrane using a transblot chamber with Tris buffer (0.025 M Tris-HCl, 0.192 M glycine, and 20% MeOH). The membrane was blocked for 1 h at room temperature with 5% non-fat milk in 0.1% TBS-Tween

Table 3. Effect of PJ34 and siRNA duplexes targeting PARP-1 on Ang II-induced PARP-1 activation in RMCs determined by colorimetric assay

Group	PARP-1 activity, U/mg protein
1	0.033989 ± 0.005841
2	0.052223 ± 0.008282 ^a
3	0.033701 ± 0.005180 ^b
4	0.032065 ± 0.005224 ^c

PARP-1 activity in RMCs determined by colorimetric assay: 1 = RMC treated with vehicle (blank), 2 = RMC treated with Ang II (0.1 μM), 3 = RMC pretreated with PJ34 (3 μM) followed by Ang II (0.1 μM) treatment, 4 = RMC pretreated with PARP-1 siRNA (50 nM) followed by Ang II (0.1 μM) treatment.

Data are expressed as mean ± SD from three independent experiments.

^a p < 0.05 (2 vs. 1); ^b p < 0.05 (3 vs. 2); ^c p < 0.05 (4 vs. 2).

20. The membranes were incubated at 4°C overnight with a dilution of antibody of PARP-1, FN and PAI-1. After extensive washing in TBS-Tween 20, the membranes were then incubated with secondary anti-mouse IgG or anti-rabbit IgG for 1 h at room temperature. After washing, the membranes were incubated with an enhanced chemiluminescence system detection kit. Positive immunoreactive bands were quantified densitometrically, normalized by β-actin and compared with controls.

PARP Activity Assay

PARP activity was assayed using the universal colorimetric PARP assay kit (Trevigen Inc., USA), based on the incorporation of biotinylated ADP-ribose onto histone proteins. Cell lysates from RMCs containing about 50 μg of protein were loaded into a 96-well plate coated with histones and biotinylated poly-ADP-ribose, allowed to incubate for 1 h, treated with streptavidin-horse-radish peroxidase, and read at 450 nm in a spectrophotometer.

Statistical Analysis

All results are expressed as mean ± SD with n as the number of experiments. Analysis of variance (ANOVA) was used to assess the differences between multiple groups using SPSS 13.0 software (SPSS Inc., Chicago, Ill., USA). A value of p < 0.05 was considered statistically significant.

Results

Effects of Ang II on PARP-1 Protein Expression in RMCs

Figure 1 shows Ang II-induced PARP-1 expression from 12 to 48 h. The PARP-1 protein expression of the Ang II group at 12, 24, 36, or 48 h was upregulated by 158.6, 226.5, 244.9, and 180.1% (p < 0.05), compared with

Fig. 1. Effects of Ang II on PARP-1 protein expression in RMCs determined by Western blot: RMCs were exposed to Ang II (0.1 μM) for (1) 0 h, (2) 3 h, (3) 6 h, (4) 12 h, (5) 24 h, (6) 36 h, and (7) 48 h.

Fig. 2. Effect of losartan on Ang II-induced PARP-1 expression in RMCs by Western blot: (1) RMCs treated with vehicle (blank), (2) RMCs treated with Ang II (0.1 μM), (3) RMCs pretreated with losartan (10 μM) followed by Ang II (0.1 μM) treatment, and (4) RMCs treated with losartan (10 μM) alone.

control cells, respectively (p < 0.05). Therefore, we chose the optimal time to examine effects of PJ34 and siRNA duplex targeting PARP-1 on Ang II-induced PARP-1 activity and expression at 24 h.

Effect of PJ34 and siRNA Duplex Targeting PARP-1 on Ang II-Induced PARP-1 Activity in RMCs

As shown in table 3, Ang II obviously stimulated PARP-1 activity at 24 h. In addition, the PARP-1 activity of the Ang II group was upregulated by 53.6% (p < 0.05), compared with control cells. PJ34 and PARP-1 siRNA duplex both effectively inhibited the activation of PARP-1 by 35.5 and 38.6%, respectively (p < 0.05), when compared with the cells treated with Ang II alone.

Fig. 3. Effects of PJ34 and siRNA duplexes targeting PARP-1 on Ang II-induced PARP-1, PAI-1 and FN mRNA in RMCs at 12 h determined by RT-PCR (a) and real-time PCR (b), respectively. The intensity of the resultant bands are scanned and expressed as a ratio of β -actin: (1) RMCs treated with vehicle (blank), (2) RMCs treated with Ang II (0.1 μ M), (3) RMCs pretreated with PJ34

(3 μ M) followed by Ang II (0.1 μ M) treatment, (4) RMCs treated with PJ34 (3 μ M) alone, (5) RMCs pretreated with PARP-1 siRNA (50 nM) followed by Ang II (0.1 μ M) treatment, and (6) RMCs treated with scrambled siRNA (50 nM) followed by Ang II (0.1 μ M). Data are expressed as mean $\pm$ SD (n = 3). * p < 0.01 versus control; # p < 0.01 versus Ang II alone.

Fig. 4. Effects of PJ34 and siRNA duplexes targeting PARP-1 on Ang II-induced PARP-1, PAI-1 and FN mRNA in RMCs at 24 h determined by RT-PCR (a) and real-time PCR (b), respectively. The intensity of the resultant bands are scanned and expressed as a ratio of β -actin: (1) RMCs treated with vehicle (blank), (2) RMCs treated with Ang II (0.1 μ M), (3) RMCs pretreated with PJ34

(3 μ M) followed by Ang II (0.1 μ M) treatment, (4) RMCs treated with PJ34 (3 μ M) alone, (5) RMCs pretreated with PARP-1 siRNA (50 nM) followed by Ang II (0.1 μ M) treatment, and (6) RMCs treated with scrambled siRNA (50 nM) followed by Ang II (0.1 μ M). Data are expressed as mean $\pm$ SD (n = 3). * p < 0.01 versus control; # p < 0.01 versus Ang II alone.

Effect of Angiotensin Receptor in Ang II-Induced PARP-1 in RMCs

The effect of angiotensin receptor blockade on Ang II-induced PARP-1 in RMCs has not been elucidated. As shown in figure 2, Ang II-induced PARP-1 in RMCs was

markedly attenuated by the AT1 antagonist losartan (p < 0.01), suggesting that AT1 signaling is involved in Ang II-induced responses. However, losartan (10 μ M) has no effect on the basal levels of PARP-1 (fig. 2).

Fig. 5. Effects of PJ34 and siRNA duplexes targeting PARP-1 on Ang II-induced protein expression of PARP-1, PAI-1 and FN in RMCs at 12 h determined by Western blot. The intensity of the resultant bands are scanned and expressed as a ratio of β -actin: (1) RMCs treated with vehicle (blank), (2) RMCs treated with Ang II (0.1 μ M), (3) RMCs pretreated with PJ34 (3 μ M) followed by Ang II (0.1 μ M) treatment, (4) RMCs treated with PJ34 (3 μ M) alone, (5) RMCs pretreated with PARP-1 siRNA (50 nM) followed by Ang II (0.1 μ M) treatment, and (6) RMCs treated with scrambled siRNA (50 nM) followed by Ang II (0.1 μ M).

Fig. 6. Effects of PJ34 and siRNA duplexes targeting PARP-1 on Ang II-induced protein expression of PARP-1, PAI-1 and FN in RMCs at 24 h determined by Western blot. The intensity of the resultant bands are scanned and expressed as a ratio of β -actin: (1) RMCs treated with vehicle (blank), (2) RMCs treated with Ang II (0.1 μ M), (3) RMCs pretreated with PJ34 (3 μ M) followed by Ang II (0.1 μ M) treatment, (4) RMCs treated with PJ34 (3 μ M) alone, (5) RMCs pretreated with PARP-1 siRNA (50 nM) followed by Ang II (0.1 μ M) treatment, and (6) RMCs treated with scrambled siRNA (50 nM) followed by Ang II (0.1 μ M).

Effect of PJ34 and siRNA Duplex Targeting PARP-1 on Ang II-Induced mRNA of PARP-1, FN and PAI-1 in RMCs

As shown in figures 3 and 4, Ang II markedly increased PARP-1, FN and PAI-1 mRNA at 12 and 24 h, respectively ($p < 0.05$), compared with control cells. PJ34 effectively inhibited the upregulation of PARP-1, PAI-1 and FN mRNA at 12 and 24 h, respectively ($p < 0.05$), compared with the cells treated with Ang II alone. Similarly, siRNA duplex targeting PARP-1 significantly inhibited the upregulation of PARP-1, PAI-1 and FN mRNA at 12 and 24 h, respectively ($p < 0.05$). However, PJ34 alone did not affect basal levels of PARP-1, PAI-1 and FN mRNA. In addition, scrambled siRNA duplex did not affect Ang II-induced upregulation of PARP-1, PAI-1 and FN mRNA in RMCs at 12 and 24 h, respectively.

Effect of PJ34 and siRNA Duplex Targeting PARP-1 on Ang II-Induced Protein Expression of PARP-1, FN and PAI-1 in RMCs

As shown in figures 5 and 6, Ang II stimulated PARP-1, PAI-1 and FN protein expression at 12 and 24 h, respectively ($p < 0.05$), compared with control cells. PJ34 effec-

tively inhibited the upregulation of PARP-1, PAI-1 and FN protein expression induced by Ang II, respectively ($p < 0.05$), compared with the cells treated with Ang II alone. Similarly, siRNA duplex targeting PARP-1 significantly inhibited the upregulation of PARP-1, PAI-1 and FN expression induced by Ang II ($p < 0.05$). However, PJ34 alone did not affect basal protein expression of PARP-1, PAI-1 and FN, and scrambled siRNA duplex did not affect Ang II-induced PARP-1, PAI-1 and FN upregulation in RMCs.

Discussion

Renal mesangial cells play a critical role in glomerulosclerosis. Over the last decade, it has been clear that Ang II regulates the growth (including hyperplasia and hypertrophy) of mesangial cells, and increases synthesis and expression in ECM proteins [5, 11]. The ECM proteins consist of FN, collagen, laminin, and others [5, 24–26]. However, our understanding of the precise mechanism is unclear and this study focused on the intracellular signaling mechanisms involved in Ang II-induced PAI-1 and FN expression in RMCs. In this study, we have demon-

strated for the first time that the PARP-1 inhibition decreased Ang II-induced upregulation of PAI-1 and FN expression in cultured RMCs, showing direct cellular effects of PARP-1 inhibitors and knockdown by its siRNA on Ang II-induced fibrotic responses.

PARPs, as poly(ADP-ribosyl)ating enzymes, are involved in many cellular processes, including the control of repair of damaged DNA (especially single-strand breaks) and the control of gene expression/transcription [16, 27]. Inhibition of PARP activity can lead to protection against many pathophysiological outcomes [16, 27]. PARP-1-deficient mice and PARP inhibitors have been shown to significantly reduce ECM accumulation in various diseases [28–32]. All of these data coincided with our findings. In this study, we found that Ang II stimulation in RMCs led to PARP-1 activation, including upregulation of PARP-1 activity, mRNA and protein. PJ34 is a water-soluble, highly selective competitive inhibitor of PARP activity [33]. Devoid of inherent antioxidant activity, it functions by blocking the NAD⁺ binding site of PARP competitively [17, 34]. The enzymatic inhibition effect of PJ34 is much stronger than any other traditional inhibitor of PARP, which is 10,000-fold that of 3-AB [35]. In this study, PJ34 not only inhibited PARP activity, but also inhibited mRNA and protein expression of PARP-1, which is contrary to some studies [36]. However, the mechanism is still unclear. It may be involved in some unknown effects of PJ34 besides its competitive inhibition property. Further studies need to be done to explore the potential mechanism.

Our data estimated that inhibition of PARP-1 by PJ34 or its siRNA effectively attenuates Ang II-induced PAI-1 and FN expression in RMCs. It implied that PARP-1 might be a critical mediator in Ang II-induced ECM accumulation in RMCs. Several studies suggested that inhibition of PARP-1 could attenuate Ang II-induced pathological outcomes. PARP-1-deficient mice were protected from Ang II-induced cardiac hypertrophy. Therefore, PARP-1 is a nuclear mediator of Ang II-mediated cell signaling contributing to cardiac hypertrophy [37]. Ang II in rats and cultured endothelial cells induced PARP activation in a dose-dependent manner. The PARP inhibitors PJ34 or INO-1001 prevented the development of the endothelial dysfunction and restored normal endothelial function [18]. Treatment with Ang II promotes PARP-1 activation in cultured cardiac fibroblasts. Inhibition of PARP-1 by PJ34 or its siRNA oligonucleotide effectively prevented Ang II-induced increase in collagen I α 1 and III α 1, MMP-9 and TIMP-1 [32].

Since we demonstrated that PARP mediated Ang II-induced FN expression in RMCs, we further sought to un-

cover the underlying mechanism. Two main degrading systems are known to be involved, namely matrix metalloproteinases (MMPs) and the plasminogen activation system (PAS) [9–11, 38]. MMPs are a family of zinc-binding proteolytic enzymes capable of degrading components of the ECM [39, 40]. The plasminogen/plasmin system was first recognized for its fibrin-degrading activity and is the main efficient fibrinolytic system in vivo. The system consists of tissue-type (t-PA) plasminogen activators (PAs), urokinase-type (u-PA) PAs and their specific inhibitors PAI [38, 41]. PAI-1, a 50-kDa glycoprotein, is the main PAI secreted in vivo and is a potent fast-acting and irreversible inhibitor of t-PA and u-PA. It forms stoichiometric complexes with active PAs, which are subsequently endocytosed and degraded. Moreover, PAI-1 is also a component of the ECM, where it seems to bind tightly to the somatomedin B domain of vitronectin [42]. In pathological conditions in vivo, human mesangial cells overexpress PAI-1, and this upregulation may be related to the stimulation by various cytokines or growth factors such as TNF- α [43] and TGF- β [44]. In the present study, we have found the molecular mechanisms involved in a beneficial effect that PARP-1 inhibition decreased Ang II-induced upregulation of PAI-1 and FN expression in cultured RMCs. However, there is still no literature that elucidates the relationship between PARP-1 activation and PAI-1 peptides. Whether the inhibition of PAI-1 peptides is responsible for PARP-1-mediated PAI-1 expression needs to be elucidated.

In conclusion, our present data suggest that PARP-1 mediates Ang II-induced PAI-1 and FN expression in RMCs, and thus may represent a potential therapeutic target in the treatment of glomerular disease.

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