Mechanical strain increases SPARC levels in podocytes: implications for glomerulosclerosis

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Submitted 2 November 2004; accepted in final form 1 May 2005

Durvasula, Raghu V., and Stuart J. Shankland. Mechanical strain increases SPARC levels in podocytes: implications for glomerulosclerosis. Am J Physiol Renal Physiol 289: F577–F584, 2005; doi:10.1152/ajprenal.00393.2004.—Glomerular capillary hypertension is a final common pathway to glomerulosclerosis. Because podocyte loss is an early event in the development of glomerulosclerosis, it is logical that the deleterious effects of glomerular capillary hypertension involve podocyte injury. Yet, the mechanisms by which elevated intraglomerular pressure is translated into a maladaptive podocyte response remain poorly understood. Secreted protein acidic and rich in cysteine (SPARC) is a matricellular protein activated in various disease states of the podocyte and accelerates renal injury, as evidenced by the milder course of experimental diabetic nephropathy in SPARC-null mice compared with diabetic SPARC wild-type mice. Accordingly, we tested the hypothesis that mechanical strain activates SPARC in podocytes and thus is a putative mediator of podocyte injury in states of intraglomerular capillary hypertension. Conditionally immortalized mouse podocytes were subjected to 10% cyclical stretch while nonstretched cells served as controls. SPARC levels were measured in whole cell lysate and cell media. Immunostaining was performed for SPARC in an experimental model of glomerular capillary hypertension. Our results demonstrate cyclical stretch of podocytes markedly increased SPARC levels in cell lysate, through activation of p38, as well as secreted SPARC. Relevance was shown by demonstrating increased podocyte staining for SPARC in the uninephrectomized spontaneously hypertensive rat. In conclusion, we have made the novel observation that mechanical strain activates SPARC in podocytes and thus is a putative mediator of podocyte injury in states of intraglomerular capillary hypertension. We speculate that the increase in SPARC may be maladaptive and lead to a progressive reduction in podocyte number, thus fueling the future development of glomerulosclerosis.

METHODS

Cell culture. Experiments were performed using early passage growth-restricted, conditionally immortalized mouse podocytes (gift...
from Dr. P. Mundel) as we previously reported (10, 17, 30). When grown under permissive conditions (in the presence of α-interferon at 33°C), cells proliferate and display characteristics of undifferentiated podocytes. However, under growth-restricted conditions (absence of α-interferon at 37°C), proliferation is markedly reduced and cells undergo cytoskeletal rearrangement with the formation of arborizing cellular processes and express podocyte-specific proteins, resembling the morphological appearance of mature differentiated podocytes in vivo (26). Cells were grown on collagen type-1-coated plates in RPMI 1640 media containing 10% FBS (Summit Biotechnology, Ft Collins, CO), penicillin (100 U/ml), streptomycin (100 μg/ml), glucose (2 mmol/l), sodium pyruvate (1 mM, Irvine Scientific, Santa Ana, CA), HEPES buffer (10 mM, Sigma Chemical, St. Louis, MO), and sodium bicarbonate (0.075%, Sigma). Cells were growth restricted for greater than 10 days at 37°C in 95% air-5% CO2.

Experimental design for inducing mechanical strain of cultured podocytes. Growth-restricted conditionally immortalized podocytes were seeded onto flexible six-well plates coated with bovine collagen type-1 (Flexcell International, Hillisborough, NC) at a density of ~110,000 cells per well yielding an initial confluence of ~25%. Cells were allowed to adhere and further differentiate for 48 h, at which time culture plates were loaded onto a computer-assisted stretch apparatus (FlexCell Strain Unit 3000T) as we previously reported (31). Intermittent negative pressure was applied to the biomembrane by a vacuum, resulting in cyclical stretch and relaxation of the adherent cell layer. Based on prior studies by our group (10), a regimen of 60 cycles of stretch and relaxation per minute with an amplitude of 10% biaxial surface elongation was uniformly applied across the membrane. Cells grown under identical conditions (within the same incubator as the Strain unit), but not exposed to stretch, served as controls.

Measuring mRNA levels. To verify SPARC mRNA expression in conditionally immortalized mouse podocytes, semiquantitative RT-PCR was performed. Total RNA was harvested from growth-restricted cells using the TRIzol method (Sigma) as previously reported (31). Two micrograms of total RNA were reverse transcribed into cDNA using the oligo(dT) method (GIBCO BRL Superscript First Strand Synthesis System, Life Technologies, Rockville, MD) in 20-μl reaction volume; 1.0 μl of reaction volume was used for conventional PCR in a reaction volume of 50 μl, containing 1.5 mM MgCl2. Each PCR cycle involved an annealing step of 1 min, followed by a replication step of 2 min. Total RNA isolated from primary culture mouse mesangial cells was used as a positive control. The following primer sequences and annealing temperature were used: SPARC: 5’-GATGAGGTTGTCTGGCCACGCTATGAGGCCCTC-3’, 3’-GAACCGAATAGACCTAGTGGGGTCGACACCCAC-5’ (60°C).

To determine the effects of mechanical strain on SPARC mRNA expression, real-time PCR was performed, based on a quantitative colorimetric assay as recently described (19). PCR conditions were 2 min at 50°C, 10 min at 95°C, followed by 40 cycles at 95°C for 15 and 60 s for 1 min. A sample of stock cDNA (synthesized from pooled cDNA using the oligo(dT) method (GIBCO BRL Superscript First Strand Synthesis System, Life Technologies, Rockville, MD)) in 20-μl reaction volume, containing 1.5 mM MgCl2. Each PCR cycle involved an annealing step of 1 min, followed by a replication step of 2 min. Total RNA isolated from primary culture mouse mesangial cells was used as a positive control. The following primer sequences and annealing temperature were used: SPARC: 5’-GATGAGGTTGTCTGGCCACGCTATGAGGCCCTC-3’, 3’-GAACCGAATAGACCTAGTGGGGTCGACACCCAC-5’ (60°C).

The protein levels of SPARC were measured by immunoblotting studies of wild-type and knockout mesangial cells confirmed in preincubation studies with SPARC protein (34) as well as immune blotting studies of wild-type and knockout mesangial cells (2). Following three wash cycles with TBST, membranes were incubated with an anti-rabbit alkaline phosphatase-conjugated secondary antibody (1:2,000 dilution, Promega, Madison, WI) for 60 min at room temperature. The chromagen 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium (Sigma) was used for detection of the resultant bands. Densitometric quantitation was performed using ImagePro-Plus software (Media Cybernetics, Silver Spring, MD) and results were corrected to GAPDH levels, used as a housekeeper, to correct for any potential errors in loading. Recombinant human SPARC (gift from Dr. H. Sage) was used as a positive control.

Measuring secreted SPARC. Because SPARC is also secreted, cell media from stretched and nonstretched growth-restricted podocytes were collected at 18 and 24 and 48 h and centrifugation was performed to pellet cell debris. The supernatant was then transferred to Centricon-10 filter columns (Millipore, Bedford, MA) and concentrated to a final volume of ~250 μl supplemented with protease inhibitors. The content of fetal calf serum in the media was decreased to 0.5% (a level that, in our experience, does not appear to have deleterious effects on the podocyte line utilized) to prevent overload of the concentrated sample with serum proteins. Phenol red was omitted from the cell media thereby permitting determination of protein concentration using the RC DC assay (Bio-Rad Laboratories, Hercules, CA). Western blot analysis was performed using a goat polyclonal antibody specific for murine SPARC only (R&D Sytems, Minneapolis, MN) thus avoiding potential interference by bovine SPARC that may have been present in fetal calf serum. Densitometric quantitation was performed as previously outlined and, to correct for any potential discrepancy in cell number resulting from different experimental conditions, results were adjusted for total protein content of cell lysate.

Tissue levels of SPARC in a disease model of glomerular capillary hypertension. To ensure that the cell culture results also occurred in vivo, the uninephrectomized spontaneously hypertensive rat (SHR) model of glomerular capillary hypertension was utilized. Unilateral nephrectomy was performed on SHR rats at 5 wk of age. Animals were killed 7 and 10 wk following nephrectomy (time points at which elevated intraglomerular pressures have been documented), and the remaining kidney was harvested, fixed in Methacarn, and immunostaining was performed for SPARC. Tissue from age-matched sham-operated rats served as controls. Death of animals for kidney retrieval is done by isoflurane anesthesia followed by cardiac exsanguination and is in accordance with protocols approved by the University of Washington Animal Care Committee.

Briefly, tissue sections were deparaffinized in Histoclear (National Diagnostics, Atlanta, GA), rehydrated with ethanol, and treated with hydrogen peroxide to neutralize endogenous peroxidase. Tissue sections were incubated with primary rabbit polyclonal anti-SPARC

**AJP-Renal Physiol** • VOL 289 • SEPTEMBER 2005 • www.ajprenal.org
antibody 5944 overnight at 4°C, followed by a biotinylated goat anti-rabbit secondary antibody (1:100 dilution, Promega) for 60 min at room temperature, followed by ABC reagent (Vector Laboratories) for 20 min at room temperature. Color development was achieved by incubating in DAB solution at 37°C for 10 min and counterstaining in methyl green for 2 min. Substitution of the primary antibody with an irrelevant rabbit IgG served as a negative control. SPARC staining was measured using Optimus 6.5 System (Media Cybernetics) and expressed as the percentage of glomerular area staining positive for SPARC. A minimum of 20 glomeruli were examined per tissue section, and a total of 6 animals were studied in each group at each time point.

To confirm increased intensity of SPARC staining in podocytes, double staining was performed for SPARC and WT-1, a transcription factor expressed exclusively by the podocyte in the adult kidney where it is important in maintaining podocyte differentiation (37). Deparaffinization of tissue sections was carried out as outlined, and staining was performed using a primary polyclonal rabbit anti-WT1 antibody overnight at 4°C. Following application of biotinylated secondary antibody and ABC reagent as above, color development was performed in DAB supplemented with nickel to optimize nuclear staining pattern of WT1. Tissue sections were then incubated in 4% rabbit serum for 60 min at room temperature to saturate available binding sites of the secondary anti-rabbit antibody, followed by goat anti-rabbit F(ab) fragments (Jackson ImmunoResearch Laboratories, West Grove, PA) overnight at 4°C to mask the rabbit anti-WT1 primary antibody. Staining for SPARC was then performed as previously outlined.

MAPK signaling studies. Western blot analysis was used to determine which signaling pathways are activated in response to mechanical strain, utilizing antibodies that recognize the phosphorylated forms of p38, ERK, and SAPK/JNK obtained from Cell Signaling Technologies (Beverly, MA). To demonstrate the potential role of these specific pathways in mediating stretch-induced changes in SPARC, studies of growth-restricted podocytes were repeated in the presence of SB-202190 (selective inhibitor of p38 pathway), SP-600125 (selective inhibitor of JNK pathway), PD-98059 (inhibitor of ERK pathway), or DMSO (vehicle). Cells were preincubated in the presence of MAPK inhibitors (all obtained from Sigma) at a final concentration of 10 μM for 60 min before initiating cyclical stretch as previously outlined, and SPARC was measured by Western blot analysis.

Statistical analysis. Unless otherwise noted, all experiments were repeated on at least three separate occasions. Western blots were run using protein harvested on each occasion, and densitometric analysis was performed in triplicate on each blot. The results were pooled and presented graphically with error bars representing the standard deviation. Statistical analysis on data obtained was performed using paired t-test or ANOVA with a Bonferroni-Dunn correction (Statview 5.0, Abacus Concepts, Berkeley, CA). A P value <0.05 was considered statistically significant.

RESULTS

Conditionally immortalized mouse podocytes synthesize SPARC. We first ensured that podocytes grown in culture synthesize SPARC. Conditionally immortalized mouse podocytes were growth restricted for 14 days, and total RNA and protein were isolated from cell lysate. RT-PCR was used to determine SPARC mRNA expression, and the results are shown in Fig. 1A. While substitution of cDNA with water as a negative control produced no bands, the predicted 300-bp band was seen in podocytes; cDNA from mouse mesangial cell was used as a positive control for SPARC. A: RT-PCR was used to demonstrate SPARC mRNA expression in cultured podocytes. While substitution of cDNA with water as a negative control produced no bands, the predicted 300-bp band was seen in podocytes; cDNA from mouse mesangial cell was used as a positive control for SPARC. B: Western blot analysis confirmed the presence of SPARC in whole cell lysate of podocytes in culture. Recombinant human SPARC was used as a positive control. C: subcellular localization of SPARC in cultured podocytes by immunofluorescence staining. While substitution of the primary antibody with an irrelevant rabbit IgG produced no staining (left), the anti-SPARC 5944 primary antibody revealed a predominantly granular pattern throughout the cytoplasm with extension into cell processes (right).

Mechanical strain increases SPARC expression in podocytes in vitro. Glomerular capillary hypertension characterizes many forms of chronic kidney disease and perpetuates further podocyte injury. We were interested in determining the effects of elevated intraglomerular pressure on the levels of SPARC in podocytes. In an effort to mimic the mechanical strain experienced by podocytes in vivo, growth-restricted podocytes were exposed to cyclical stretch of 10% amplitude. As shown in Fig. 2A, cyclical stretch resulted in a marked increase in SPARC protein levels at 24 and 48 h compared with static controls. Densitometric analysis corrected for the housekeeping gene GAPDH confirmed a 5.9 (P < 0.01)- and 2.0-fold (P < 0.01) increase, respectively, in SPARC levels. Real-time PCR was performed to determine whether the stretch-induced increase in SPARC is under transcriptional control. As shown in Fig. 2B, mechanical strain resulted in a 1.4- and 1.6-fold increase in SPARC mRNA expression at 6 and 24 h, respectively, compared with nonstretched controls (P < 0.001). Taken together, these data suggest that the induction of SPARC in response to mechanical strain is under transcriptional regulation.
Mechanical strain increases SPARC secretion by cultured podocytes. As a member of the matricellular protein family, many of the effects of SPARC are mediated at the interface between cells and matrix constituents. We were therefore interested in determining whether mechanical strain leads to an increase in secretion of SPARC by podocytes in culture. Cell media were collected from stretched and nonstretched podocytes and Western blot analysis was performed for murine SPARC. As shown in Fig. 3, a marked increase in SPARC was detected in the media harvested from stretched cells compared with nonstretched control cells. To correct for any potential discrepancy in cell number under different experimental conditions, densitometric analysis was performed and results were adjusted for total protein content of cell lysate. As shown in Fig. 3, mechanical strain resulted in a 2.6-, 2.2-, and 2.3-fold increase in SPARC secretion at 18, 24, and 48 h, respectively, compared with nonstretched control cells (P < 0.01).

Podocyte levels of SPARC are increased in an experimental model of glomerular capillary hypertension. To demonstrate the relevance of our cell culture studies to the in vivo setting, we determined SPARC levels in an experimental model of glomerular capillary hypertension. The uninephrectomized SHR is a well-characterized noninflammatory model of glomerular capillary hypertension, resulting in progressive glomerulosclerosis and proteinuria. Micropuncture studies have reliably demonstrated elevated glomerular pressures beginning ~5 wk following uninephrectomy (11). Accordingly, Methacarn-fixed kidney sections were obtained 7 wk following uninephrectomy in SHR rats. As shown in Fig. 4, low levels of SPARC were detected in the glomerulus of a sham-operated control (A) or a control kidney excised before the development of increased glomerular pressure (B). In contrast, a marked increase in intensity of staining for SPARC is evident in uninephrectomized SHR rat at 7 wk (C, arrows) and is even more pronounced at 10 wk (D, arrows) following uninephrectomy. Specifically, when expressed as percentage of glomerular area (Fig. 4E), an 8.2- and 9.3-fold increase in SPARC staining was observed at 7 and 10 wk in the uninephrectomized SHR rats compared with control animals (P < 0.001). Specificity of the antibody was confirmed by the absence of staining following substitution of the primary antibody with an irrelevant rabbit IgG (data not shown).

To confirm increased intensity of SPARC staining in podocytes, double staining was performed for SPARC and WT-1, a transcription factor expressed exclusively by the podocyte in the adult kidney where it is important in maintaining podocyte differentiation. As shown in Fig. 5, nuclear staining for WT-1 (arrowhead, A) colocalizes with SPARC-positive cells in the glomerulus (arrows, B). Taken together, these data are consistent with our in vitro findings and suggest that podocytes are responsive to mechanical strain in states of elevated glomerular capillary pressures, resulting in an increase in SPARC production.
Stretch-induced upregulation of SPARC is p38 dependent. Having demonstrated that mechanical forces increase podocyte levels of SPARC in culture and experimental disease, we determined the signaling pathways involved. Western blot analysis for the phosphorylated (activated) forms of p38, ERK, and JNK was performed on whole cell lysate from stretched and nonstretched control cells. As shown in Fig. 6, when adjusted for total p38 levels as a loading control, densitometric analysis revealed a 2.4 (P < 0.02)-, 2.7 (P < 0.02)-, and 1.3-fold (P = 0.06) increase in phospho-p38 at 5, 20, and 40 min, respectively, in stretched cells compared with nonstretched controls. In contrast, mechanical strain did not alter the level of activation of JNK or ERK pathways in podocytes (results not shown).

The p38 MAPK family plays an important role in cellular responses to a variety of external stressors and has been implicated in mediating podocyte injury. To prove a role for p38 in mediating stretch-induced increase in SPARC, growth-restricted podocytes were preincubated for 60 min in media containing SB-202190 (10 μM), a selective inhibitor of the p38 pathway (9), and exposed to pulsatile strain as previously outlined. As shown in Fig. 7, inhibition of the p38 pathway by SB-202190 completely abrogated the increase in SPARC induced by mechanical strain at 48 h. In contrast, incubation with...
an equivalent volume of DMSO (vehicle for the p38 antagonist) had no demonstrable effect on SPARC levels, confirming specificity of action of SB-202190. While we did not observe activation of the ERK or JNK pathways by mechanical stretch, for the sake of completeness, preincubation studies were also performed in the presence of the selective inhibitors SP-600125 and PD-98059, respectively. Preincubation with inhibitors of the JNK pathway or ERK pathway did not have any appreciable effect on preventing the stretch-induced increase in SPARC (data not shown). Taken together, these data suggest that the upregulation of SPARC in response to mechanical strain occurs in a p38-dependent fashion.

**DISCUSSION**

A progressive reduction in podocyte number leads to the development of glomerulosclerosis (22). While the podocyte is a primary target of injury in many forms of glomerular disease such as membranous nephropathy, focal segmental glomerulosclerosis, and diabetic nephropathy, podocytes may be secondarily injured by mechanical forces resulting from glomerular capillary hypertension. Podocytes indeed are mechanosensitive as our group and others previously demonstrated (12). Exposure to excess levels of mechanical strain initiates a series of maladaptive responses. In contrast to mesangial cells that proliferate in response to mechanical forces (18), we showed that cyclical stretch is antiproliferative for podocytes in culture (31). Furthermore, we recently demonstrated that mechanical strain causes apoptosis of podocytes through activation of a local angiotensin system (10). Thus independent of the initial glomerular insult, elevated intraglomerular pressure in states of reduced nephron mass leads to distention of the capillary tuft, resulting in further podocyte loss, ultimately fueling the future development of glomerulosclerosis. Therefore, understanding mechanisms by which mechanical forces injure podocytes is of paramount importance.

With the use of a combined in vitro-in vivo approach, the major finding of this study was that mechanical forces characteristic of states of glomerular capillary hypertension lead to increased levels of SPARC in podocytes. Pulsatile strain experienced by podocytes was simulated using vacuum pressure to induce repetitive cell deformation. A regimen of 60 cycles per minute of 10% biaxial elongation was deemed a reasonable approximate of the degree of stretch experienced by podocytes in vivo as recently discussed (10). Exposure of conditionally immortalized mouse podocytes to cyclical stretch markedly increased SPARC levels in whole cell lysate as well as secreted SPARC into the extracellular milieu. Furthermore, mechanical strain increased SPARC mRNA levels, suggesting the stretch-induced increase in SPARC is under transcriptional regulation.

To demonstrate relevance of these findings to the disease state, immunohistochemistry for SPARC was performed in the uninephrectomized SHR. While the SHR strain is considered an animal model of essential hypertension with eventual renal injury restricted to the medullary nephrons, surgical reduction of nephron mass by uninephrectomy results in accelerated renal disease characterized by progressive proteinuria and widespread glomerulosclerosis (11, 39). Furthermore, intraglomerular capillary pressure predictably increases 5 wk following uninephrectomy and is a prerequisite for the subsequent development of glomerular injury (39). The uninephrectomized SHR rat is therefore a useful experimental model of glomerular capillary hypertension. At 7 wk following uninephrectomy, intensity of SPARC staining was increased in a podocyte distribution compared with sham-operated controls. Furthermore, studying different time points in the same animal revealed that before the development of glomerular capillary pressure (i.e., at the time initial nephrectomy was performed) glomerular staining for SPARC was minimal. In contrast, 10 wk following uninephrectomy (a time point at which intraglo-
meral capillary pressure is predictably elevated), intensity for SPARC staining in podocytes was much greater. Taken together, our data implicate that mechanical strain represents a novel mechanism responsible for increased SPARC production by podocytes.

Furthermore, we demonstrated that mechanical strain-induced increase in SPARC is dependent on activation of the p38 MAPK signaling pathway, an important regulator of cellular responses to external stressors. A role for p38 in regulating SPARC has been shown in endothelial cells following stimulation with VEGF. In our study, cyclical stretch increased activated p38 levels and preincubation with a selective inhibitor of p38 abrogated stretch-induced increase in SPARC levels in podocyte. Our findings are consistent with recent data published by Martineau et al. (25a), who demonstrated the role of p38 pathway in mediating stretch-induced COX-2 and PG EP4 expression in podocytes.

The role of SPARC in mediating glomerular disease is likely dependent on the site and type of injury. Studies from Pichler et al. (33) demonstrate that SPARC may be protective in disease states associated with a primarily mesangio proliferative response. An increase in SPARC was demonstrated by day 5 in the Thy.1 model, which coincided with resolution of the cellular proliferative response, due to antagonism of PDGF-induced mitogenic response (33). However, while SPARC may ameliorate injury in mesangial disease states, evidence suggests that it may exacerbate renal injury in disease states where the podocyte is the primary target. A recent study by Taneda et al. (38) examining the role of SPARC in the pathogenesis of chronic diabetic nephropathy demonstrated a marked increase in glomerular staining of SPARC predominantly restricted to the podocyte. Furthermore, when diabetes was induced in SPARC-null mice, the disease course was attenuated compared with wild-type counterparts, as evidenced by a reduction in albuminuria and degree of glomerulosclerosis. Indeed, SPARC levels have also been shown to correlate with severity of clinical diabetic nephropathy (20).

How could increased levels of SPARC fuel the development of podocytopenia and future glomerulosclerosis? We speculate that SPARC may lead to a reduction in podocyte number though its known antiproliferative effects. Although the podocyte is considered a terminally differentiated cell with limited proliferative capacity in vivo, it is reasonable to speculate that low levels of proliferation must be taking place to replace senescent cells shed in the urine, thereby maintaining podocyte number (40). Thus any process that compromises the limited proliferative capacity of the podocyte will result in the development of podocytopenia. We previously demonstrated that mechanical strain compromises the proliferative capacity of podocytes through decreased expression of cyclins D1, A, and B1 with a concomitant reduction in cdk2 activity (31). Beyond its function to antagonize mitogenic growth factors such as PDGF and VEGF in the extracellular milieu, as a constituent of nuclear matrix (16), SPARC may also act intracellularly to directly affect proliferation. Consistent with this notion is the recent finding that primary mesenchymal cells isolated from SPARC-null mice have higher rates of proliferation associated with markedly elevated levels of cyclin A compared with wild-type cells (4).

It is possible that the deleterious effects of SPARC are mediated through stimulation of TGF-β. While TGF-β is well known as a critical determinant of chronic fibrosis (41), recent studies in the transgenic mouse model demonstrated that TGF-β is directly involved in podocyte injury and subsequent development of glomerulosclerosis (36). Indeed, our group made the novel observation that TGF-β induces podocyte apoptosis in a p21-dependent fashion (Wada T, Pippin JW, Terada Y, and Shankland S, unpublished observations). A reciprocal relationship between SPARC and TGF-β has been demonstrated in many tissue systems (3, 14, 15). Recent studies by Schiemann et al. (35) showed that the antiproliferative effects of SPARC are mediated by TGF-β activation in an epithelial cell line. Furthermore, we showed that mechanical strain increases TGF-β expression in cultured podocytes (10).

Taken together, these observations raise the possibility that increased levels of SPARC in states of glomerular capillary hypertension may directly target events at the level of cell cycle to limit proliferation of podocytes.

In conclusion, using a combined in vitro-in vivo approach, we made the novel observation that mechanical strain of the podocyte, characteristic of disease states associated with glomerular capillary hypertension, increases SPARC via activation of the p38 MAPK pathway. We speculate that the increase in SPARC may be maladaptive and lead to a progressive reduction in podocyte number, thus fueling the future development of glomerulosclerosis.

**GRANTS**

This work was supported by Public Health Service Grants DK-051096, DK-60525, DK-56799, and DK-062759. S. J. Shankland is an Established Investigator of the American Heart Association. R. V. Durvasula is supported by a Mentored Career Development Award from National Institutes of Health (K08DK62759).

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